

Two new species of *Thelohanellus* Kudo, 1933 (Cnidaria: Mixosporea) from minor carps (*Labeo* spp. and *Cirrhinus reba*) of Tripura state, India

Remya Madhavan¹, Probir K. Bandyopadhyay¹ and B. Santosh²

¹ Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani, West Bengal, India

² Vizhinjam Research Centre of Central Marine Fisheries Research Institute, ICAR, Vizhinjam, Thiruvananthapuram, India

Summary

Studies of fish myxosporeans have become widespread because of the large scale mortalities caused by these organisms. Kudo (1933) separated the unicapsulated myxosporean species from bicapsulated ones and erected a new genus *Thelohanellus* for accommodating unicapsulated myxosporeans. In this paper, we describe two new myxosporean species, *Thelohanellus tripurensis* sp. n. and *Thelohanellus batae* sp. n., from the economically important native minor carps of the genera *Labeo* Cuvier and *Cirrhinus* Oken, namely *L. bata*, *L. calbasu*, *L. gonius* and *C. reba*, inhabiting the north-eastern Indian state of Tripura of total area of 10,491 sq. km.

Key words: Myxozoa, *Thelohanellus*, systematics, Tripura, minor carps

Introduction

The Myxosporean group was discovered in the first half of the nineteenth century. Bütschli (1881) termed these parasites as Myxosporidia for the first time. The first classification of Myxosporeans based on characteristics of spore was done by Thelohan (1892, 1895). Myxozoans with one iodophilous vacuole and one or two polar capsules were incorporated in the genus *Myxobolus* Bütschli, 1882 by Thelohan (1892). Later on Kudo (1933) isolated the unicapsulated species from bicapsulated ones and erected a new genus *Thelohanellus*, for accommodating the unicapsulated Myxozoan species, and there on the original *Myxobolus* Bütschli, 1882

included only bicapsulated forms of Myxozoan species.

Two new myxosporean species (*Thelohanellus tripurensis* sp. n. and *Thelohanellus batae* sp. n.) were found on fins and gills of carps collected in natural and artificial water basins of four different districts of Tripura (latitude 22° 51'–24° 32' N and longitude 90° 10'–92° 21' E). The new species are described here according to guidelines of Lom and Dyková (1992).

Material and methods

The host fishes were collected from the natural habitat represented by the principal rivers Gumti,

Mannu, Dhalai, Khowai, the Dumbur reservoir and also from the aquaculture ponds of all four districts of Tripura. Identifications of hosts were based on Jhingran (1975), Talwar and Jhingran (1991) and Jayaram and Dhas (2000). After recording the standard length and body weight of the host, different organs were removed and carefully examined for protozoan parasites.

The sporogonic plasmodia were isolated with sterile forceps and smeared on grease-free glass slides in 0.5% NaCl solution drops, covered with cover slips, and examined in bright field of the light microscope (LM) Leica DM 1000. 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 detection of any external mucous coating around the spores.

Following fixation in acetone-free absolute methanol, the air-dried smears were stained with Giemsa solution for making permanent preparations. The measurements based on twenty fresh spores treated with Lugol's iodine were done with the help of the calibrated ocular micrometer. Measurements of all the parameters are in μm and represented as mean \pm SD followed in parenthesis by the range. Drawings were made from fresh or stained material with the help of a mirror type camera lucida and the computer programme Corel Draw, Ver. 14.

The abbreviations used in this paper are as follows: CV – covariance; DIV – diameter of iodophilous vacuole; LLPC – length of large polar capsule; LPC – length of polar capsule; LS – length of the spore; LSPC – length of small polar capsule; N – Number of measurements; WLPC – width of large polar capsule; WPC – width of polar capsule; WS – width of spore; WSPC – width of small polar capsule.

Results and discussion

THELOHANELLUS TRIPURENSIS SP. N. (FIG. 1, PLATE 1)

Plasmodia. Plasmodia or cysts are found attached on the inner sides of the dorsal, pelvic and pectoral fins. They are round to slightly cylindrical in shape and creamy white in colour.

