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Description of new species of *Myxobolus* Bütschli, 1882 and *Thelohanellus* Kudo, 1933 from the *Cirrhinus mrigala* Hamilton, 1822 an edible fish of India

Sukanta Majumder[1], P.K. Bandyopadhyay[2]
[1](Department of Zoology, Siliguri College, India)
[2](Parasitology Laboratory, Department of Zoology, University of Kalyani, India)
Corresponding Author: Sukanta Majumder

Abstract: *Myxobolus* Bütschli, *1882 and Thelohanellus* Kudo, *1933 are important groups of Myxozoan parasitic protozoa infecting a large number of freshwater fish species worldwide. Myxozoan parasites cause gill myxoboliasis in fishes throughout different geographical areas. The severity of Myxobolus and Thelohanellus infection leads to heavy loss in production of fishes. During the survey of the fish parasites, one species from each of the genus Myxobolus and Thelohanellus which appears to be new have been isolated from the gills of a freshwater edible fish, Cirrhinus mrigala* Hamilton, *1822 from West Bengal, India which has been described here. The isolated mature Myxobolus spores have a length of 10.75µm ±0.23 µm (10.40µm -11.02µm) with a width of 4.90 µm ±0.19µm (4.59µm -5.20µm). The spore is tear-shaped with anterior end bluntly pointed and greatly rounded posterior end. Towards the anterior portion the spore takes a nearly bottle-neck shaped with a small knob like structure. Both the polar capsules are similar, pear shaped which measures of 4.86µm ±0.31µm (4.28µm -5.30µm) in length and width of 2.05µm ±0.08µm (1.94µm -2.14µm). The isolated mature Thelohanellus spores have a length of 17.33µm ±0.35µm (16.89µm -17.72µm) with a width of 7.76µm ±0.21µm (7.46µm -8.02µm). They are elongated tear shaped with bluntly pointed anterior end and rounded posterior end. The single polar capsule measures 13.34µm ±0.14µm (13.14µm -13.56µm) in length and width of 4.68µm ±0.09µm (4.54µm -4.82µm) and is elongated tear shaped with slightly pointed anterior end.*

Keywords: *Cirrhinus mrigala - Myxozoan parasite – Myxobolus - Thelohanellus – Spores – Polar capsules - West Bengal*

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I. Introduction

Myxosporeans are considered to be one of the most abundant and diverse group of fish parasites found in different geographical areas (Landsberg & Lom, 1991, Lom and Dykova, 1992). *Myxobolus* Bütschli, 1882 comprises more than 1200 available species commonly found in fish (Lom and Dyková, 1992). Myxozoan parasites cause gill myxoboliasis in fishes throughout different geographical areas. Among them, the genus *Myxobolus* Bütschli, 1882 is the largest group of the Myxobolidae family containing 744 described species (Eiras et al.2005) and has been reported as an important pathogen in fish. Currently, the myxozoan spores having two polar capsules, with or without iodinophilus vacuoles and generally two sporogenic nuclei are placed under the Genus *Myxobolus* Bütschli, 1882 (Kudo, 1933). *Thelohanellus* Kudo, 1933 is the second most prevalent genus after *Myxobolus* Bütschli, 1882 having 60 valid species (Lom and Dyková, 2006). Spores of the genus *Thelohanellus* are tear to pyriform in shape, broadly ellipsoidal in valvular view and slim in sutural view. Spores contain a pyriform polar capsule with a 1–2 coils of polar filament. Sporoplasm contains two sporoplasmic nuclei.

During the present study, myxosporean spores belonging to two genera, namely *Myxobolus* and *Thelohanellus* have been identified from *Cirrhinus mrigala* Hamilton, 1822. The species have been described according to the guidelines of Lom and Arthur (1989) and Lom and Dykova (1992).

II. Materials and methods

Host fishes were collected from the Naihati fish farm, North 24 Parganas, West Bengal, India and brought alive to the laboratory and cultured in the vats near the laboratory. Host fishes were examined time to time. The fishes were dissected and the gill smears were taken in the grease free slides (1 mm) and air dried. The slides were then fixed in methanol for 2–8 min. Then these slides were taken out and Giemsa stain was put on it. This Indian ink method of Lom and Vavra´ (1963) was employed to identify the myxozoan spore and for permanent preparation. It was noticed that each slides were fully dipped into this stain. Staining was done for

about 25–30 min and then washed in tap water. The slides were then covered with coverslips and mountant with DPX. The slides were then observed under microscope. Sporogonic plasmodia, when found, were carefully removed with sterile needles, smeared on clean grease free slides with drops of distilled water, covered with cover slips and sealed with DPX for examination of fresh spores under the oil immersion lens of Olympus KH phase contrast microscope. Some of the fresh smears were treated with various concentrations of KOH (2–10 %) for the extrusion of polar filament.

