

TWO NEW *THELOHANELLUS* KUDO, 1933 (MYXOZOA: MYXOSPOREA: BIVALVULIDA) INFECTING FINS OF MAJOR CARPS IN WETLANDS OF PUNJAB

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(E-mail: *harpreet_bimbira@yahoo.com; **ranjitsrana@gmail.com)**ABSTRACT**

A survey of parasites of freshwater fishes in Ropar and Kanjali wetlands of Punjab (India) revealed the presence of two new myxosporean species belonging to the genus *Thelohanellus* Kudo, 1933 parasitizing caudal fins from various freshwater fishes. Spores of the first species, *T. deri* sp. nov. (10.0x5.0µm) are elongately pyriform in valvular view having sharply pointed anterior end and broad rounded, bulb-like posterior end demarcated by a small constriction located medioposteriorly. Polar capsule is broadly pyriform in shape (5.4x3.6µm) with sharply pointed anterior end and broad rounded posterior end and is situated anteriorly in the spore body cavity. Spores of the second species, *T. halderi* sp. nov. (12.6x6.2µm) elongated oval in valvular view having blunt rounded anterior end with a distinct pore and rounded posterior end. Polar capsule is oblong oval to spherical in shape (7.0x4.8µm) situated at the anteriomedian position in the spore body cavity. Lateral sides of the spore are parallel to each other throughout the spore length.

KEY WORDS: caudal fins, Kanjali, Ropar, spore, wetlands**INTRODUCTION**

Wetlands of Punjab (included in Ramsar list of Wetlands of international importance) with their vast expanse of water bodies have a rich freshwater fish fauna which form the major source of food fish in North India. Ramsar convention has defined wetlands as “areas of marsh, peat land or water, whether natural or artificial, temporary or permanent, with water that is static or flowing, fresh, brackish or salty including area of marine water, the depth of which at low tide does not exceed six meter”. In Punjab, there are 12 natural, 10 man-made wetlands covering 15,500 Ha area and only 3 main wetlands are included in Ramsar list of International importance i.e., Harike, Kanjali and Ropar wetlands. These wetlands have extremely rich biodiversity as they support a variety of plant and animal life. Kanjali wetland with an area of 185 Ha support diversity of resident and migratory birds, nurture large number of fish fauna with as many as 17 species of fishes. Ropar wetland is an important habitat of many species and has tremendous ecological value, spread over 1365 Ha, this wetland support as many as 35 species of fishes. These fishes are vulnerable to various parasitic infections, out of which Myxozoa is emerging as the major group. They cause production loss and deaths and some fish have to be discarded because they are unsightly and not considered to be fit for human consumption.

Myxosporeans are the abundant and diversified group of parasites infecting fishes, amphibians, reptiles as well as human beings (Boreham *et al.* 1998). Phylum Myxozoa include 4 malacosporean and 2,180 myxosporean species to a total of 62 genera (Lom and Dykova, 2006). However, three more genera (*Soricimyxum*, *Gadimyxia*, *Thelohanelloid*) with type species *S. fegati* Prunescu *et al.* (2007) from liver of *Sorex araneus*; *G. atlantica* Koie *et al.* (2007) from urinary system of *Gadus morhua* and *T. bengalensis* Sarkar (2009) from gall bladder of *Arius sagor* have been described subsequently. Phylum Myxozoa has been studied by only limited number of workers in Indian subcontinent. In north India, Gupta and Khera (1987, 1988a,b,c,d, 1989a,b, 1990, 1991) recorded 25 species belonging to genera *Myxobolus*, *Henneguya*, *Myxidium*, *Thelohanellus* and *Unicauda* infecting freshwater fishes.

Recently, Kaur and Singh (2008, 2009, 2010a,b, 2010/2011, 2011a,b,c,d,e,f, 2012a) have contributed 17 new species to the genus *Myxobolus* and 1 new species of the genus *Triangula* from freshwater fishes in wetlands of Punjab. Kaur and Singh (2012b) also compiled and published a synopsis of 131 nominal species of *Myxobolus* Butschli, 1882 (Myxozoa: Myxosporae: Myxobolidae) reported from India and a revised dichotomous key of 59 genera of the Phylum Myxozoa (class Myxosporae).