Spores. Fully mature spores have size dimension $17.0\text{--}18.0$ (17.4 ± 0.31) \times $9.5\text{--}11.0$ (10.1 ± 0.68) μm . The spores are egg-shaped in valvular view and lenticular in sutural view. They have slightly pointed anterior end and purely rounded posterior portion without any notches. The two shell valves are equal in thickness and smooth surfaced without any peripheral folds.

The polar capsule is found in the anterior portion of the spore cavity and is oval in shape. It measures $6.0\text{--}6.5$ (6.3 ± 0.18) μm in length and $4.1\text{--}4.7$ (4.4 ± 0.24) μm in breadth. About five to six spiral coils of the polar filament could be counted inside the polar capsule. The sporoplasm is thickly packed with homogenous granular particles. There is an iodophilous vacuole, $3.4\text{--}5.3$ (4.3 ± 0.82) μm in diameter, present towards the posterior part of the sporoplasm which is very conspicuous on treatment with Lugol's iodine solution.

The present species has close resemblance to *Thelohanellus bengalensis* Sarkar and Choudhury, 1986 reported from the gall bladder of *Catla catla* (Sarkar and Choudhury, 1986), *T. sanjibi* Sarkar and Ghosh, 1990 from the kidney of *Mystus guleo* (Sarkar and Ghosh, 1990), *T. sudevi* Sarkar and Ghosh, 1990 observed from the kidney of *Amblypharyngodon mola* (Sarkar and Ghosh, 1990) and *T. caudatus* Pagarkar and Das, 1993 from the caudal and anal fin rays of *Labeo rohita* (Pagarkar and Das, 1993).

The present species has larger spore size dimensions than *Thelohanellus bengalensis* (LS: 10.9; WS: 6.6; LPC: 5.4; WPC: 3.4). Moreover, the presence of iodophilous vacuole in the present specimen shows that it is really a new species distinct from *T. bengalensis*. The present species also exhibits superficial resemblance and affinity to *T. sanjibi* (LS: 12.5; WS: 8.3; LPC: 4.5; WPC: 4.0), *T. sudevi* (LS: 14.1; WS: 5.9; LPC: 5.2; WPC: 2.7) and *T. caudatus* (LS: 13.0–14.0; WS: 8.5–9.5; LPC: 7.0–7.5; WPC: 5.0–5.5); however on detailed morphometric comparison it was observed that these species are remarkably smaller in dimension than the present species.

In view of these differences with closely related species, the present myxozoan should be established as a new species. Hereby, it is named as *Thelohanellus tripurensis* sp. n. (Table 1).

Taxonomic summary.

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

Type locality: Ambassa, Dhalai district, Tripura.

Type specimen: Holotype on slide TH/LC/PR/09 and paratype on slide TH/LC/PR/11, Collection of Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani, West Bengal, India.

Prevalence: 1.7

Mean intensity: 1.05%

Infection loci: Pectoral, dorsal and caudal fins.

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

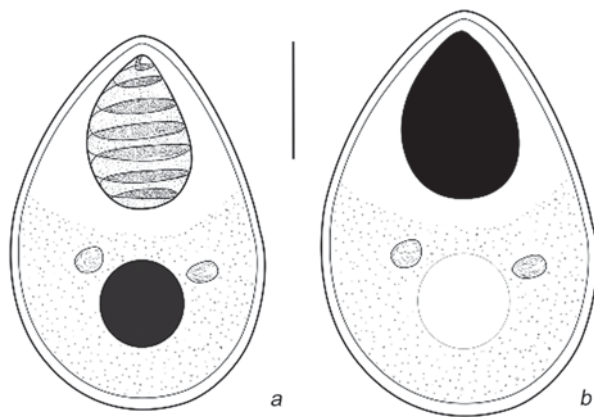


Fig. 1. Line drawing (Camera Lucida) of *Thelohanellus tripurensis* sp. n. spores. a – Fresh mature spore in valvular view showing iodophilous vacuole (Lugol's iodine stained); b – fixed spore in valvular view (Giemsa stained). Scale bar: 5 μm.