Some fresh smears containing the spore were treated with Lugol's Iodine solution for the detection of iodinophilus vacuoles. Measurements based on twenty fresh spores (stained with both Lugol's Iodine and Geimsa) were done with a calibrated ocular micrometer. All measurements are presented in micrometer (lm) as mean ± SD followed by parentheses by the range. Stained spores were examined under the oil immersion lens of Olympus KH phase contrast microscope. Photomicrographs of the stained spores were also taken with the help of an Olympus KH phase contrast microscope fitted with Olympus camera in 100X magnification.

Abbreviations:
LS- Length of the spore
WS- Width of the spore
LPC- Length of the polar capsule
WPC- Width of the polar capsule
SD- Standard Deviation
SE- Standard Error
CV- Coefficient of Variance

III. Results

Taxonomic Summary of *Myxobolus bulbulus* sp. n.
Type host: - *Cirrhinus mrigala* Hamilton
Locality: - Naihati (22.90º N and 88.42º E), North 24 Parganas, West Bengal, India.
Type specimens: - Slide No. MB/PA/KU/13-14 containing Holotype and four slides MB/PAKU/15-18 containing Paratype have been deposited in the collection of Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, W.B.
Symbiotype:- The host specimen bearing no. CM/PARA/08-14 deposited in the museum of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, W.B.
Site of infection:- Gill lamellae.
Prevalence of infection:- 14 out of 22 hosts examined (63.64 %).
Spore description
Plasmodia- These were isolated from the gills of the infected fish. Creamy yellow plasmodia are ovoid to elongate in shape, and isolated from the gill lamellae of the infected fishes (2-3 mm in diameter). It contains both late developmental stages and mature spores.
Spore- The spore is tear-shaped with anterior end bluntly pointed and greatly rounded posterior end. The spore takes a nearly bottle-neck shape with a small knob like structure towards the anterior portion. Two polar capsules are pyriform and pear shaped. The polar filament is thin and makes 8-9 tightly packed coils inside each polar capsule. The granular and homogenous mass of cytoplasm fills the extra capsular region of the spore cavity. The sporoplasm contains two very minute nuclei. The measurements are given in the Table 1.
Spore index:
LS: WS = 1: 0.456
LPC: WPC = 1: 0.422
LS: LPC = 1: 0.452
WS: WPC = 1: 0.418

Table 1: Statistical analysis of measurements of the spores of *Myxobolus bulbulus* sp. n.

Measurements	Range (µm)	Mean (µm)	SD	SE	CV%
Length of the spore (LS)	10.40-11.02	10.75	0.23	0.13	0.02
Width of the spore (WS)	4.59-5.20	4.90	0.19	0.16	0.04
Length of the polar capsule (LPC)	4.28-5.30	4.86	0.31	0.17	0.07
Width of the polar capsule (WPC)	1.94-2.14	2.05	0.08	0.16	0.03