There are very few numbers of species of the genus *Thelohanellus* reported all over the world. In a monograph, Lom and Dykova (1992) enlisted 39 species of this genus. Basu and Haldar (1999) described a new species of *Thelohanellus* from gills of hybrid carps and gave a checklist of its different species described from Indian fishes. Basu *et al.* (2006) provided a synopsis of 32 indian species belonging to the genus *Thelohanellus* including one new species- *T.*

disporomorphus infecting Indian major carp, *Cirrhina mrigala*. Kalavati and Nandi (2007) gave a compilation of 27 species of genus *Thelohanellus* infecting Indian fishes.

Recently, Singh and Kaur (2012a) has recorded two new species of genus *Thelohanellus* i.e. *T. kalavate* and *T. kalbensi* from caudal fin of *Cirrhina reba* and gills of *Labeo calbasu* respectively. Singh and Kaur (2012b) also studied the biodiversity of the myxozoan parasites in these wetlands and recorded 36% infection in carp fishes infected with genus *Myxobolus*, *Thelohanellus*, *Triangula* and *Neothelohanellus*.

Genus *Thelohanellus* is characterized by pyriform or broadly ellipsoidal spores (valvular view), which look slimmer in sutural view. Spores always have smooth valves and single pyriform polar capsules, with a single coil of polar filament or subspherical polar capsules with two coils. Sporoplasms are binucleate, mostly with a spherical polysaccharide inclusion (Lom and Dykova, 2006). These parasites are histozoic and infect freshwater fish.

During the present study on the fishes of Ropar and Kanjali wetlands of Punjab (India), two new species, *T. deri* sp. nov. and *T. halderi* sp. nov. collected from caudal fins of *L. dero* and *C. mrigala* respectively. The description has been prepared in accordance with the guidelines of Lom and Arhtur (1989). A variety of other freshwater fishes were also collected and examined which include *Cyprinus carpio*, *Catla catla*, *Amblypharyngodon mola*, *Labeo bata*, *Labeo dero* and *Mystus seenghala*. Spores have been identified according the keys given by Kaur and Singh (2012a), and have been placed under the genus *Thelohnaellus*. The description has been prepared in accordance with the guidelines of Lom and Arhtur (1989).

MATERIALS AND METHODS

Fishes collected from Ropar and Kanjali wetlands were brought to the laboratory and examined for myxozoan infections. Plasmodia when found were removed and teased on slide and covered with cover slip and examined under the oil immersion for the presence of myxospores. Fresh spores were treated with 8% KOH solution for the extrusion of polar filaments. For permanent preparation, air-dried smears were stained with Ziehl- Neelsen and Iron-haematoxylin. Drawings were made from stained material with the aid of camera lucida.

Measurements of spores were done with the aid of a calibrated ocular micrometer. All measurements are presented in μm as range values followed by mean \pm SD in parentheses. The abbreviations used in the paper are as follows: -LS: Length of spore; WS: Width of spore; LPC: Length of polar capsule; WPC: Width of polar capsule; NC: Number of coils of polar filaments; SD: Standard deviation.

RESULTS AND DISCUSSION

SP. I

Thelohanellus deri sp. nov. (Figure 1,2)

Plasmodia

Small, creamy-white rounded, present on the caudal fin, 3-4 in number and measure 0.8-0.9 mm in diameter. 9-10 spores are present per plasmodium.