THELOHANELLUS BATAE SP. N. (FIG. 2, PLATE 2)

Plasmodia. Plasmodia remarkably elongated and oval in shape are found encysted on the gill filaments of the host fish.

Spores. The spores are pyriform in shape and strikingly elongated with rounded posterior and a blunt anterior proximity. There are no markings or folds on the valves. Mature spores measure 32.5–35.0 (33.7 ± 0.82) μm in length and 11.0–14.0 (12.6 ± 1.15) μm in breadth.

Large sized, pyriform polar capsule measuring 21.5–23.0 (22.4 ± 0.59) μm in length and 7.5–8.5 (8.0 ± 0.34) μm in breadth occupy a major portion of the spore cavity. There are about thirteen to sixteen coils of the polar filament. The filament extruded readily after treatment with 5 % KOH solution. Polar filaments measure 35.0–43.4 (37.2 ± 4.03) μm in length. Mucous envelope and iodophilous vacuole were not observed in samples treated with Indian ink and Lugol's iodine, respectively. Sporoplasm constitutes fairly small portion of the spore cavity, given large size of the polar capsule within the cavity.

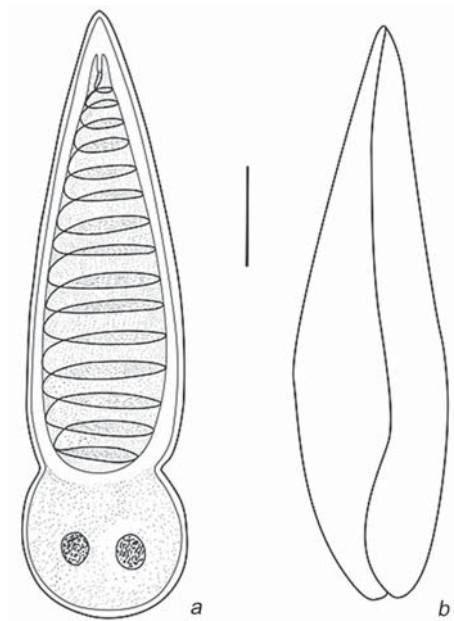


Fig. 2. Line drawing (Camera Lucida) of *Thelohanellus batae* sp. n. spores. a – Fresh mature spore in valvular view (Lugol's iodine stained); b – fresh mature spore in sutural view (Lugol's iodine stained). Scale bar: 5 μm.

Comparative study of morphological and morphometric characteristics of the spore and polar capsule with the closely related species shows that the present species has maximum affinity with the spores of *T. rohita* Southwell and Prashad, 1918 reported from gills of *Labeo rohita* (Southwell and Prashad, 1918) and *T. andhrae* Qadri, 1962 found on the gills of *Labeo fimbriatus* (Qadri, 1962). However both species, *T. rohita* (LS: 30.0–33.0; WS: 10.0–13.0; LPC: 16.0–20.0; WPC: 7.0–8.0) and *T. andhrae* (LS: 11.2–14.5 WS: 4.5–5.5; LPC: 6.0–8.0; WPC: 2.0–2.5), are much smaller in size compared to the present species. Moreover, a characteristic feature of *T. andhrae*, the polar capsule adhering to the spore wall, is absent in the new species.

Therefore, taking into account all the differences with the closely related species the present species is designated as a new species, *Thelohanellus batae* (Table 2).

Table 1. Morphometric parameters of *Thelohanellus tripurensis* sp. n.

Morphometric parameters	N (number of measurements)	Range (μm)	X (μm)	SD (μm)	SE (μm)	CV(%)
LS	20	17.0–18.0	17.4	0.31	0.07	1.78
WS	20	9.5–11.0	10.1	0.68	0.15	6.73
LPC	20	6.0–6.5	6.3	0.18	0.04	2.86
WPC	20	6.0–6.5	6.3	0.18	0.04	2.86
DIV	20	6.0–6.5	6.3	0.18	0.04	2.86