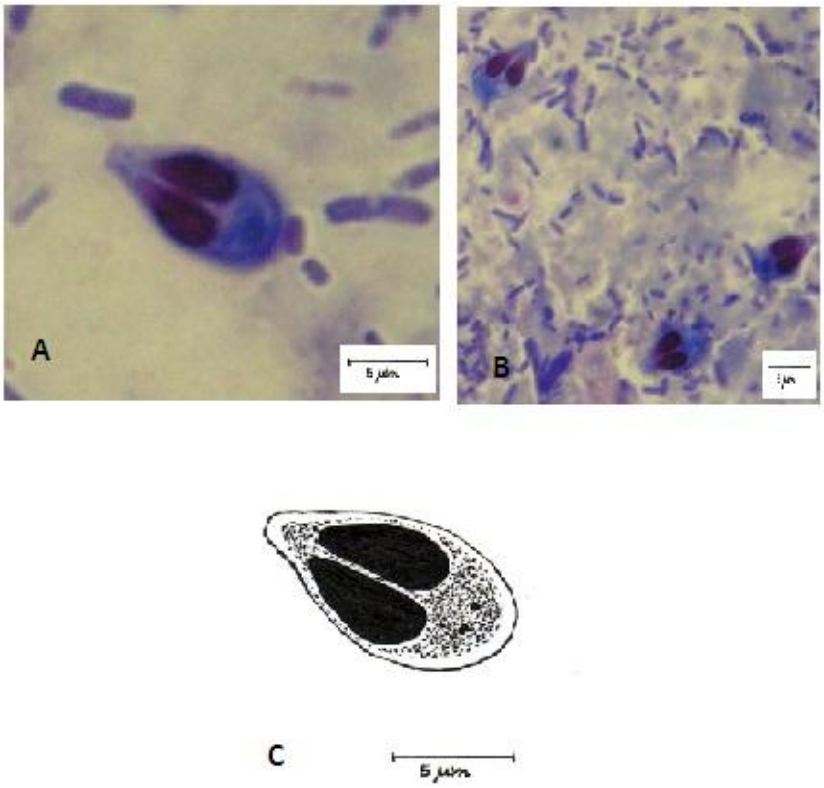

Fig 1: A-B- Photomicrographs of *Myxobolus bulbulus* sp. n. (Scale bar 5 µm); C- Camera lucida drawing of *Myxobolus bulbulus* sp. n. (Scale bar 5 µm)

Specific epithet The name of the species has been given due to its small bulb like structure.

Taxonomic Summary of *Thelohanellus haldari* sp. n.
Type host: - *Cirrhinus mrigala* Hamilton
Locality: - Naihati (22.90º N and 88.42º E), North 24 Parganas, West Bengal, India.
Type specimens: - Slide No. TH/PA/KU/10-11 containing Holotype and four slides TH/PA/KU/12-15 containing Paratype have been deposited in the collection of Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, W.B.
Symbiotype:- The host specimen bearing no. CT/PARA/04-10 deposited in the museum of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, W.B.
Site of infection:- Gill lamellae.
Prevalence of infection:- 08 out of 22 hosts examined (36.36 %).
Spore description
Plasmodia- The plasmodia are creamy white in color. They are rod shaped and are found attached with the gill lamallae. It contains both late developmental stages and mature spores.
Spore- Mature spores are elongated tear shaped with bluntly pointed anterior end and rounded posterior end. The single polar capsule is elongated tear shaped with slightly pointed anterior end. The polar filament makes 14-16 coils inside the capsule. Two small nuclei and a large rounded iodinophilous vacuole are present within the sporoplasm. There are no bulbular structures or mucous membrane at the posterior extremity of the spore. The measurements are given in Table 2.
Spore index
LS: WS = 1: 0.448
LPC: WPC = 1: 0.351
LS: LPC = 1: 0.769
WS: WPC = 1: 0.603

Table 2: Statistical analysis of measurements of the spores of *Thelohanellus haldari* sp. n.

Measurement	Range(µm)	Mean(µm)	SD	SE	CV%
Length of the spore (LS)	16.89-17.72	17.33	0.35	1.30	2.2
Width of the spore (WS)	7.46-8.02	7.76	0.21	0.85	2.7
Length of the polar capsule (LPC)	13.14-13.56	13.34	0.14	1.14	1.0

Width of the polar capsule (WPC)	4.54-4.82	4.68	0.09	0.63	2.1
Diameter of the iodinophilus vacuole (DIV)	3.15-3.42	3.26	0.24	0.46	1.2