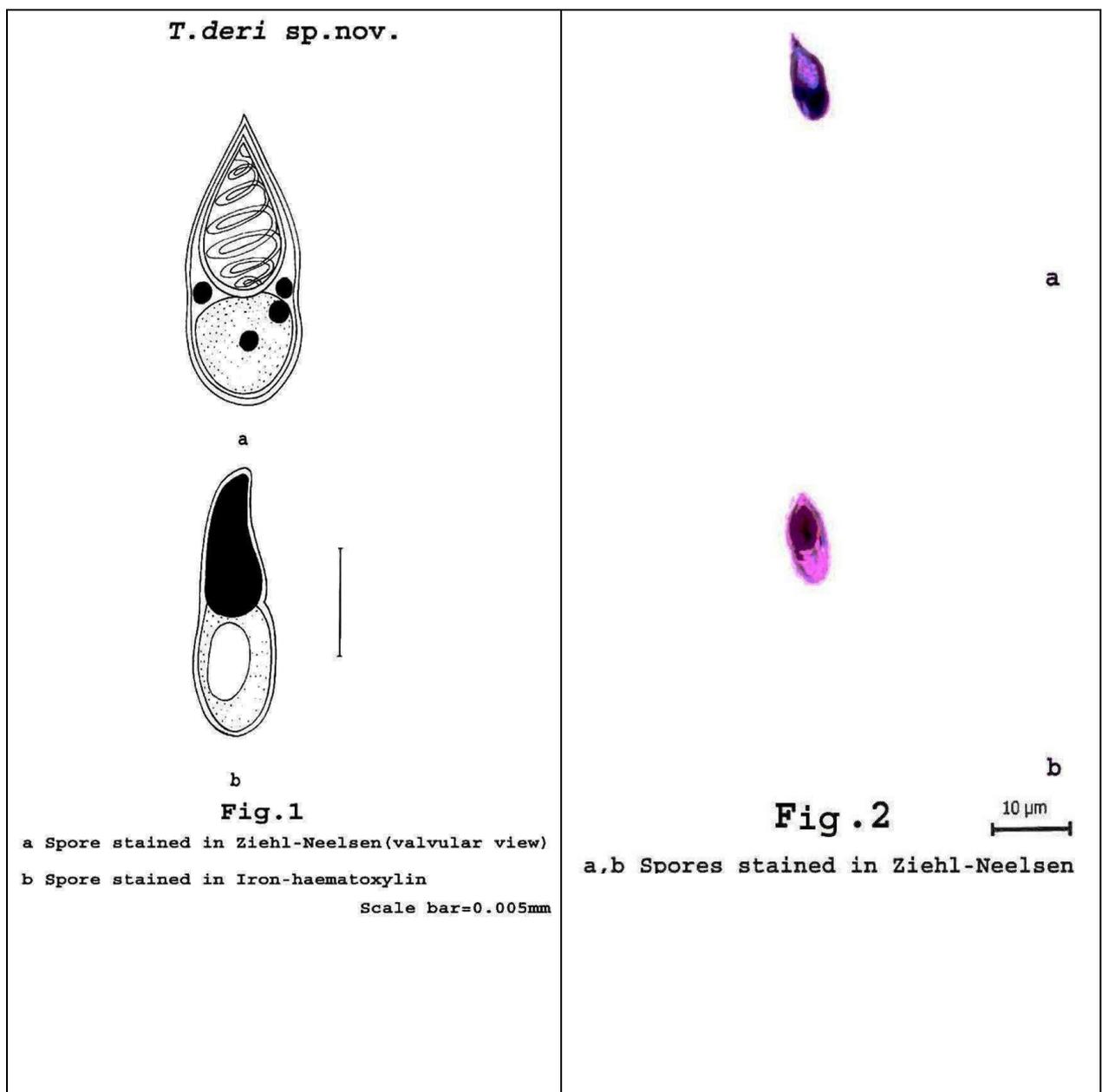
Table 1. Measurements (μm) and ratio of *T. deri* sp. nov.