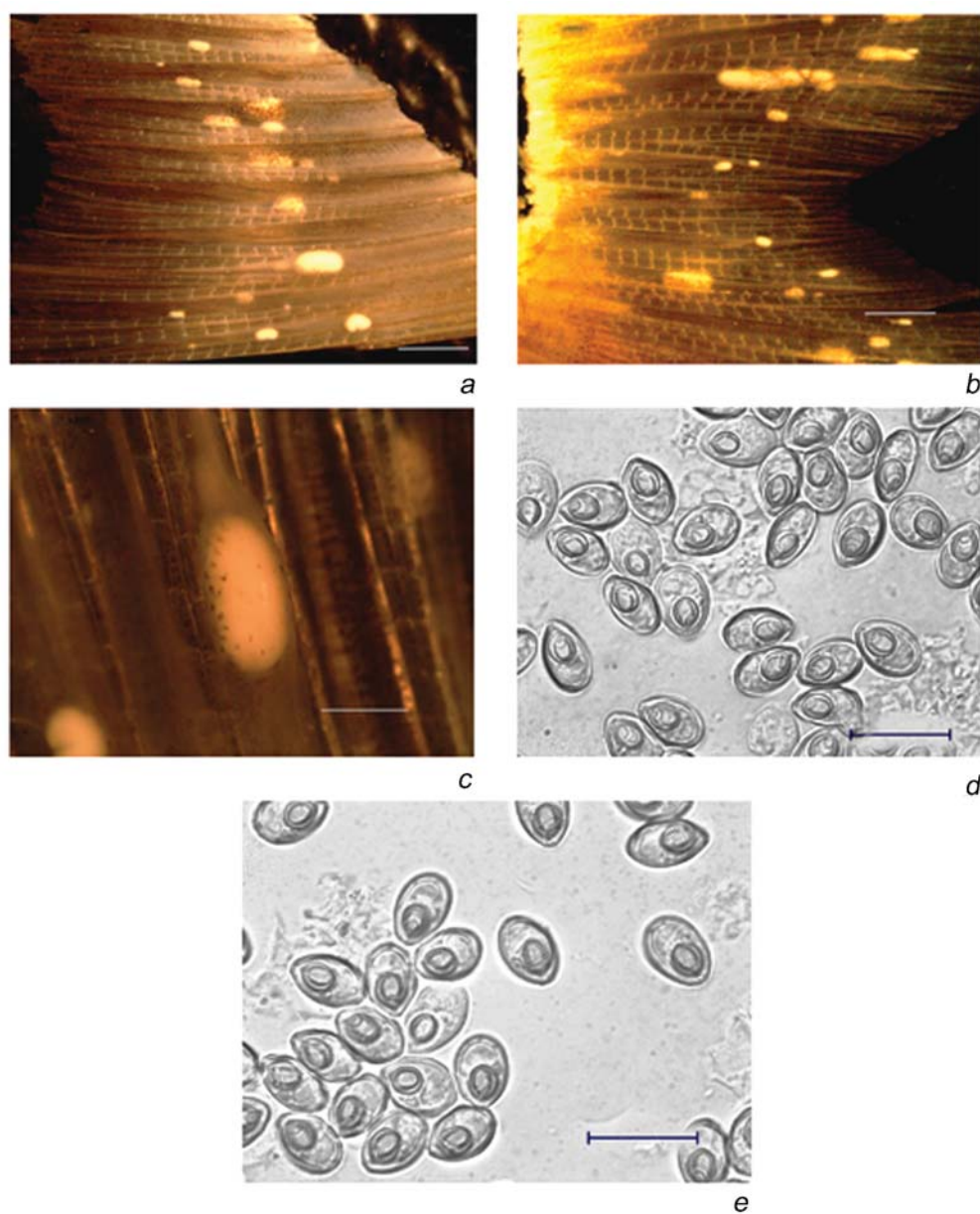


Plate 1. Photomicrographs of plasmodia and cysts of *Thelohanellus tripurensis* sp. n. a – attached to the dorsal fin of *Labeo calbasu*, b – attached to the caudal fin of *Labeo gonius*; c – enlarged view of the cyst; d, e – spores show variations in appearance. Scale bars: a, b – 700 µm, c – 120 µm, d, e – 20 µm.

Table 2. Morphometric parameters of *Thelohanellus batae* sp. n.