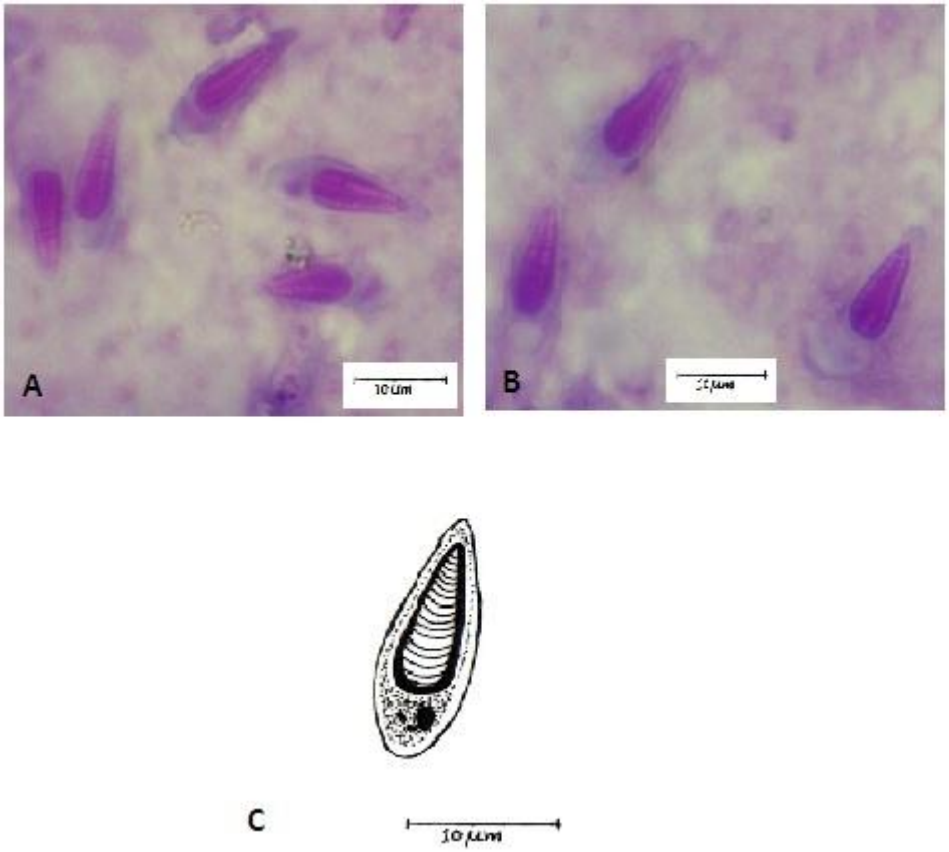

Fig 2: A-B- Photomicrographs of *Thelohanellus haldari* sp. n. (Scale bar 10 µm); C- Camera lucida drawing of *Thelohanellus haldari* sp. n. (Scale bar 10 µm)

Specific epithet The name of the species has been given to commemorate Late Professor D. P. Haldar, renowned Protozoologist, for his outstanding contribution in the field of Fish Parasitology.

IV. Discussion

Taxonomic affinity of *Myxobolus* n.sp.

The Myxozoan parasite under discussion having two polar capsules, with two sporogenic nuclei justifies its inclusion under the family Myxobolidae and genus *Myxobolus* Bütschli, 1882 as described by Kudo (1933). The present species resembles a number of Myxobolus species in morphology or morphometry of the spores and polar capsules. These species includes *M. bhadurius* (Sarkar, 1985(a)) Landsberg & Lom, 1991; *M. braziliensis* Casal et al., 1996; *M. chinsurahensis* Basu & Haldar, 2003; *M. distichodi* Kostoı¨ngue´ & Toguebaye, 1994; *M. mugilii* Haldar et al., 1996; *M. perforata* Ali et al., 2002.

The present species is larger in comparison to *M. braziliensis*, *M. chinsurahensis* and *M. perforata* while it is smaller than *M. mugilii* in respect of its length. The present species shows similarity in length with *M. bhadurius* and *M. distichodi*. Whereas, M. braziliensis, *M. chinsurahensis*, *M. perforata*, *M. mugilii*, *M. bhadurius*, *M. distichodi* are larger in relation to the width of the spore than the present species. The length of polar capsule is bigger in case of *M. bhadurius*, *M. braziliensis*, *M. mugilii* and *M. perforata* while it is smaller in *M. chinsurahensis* and *M. distichodi* in comparison to present form. The width of the polar capsule is larger in *M. bhadurius*, *M. mugilii* and *M. perforata* but resembles closely with *M. chinsurahensis*. Where as it is smaller in case of *M. braziliensis* and *M. distichodi* than that of the present form. Furthermore, the shape of the spore also varies. It is oval and somewhat elongated in shape in all the compared species where as it is tear shaped with anterior end bluntly pointed and greatly rounded posterior end in the present form. The size of the spore of the present form resembles closely to *M. perforata*, in respect to its length and morphometry but the anterior portion the spore takes a nearly bottle-neck shaped with a small knob like structure in the present one. The overall comparison is shown in table 3.

Table 3: Taxonomic affinity of *Myxobolus bulbulus* sp. n.

Name of the Species	**Host**	**Site Infection**	**Length of Spore (µm)**	**Width of Spore (µm)**	**Length of Polar Capsule (µm)**	**Width of Polar Capsule (µm)**
M. bhadurius (Sarkar, 1985(a)) Landsberg & Lom, 1991	Gall-bladder	*Wallago attu*	10.6 (8.8-11.2)	6.3 (4.8-6.7)	5.3 (4.0-6.4)	2.8 (2.4-3.2)
M. braziliensis Casal et al., 1996	Base of secondary gill lamellae	*Bunocephalus coracoideus*	10.2 (9.4-10.9)	5.2 (4.7-5.9)	5.3 (5.0-5.4)	1.4 (1.4-1.4)
M. chinsurahensis Basu & Haldar, 2003	Scales	*Anabus testudineus*	8.4 (8.0-9.7)	5.4 (5.1-6.1)	4.4 (3.9-6.6)	2.1 (1.8-2.5)
M. distichodi Kostoı¨ngue´ & Toguebaye, 1994	Gill, Intestine, Liver	*Distichodus engycephalus*	10.6 (10-11)	5.7 (5-6)	4.4 (4-5)	1.8 (1.5-2)
M. mugilii Haldar et al., 1996	Gills	*Mugil cephalus*	11.7 (8.1-16.3)	5.5 (4.0-7.3)	6.1 (2.4-8.1)	2.7 (1.6-4.0)
M. perforata Ali et al., 2002	Internal surface	*Hydrocynus forskalii*	10.4 (9.9-11.3)	5.2 (4.5-5.9)	5.2 (4.0-5.4)	2.4 (1.2-2.7)
Myxobolus n. sp. present study	Gill lamellae	*Cirrhinus mrigala*	10.75 (10.40-11.02)	4.90 (4.59-5.20)	4.86 (4.28-5.30)	2.05 (1.94-2.14)