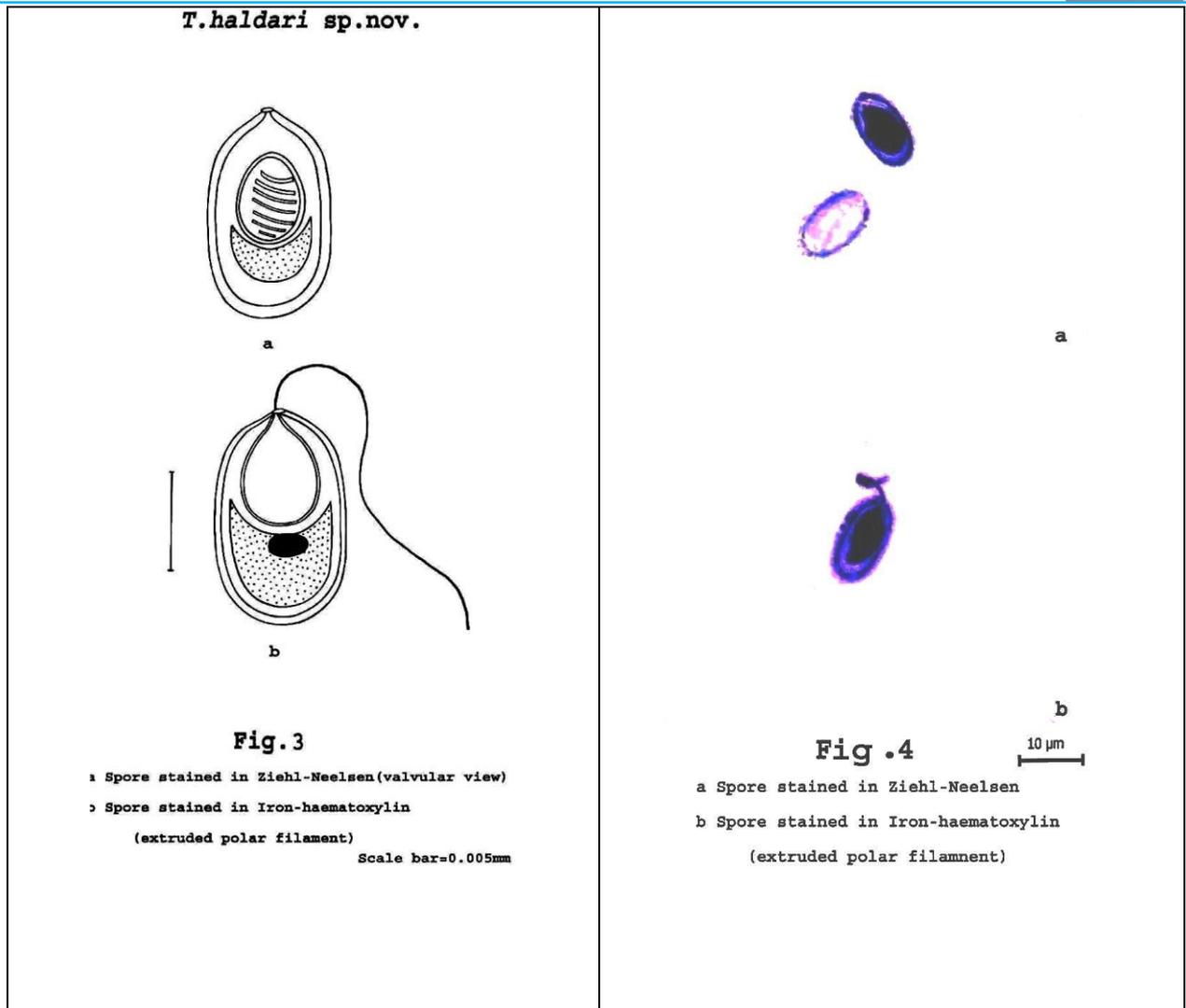
Characters	Range	Mean Values	SD
LS	9.5-10.5	10.0	0.70
WS	4.7-5.3	5.0	0.42
LPC	5.0-5.9	5.4	0.63
WPC	3.0-4.2	3.6	0.84
Ratio: LS/WS		2	
NC		6-7	
Parietal Folds		absent	

Spores description (Table 1)

(Measurements based on 10-11 spores in frontal view)