Morphometric parameters	N (number of measurements)	Range (µm)	X (µm)	SD (µm)	SE (µm)	CV(%)
LS	20	32.5-35.0	33.7	0.82	0.18	2.43
WS	20	11.0-14.0	12.6	0.15	0.26	9.13
LPC	20	21.5-23.0	22.4	0.59	0.13	2.63
WPC	20	7.5-8.5	8.0	0.34	0.08	4.25
LPF	20	35.0- 43.4	37.2	4.03	0.90	10.83

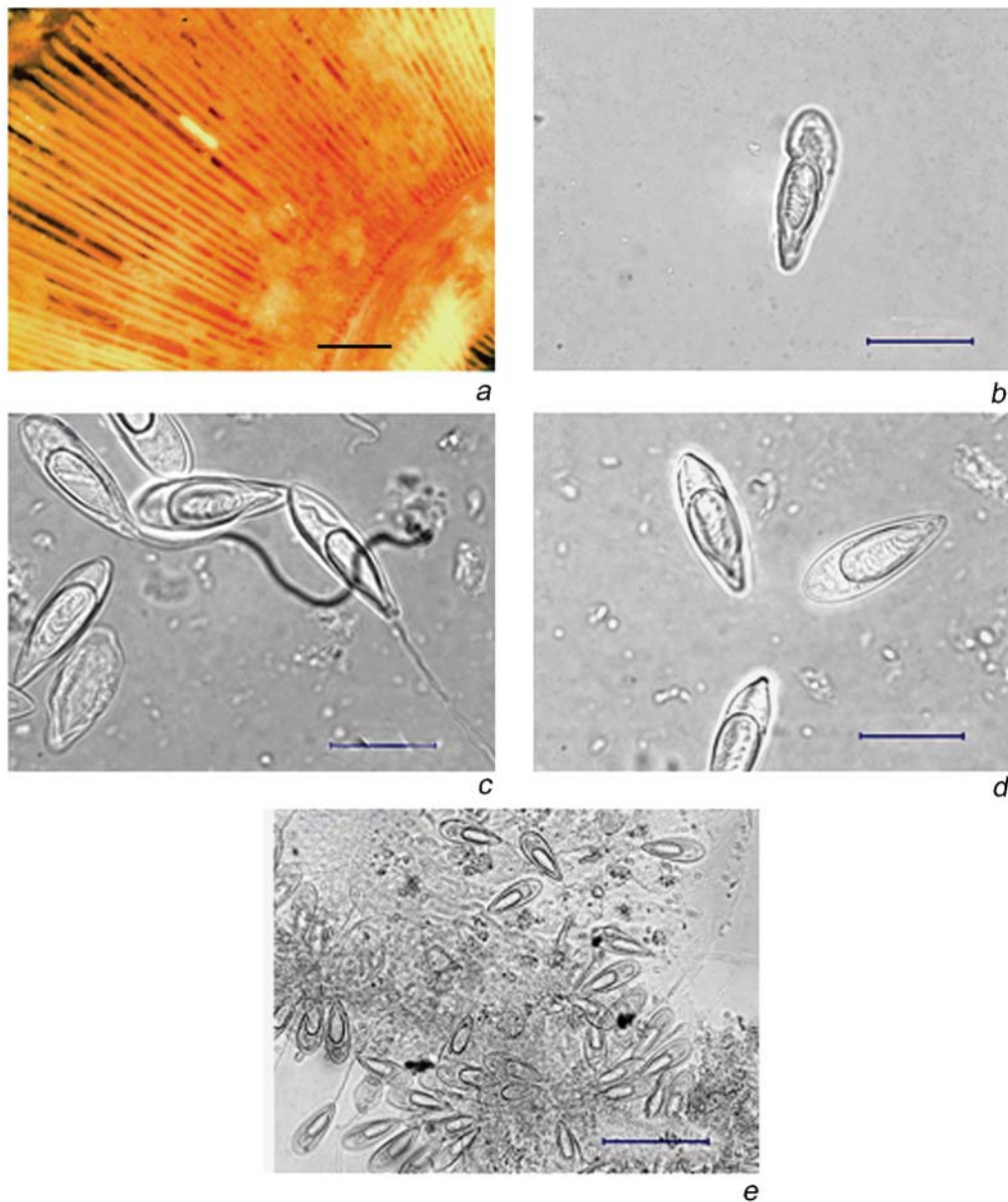


Plate 2. Photomicrographs of *Thelohanellus batae* sp. n. a – Cyst attached to the middle region of gill of *Labeo bata*; b-e – fresh spores show variations in appearance. Scale bars: a – 700 μm , b, c, d – 20 μm , e – 50 μm .

Taxonomic summary.

Type host: *Labeo bata* (Hamilton, 1822).

Type locality: Udaipur, South Tripura.

Type specimen: Holotype on slide TH/LC/PR/09 and paratype on slide TH/LC/PR/11, Collection of Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani, West Bengal, India.

Prevalence: 1.7

Mean intensity: 1.05%

Infection loci: Pectoral, dorsal and caudal fins.

Etymology: The specific name *batae* denotes the host *Labeo bata* from which the parasite was obtained.

Acknowledgements

The Head of the Department of Zoology, University of Kalyani and the authorities of ICAR, Tripura Centre are humbly acknowledged for providing the necessary laboratory facilities.

References

- Bütschli O. 1881. Myxosporidien. Zool. Jb. 1, 162–164.
- Jayaram K.C. and Dhas J.J. 2000. Revision of the genus *Labeo* from the Indian region with a discussion on its phylogeny and zoogeography (Pisces: Cypriniformes, Cyprinidae, Cyprininae). Records of Zoological Survey of India. Occ. Paper No.183, 1–143.
- Jhingran A.G. 1975. Fish and fisheries of India. Hindustan Publishing Corporation, Delhi.
- Kudo R.R. 1933. A taxonomic consideration of Myxosporidia. Trans. Amer. Microsc. Soc. 52, 195–216.