Considering all these aspects, the present *Myxobolus* species under description is new to science and hence, it is designated as *Myxobolus bulbulus* sp. n.

Taxonomic affinity of *Thelohanellus haldari* sp. n.

The Myxozoan parasite under discussion having single polar capsule, with two sporogenic nuclei justifies its inclusion under the family Myxobolidae and genus *Thelohanellus* Kudo 1933 as described by Kudo (1933). The present species resembles a number of *Thelohanellus* species in morphology or morphometry of the spores and polar capsule. These species includes *T. avijiti* Basu & Haldar, 2003; *T. habibpuri* Acharya and Dutta, 2007; *T.imphalensis* Hemananda et al., 2010 and 2011; *T. niloticus* Ghaffar et al., 2012; *T. seni* Chakravarty and Basu, 1948; *T. shortii* Qadri, 1967.

The present species is larger in comparison to *T. avijiti, T. habibpuri, T. seni and T. shortii* while it is smaller than *T.imphalensis and T. niloticus* in relation to its length. On the other hand, *T. avijiti, T. habibpuri, T.imphalensis, T. niloticus and T. seni* are larger in relation to width of the spore than that of the present species. Whereas, the present species is larger, in relation to the width of the spore, than that of *T. qadri*. The length of polar capsule in the present species is bigger than the other compared species. Whereas the width of the polar capsule is smaller in *T. avijiti, T.imphalensis and T. shortii* but resembles closely with *T. habibpuri ,T. niloticus and T. seni* . Furthermore, the shape of the spore also varies. The present species is elongated in shape similar to all the compared species where as it has anterior end slightly blunt pointed and rounded posterior end. The size of the spore of the present form resembles with *T. avijiti, T. habibpuri, T. seni and T. shortii*, in respect to its length and morphometry but the polar capsule is elongated tear shaped in comparison to the rounded in those compared species. On the other hand, the present species shows similarity in respect of length of the polar capsule with *T.imphalensis and T. niloticus* but the spore size differs in case of that two species than that of the present form. The overall comparison is shown in table 4.

Table 4: Taxonomic affinity of *Thelohanellus haldari* sp. n.

Name of the Species	**Host**	**Site Infection**	**Length**	**Width**	**Length of Polar Capsule (µm)**	**Width (µm)**
T. avijiti Basu and Halder, 2003	Dorsal fin	*Labeo rohita*	14.0	9.7	6.0	4.0
T. habibpuri Acharya and Dutta, 2007	Pectoral fin	*Labeo rohita*	13.9 (13.0-14.3)	8.5 (8.0-9.0)	6.0 (6.0-6.5)	4.9 (4.1-5.0)
T. imphalensis Hemananda et al., 2010/2011	Gills	*Labeo rohita*	21.33 (20.4-22.1)	9.43 (8.	10.79 (10.2-	3.78 (3.40-4.25)

				5-10.2)	11.05)	
T. niloticus Ghaffar et al., 2012	Gills	*L. niloticus*	23.3	13.4	11.7	4.7
T. seni Chakravarty and Basu, 1948	Branchiae	*Catla catla*	12.48-14.94	8.56	6.42	4.52
T. shortii Qadri, 1967	Fin	*Labeo fimbriatus*	12.53 (11.42-12.85)	6.91 (6.42-7.14)	7.07 (6.42-7.95)	4.2 (3.5-4.28)
Thelohanellus n. sp. present study	Gill lamellae	*Cirrhinus mrigala*	17.33 (16.89-17.72)	7.76 (7.46-8.02)	13.34 (13.14-13.56)	4.68 (4.54-4.82)

Considering all these points, the present *Thelohanellus* species under description is new to science and hence it is designated as *Thelohanellus haldari* sp. n.

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