The spores are histozoic, measure 10.0x5.0 μm , elongately pyriform in valvular view having sharply pointed anterior end and broad rounded, bulb-like posterior end demarcated by a small constriction located medioposteriorly. Shell valves are thin, smooth, symmetrical and measure 0.4 μm in thickness. Parietal folds are absent. Polar capsule measure 5.4x3.6 μm , broadly pyriform with sharply pointed anterior end and broad rounded posterior end and is situated anteriorly in the spore body cavity. It occupy more than half of the spore body cavity and contain 6-7 coils of polar filament arranged obliquely to the polar capsule axis. Two capsulogenic nucleus measuring 0.7-0.8 μm in diameter are present beneath the polar capsule. Sporoplasm is agranular, homogenous and occupies whole of the extracapsular space behind the polar capsule. Sporoplasm contain two sporoplasmic nuclei measuring 0.9-1.0 μm in diameter. An iodophilous vacuole is absent.





Taxonomic summary of *T. deri* sp. nov.

Type host	: <i>Labeo dero</i> (Ham.) vern. gid
Type locality	: Ropar wetland, Punjab, India
Type specimen	: Paratypes are spores stained in Ziehl-Neelsen and Iron-haematoxylin, deposited in the museum of department of Zoology, Punjabi University, Patiala, India. Slide no. C/I/ZN 10.05.2009 and C/I/IH 10.05.2009
Site of Infection	: Caudal fin
Prevalence of infection	: 20% (2/10)
Clinical symptomatology	: None
Etymology	: The specific epithet <i>deri</i> has been given after the specific name of the host fish

Differential diagnosis

The studied species under study was compared to 18 representatives of the genus *Thelohanellus* infecting fish (Table 2). It differs from all of them by morphometric characters.

Table 2. Comparative description of *T. deri* sp. nov. with morphologically similar species (measurements are in micrometer)

Species	Host	Site of infection	Locality	Spore	Polar capsule
<i>T. deri</i> sp. nov. (present study)	<i>Labeo dero</i>	fin	Ropar wetland, Punjab (India)	10.0x5.0	5.4x3.6
<i>T. niloticus</i> Gurley (1893)	<i>L. niloticus</i>	skin of head	Nile (Egypt)	5.0x3.5	-
<i>T. rohita</i> (Southwell and Prashad, 1918) Chakravarty, 1943	<i>L. rohita</i> , <i>L. bata</i>	gills	West Bengal (India)	30.0-33.0x 10.0-13.0	16.0-20.0x 7.8-24.0
<i>T. shortii</i> Qadri (1967)	<i>L. fimbriatus</i>	fin	Andhra Pradesh (India)	11.42- 12.85(12.53)x 6.42-7.14(6.91)	6.42-7.95(7.07) x 3.5-4.28(4.2)
<i>T. batae</i> Lalitha Kumari (1969)	<i>L. bata</i>	gill filaments	Andhra Pradesh (India)	12.3x6.2	7.7x3.0
<i>T. potaili</i> Lalitha Kumari (1969)	<i>L. potail</i>	fin	Andhra Pradesh (India)	13.0x8.2	5.9x4.3
<i>T. jiroveci</i> Kundu and Haldar (1981)	<i>L. bata</i>	branchiae	West Bengal (India)	Macrospore: 35.0x13.0 Microspore: 16.3x6.8	Macrospore: 18.4x7.0 Microspore: 7.3-4.1
<i>T. chilensis</i> Kalavati and Vaidehi (1991)	<i>L. rohita</i>	gall bladder	Orissa (India)	26.7x8.7	17.54x7.01
<i>T. caudatus</i> Pagarkar and Das (1993)	<i>L. rohita</i>	between rays of caudal fin and anal fin	West Bengal (India)	13.8x9.0	7.02x5.07
<i>T. assambai</i> Fomea et al. (1994)	<i>Labeo</i> sp.	-	Africa	10.5x6.0	7.5x2.7
<i>T. costae</i> Sakiti (1997)	<i>L. senegalensis</i>	gill	Benin (Africa)	8.5–10.5 ((9.4) x 5.0–6.5(5.6)	4.0–5.5(4.8) x 2.0–3.0(2.6)
<i>T. orissae</i> Haldar et al. (1997)	<i>Cirrhina mrigala</i>	gills	Orissa (India)	Macrospore: 13.85x4.8 Microspore: 7.29x3.11	Macrospore: 6.34x2.36 Microspore: 3.72x2.32
<i>T. bifurcata</i> Basu and Haldar (1999)	<i>Catla catla</i> x <i>L. rohita</i>	gill lamellae	West Bengal (India)	34.89x9.21	25.3x6.6
<i>T. ndjamenaensis</i> Kostoingue et al. (1999)	<i>L. parvus</i>	gill	Chad (Central Africa)	10.0 11.0(10.0)x 7.0–8.0 (7.3)	4.0–5.0 (4.2)x (3.0–5.0(3.2))
<i>T. bicornei</i> Kabre et al. (2002)	<i>L. coubie</i>	intestine	Burkina Faso (Africa)	13.0–14.0(13.5) x 8.0–9.0(8.4)	6.5–8.0(7.2)x 3.5–4.0(3.7)
<i>T. avijiti</i> Basu and Haldar (2003)	<i>L. rohita</i>	dorsal fin	West Bengal (India)	14.0x9.7	6.0x4.0
<i>T. chandannagarensis</i> Basu and Haldar (2003)	<i>Catla catla</i>	gill lamellae	West Bengal (India)	12.5x6.7	5.1x3.1
<i>T. habibpuri</i> Acharya and Dutta (2007)	<i>L. rohita</i>	pectoral fin	West Bengal (India)	13.0-14.3(13.9) x 8.0-9.0(8.5)	6.0-6.5(6.0) x4.1-5.0(4.9)
<i>T. imphlaensis</i> Hemananda et al. (2010/2011)	<i>L. rohita</i>	gills	Imphal, Manipur (India)	20.4 – 22.1 ((21.33)x8.5 – 10.2 (9.43)	10.2 – 11.05 (10.79)x3.4.0 – 4.25 (3.78)