- Lom J. and Vávra J. 1963. Mucous envelopes of spores of the subphylum Cnidospora (Doflein). Vestník Československe zoologiske spolecnosti. 27, 4–6.
- Lom J. and Dyková I. (Eds). 1992. Protozoan parasites of fishes. Developments in aquaculture and fisheries. Elsevier, Amsterdam.
- Pagarkar A.U. and Das M.K. 1993. Two new species of Myxozoan, *Thelohanellus caudatus* n. sp. and *Myxobolus serrata* n. sp. from cultured carps. J. Inland Fisheries Society of India. 25, 30–35.
- Qadri S.S. 1962. New Myxosporidia from Indian freshwater fish, *Labeo fimbriatus*. II. *Thelohanellus andhrae* sp. n. Z. Parasitenkd. 21, 517–520.
- Sarkar N.K. and Choudhary S.R. 1986. *Thelohanellus bengalensis* sp. n. and *Myxidium mystusium* sp. n. (Myxozoa): two new Myxosporidia from Indian fresh water teleosts. Acta Protozool. 25, 359–362.
- Sarkar N.K. and Ghosh S. 1990. Two new Myxozoan parasites of the genus *Thelohanellus* Kudo 1933 (Myxosporidia; Myxobolidae) from freshwater fishes of West Bengal, India. New Agriculturist. 1, 35–38.
- Southwell T. and Prasad B. 1918. Notes from the Bengal fisheries laboratory No. 5. Parasites of Indian fishes with note on carcinoma in the climbing perch. Records of the Indian Museum. 15, 341–355.
- Talwar D.K. and Jhingran A.G. 1991. Inland fishes of India and adjacent countries. Vol. 1 and 2. Oxford and IBH Publ. Co., New Delhi.
- Thelohan P. 1892. Observation sur less myxosporidies et essai de classification de ces organismes. Bull. Soc. Philom. 4, 165–178.
- Thelohan P. 1895. Recherches sur les Myxosporidies. Bull. Sci. France et Belg. 26, 100–394.

Address for correspondence: Remya Madhavan. Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani, West Bengal, India; e-mail: remyamadhavan364@gmail.com