Table 3. Measurements (μm) and ratio of *T. haldari* sp. nov.

Characters	Range	Mean Values	SD
LS	12.2-13.0	12.6	0.56
WS	6.0-6.4	6.2	0.28
LPC	6.7-7.3	7.0	0.42
WPC	4.3-5.3	4.8	0.70
Ratio: LS/WS		2.0	
NC		3-5	
Parietal Folds		absent	

Table 4. Comparative description of *T. haldari* sp. nov. with morphologically similar species (measurements are in micrometer)

Species	Host	Site of infection	Locality	Spore	Polar capsule
<i>T. haldari</i> sp. nov. (present study)	<i>Cirrhina reba</i>	caudal fin	Kanjali wetland, Punjab (India)	12.6x6.2	7.0x4.8
<i>T. mrigalae</i> Tripathi (1952)	<i>C. mrigala</i>	skin on the head	West Bengal (India)	10.8-12.0x 6.3-7.2	5.4-7.2x 3.6-5.0
<i>T. nikolski</i> Akhmerov (1955)	<i>Cyprinus carpio haematopterus</i>	fin	Amur basin (Russia)	19.0- 20.0x12.0	7.0x5.0-6.0
<i>T. bengalensis</i> Sarkar and Raychaudhury (1986)	<i>Catla catla</i>	gall bladder	West Bengal (India)	10.95x6.59	5.42x3.47
<i>T. sanjibi</i> Sarkar and Ghosh (1990)	<i>Mystus gulio</i>	kidney	West Bengal (India)	12.52x8.27	4.52x4.0
<i>T. sudevi</i> Sarkar and Ghosh (1990)	<i>Amblypharyngodon mola</i>	fin	West Bengal (India)	14.05x5.87	5.17x2.65
<i>T. orissae</i> Halidar et al. (1997)	<i>C. mrigala</i>	gills	Orissa (India)	7.29x3.11	3.72x2.32

The novel species possess, elongately pyriform in valvular view having sharply pointed anterior end and broad rounded, bulb-like posterior end demarcated by a small constriction located medioposteriorly, like *T. bifurcata* in which a similar bulb-like structure is demarcated by a notch at the posterior extremity. The shape of the present species also resembles with spores of *T. chilkinsis* in which the inner wall of the shell has a distinct constriction formed by 2 indentations on either side at the posterior one third. However, the present species differ from *T. bifurcata* (LS/WS: 3.7) in having much wider spores (LS/WS: 2).

In addition, the anterior end of the polar capsule in *T. bifurcata* is elongately pyriform, bifurcated and occupies more than two thirds of the spore body cavity unlike in the present species in which it is broadly pyriform with sharp anterior end occupying more than half of spore body cavity. The present species also differ from *T. chilkinsis* in which the anterior end of the spore is narrow, flat at the tip and contains a flask-shaped polar capsule with a small neck opening 2-3 μm below the anterior tip.

In view of the above differences, the present species under study is proposed as new to the science and named as *T. deri* sp. nov.

SP. II

Thelohanellus haldari sp. nov. (Figure 3,4) Plasmodia

Small, white, rounded, present on the caudal fin, 5-7 in number and measure 0.7-0.8 mm in diameter. 10-12 spores are present per plasmodium.

Spore description (Table 3)

(Measurements based on 10-11 spores in frontal view)

The spores are histozoic, measure 12.6x6.2 μm , elongated oval in valvular view having blunt rounded anterior end with a distinct pore and rounded posterior end. Shell valves are thick, smooth, symmetrical and measure 1.5 μm in thickness. Parietal folds are absent. Lateral sides of the spore are parallel to each other throughout the spore length. Polar capsule is oblong oval to spherical in shape, measure 7.0x4.8 μm , situated at the anteriomedian position in the spore body cavity. Polar filament form 3-5 coils arranged perpendicular to the polar capsule axis. Polar filament thread-like extruding through a distinct pore at the anterior tip and measure 26.6 μm in length after eversion. Sporoplasm is agranular, homogenous occupying whole of extracapsular space behind the polar capsule. Sporoplasm contain a large nucleus measuring 2.0 μm in diameter. An iodophilous vacuole measuring 2.9 μm in diameter is present.

Taxonomic summary of *T. haldari* sp. nov.

Type host	: <i>Cirrhina reba</i> (Ham.) vern. chunni, mori, kursa
Type locality	: Kanjali wetland, Punjab, India
Type specimen	: Paratypes are spores stained in Ziehl-Neelsen and Iron-haematoxylin, deposited in the museum of department of Zoology, Punjabi University, Patiala, India. Slide no. C/I/ZN11.09.2008 and C/P/IH 11.09.2008
Site of Infection	: Caudal fin
Prevalence of infection	: 25% (3/12)
Clinical symptomatology	: None
Etymology	: The specific epithet <i>haldari</i> has been given after the name of Dr. Durga P. Haldar, an eminent worker of the department of Zoology, University of Kalyani-741 235, Nadia, West Bengal, India

Differential diagnosis

The studied species under study was compared to 6 representatives of the genus *Thelohanellus* infecting fish (Table 4). It differs from all of them by morphometric characters.

The present species have spores elongately oval in shape with blunt anterior end having a distinct pore. The spores are peculiar in having their lateral sides parallel to each other throughout the length. On the basis of shape, it is comparable with *T. mrigalae* and *T. sudevi*, however, differ from *T. mrigalae* in which the lateral sides are more or less parallel with a knob-like projection at the anterior end while the spores in *T. sudevi* are ellipsoid with acuminate anterior end and rounded posterior end.

Furthermore, the polar capsule in the present species is oblong oval to spherical in shape occupying half of the spore body cavity and is situated at anteriomedian position inside the spore body cavity which opens through a duct to the exterior in comparison to oval shaped in *T. mrigalae* and pyriform in *T. sudevi*. In view of the above differences, the present species under study is proposed as new to the science and named as *T. haldari* sp. nov.

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