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Protistology

Myxosporean species of the genus *Thelohanellus* Kudo, 1933 (Myxozoa: Myxosporea: Bivalvulida) from freshwater fishes of Punjab wetlands, India

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Summary

A study on the myxosporean infections in freshwater fishes in Harike and Kanjali wetlands of Punjab (India) has revealed the presence of three myxozoan species belonging to the genus *Thelohanellus*. Plasmodia were teased out with the help of a fine needle to liberate the spores on a clean slide. Spores were studied fresh as well as stained after fixing in Bouins fixative. *T. batae* Lalitha Kumari, 1969 was located within the wall lining of the duodenum and on the pectoral fin of the cat fish, *Wallago attu*. Earlier, this parasite was recorded from gill filaments of *Labeo bata* in Andhra Pradesh (India). Spores of *T. mrigalae* Tripathi, 1952 were found parasitizing the caudal fin of *Catla catla*. Spores of the new species, *T. thaili* sp. nov. were found infecting gill lamellae of *Catla catla*. Spores were pyriform in valvular view, tapering towards the anterior end with bluntly pointed tip. Spores measured 11.67×7.22 μm in size. Polar capsule was flask-shaped, long-necked, measuring 7.3×4.4 μm in size. It was placed eccentrically in the spore body cavity (aligned to one side of the inner wall of the spore) occupying nearly three-quarters of the spore body cavity. In the present study, new host, new site of infection and new locality for *T. batae* and *T. mrigalae* have been reported.

Key words: caudal fin, gill lamellae, Harike, Kanjali, plasmodium, thelohanelloid

Introduction

Punjab (India) has 3 main wetlands, i.e. Harike, Kanjali and Ropar wetlands which are included in Ramsar list of International importance. Harike wetland is the largest freshwater wetland (in northern India) of 4100 ha. area and is a suitable habitat for as many as 26 species of fishes. Kanjali wetland is a man made wetland covering an area of 185 ha., which nurtures up to 17 fish species. These wetlands form major natural fisheries resource in whole of the Punjab state. A large variety of these fishes in these wetlands are vulnerable to myxozoan infections, beside other parasitic infections.

Myxozoans are economically important group of microscopic metazoan parasites of marine and freshwater fishes from natural and aquaculture resource causing production losses and render them unfit for human consumption. Up to now, phylum Myxozoa include more than 2,180 species attributed

to 65 genera (Singh and Kaur, 2012a). *Thelohanellus* Kudo, 1933 is the second most prevalent genus after *Myxobolus* Bütschli, 1882 having 60 valid species (Lom and Dyková, 2006). Lom and Dyková (1992) in a monograph enlisted 39 species of the genus *Thelohanellus*. Kaur and Singh (2008, 2008-2009, 2009, 2010a, 2010b, 2010/2011, 2011a, 2011b, 2011c, 2011d, 2011e, 2011f, 2012a, 2012b) have recorded many myxosporeans infecting freshwater fishes of Punjab wetlands and described as many as 18 new species of the genera *Myxobolus* and *Triangula*. Kaur and Singh (2012a) gave a synopsis of Indian myxobolids and revised key to the phylum Myxozoa. Basu and Haldar (1999) and Basu and coauthors (2006) gave a checklist and a synopsis of the genus *Thelohanellus* enlisting 32 Indian species, respectively. Kalavati and Nandi (2007) compiled a handbook on myxosporean parasites of Indian fishes. Valuable work has been contributed by Gupta and Khera (1987, 1988a, 1988b, 1988c, 1988d, 1989a, 1989b, 1990, 1991) on these parasites in north India. More recently, Singh and Kaur (2012a, 2012b) have recorded four new species, i.e. *Thelohanellus kalavate, T. globulosa* from caudal fin of *Cirrhina reba* and *T. kalbensi, T. kanjalensis* from gills of *Labeo calbasu* and skin of snout of *Catla catla*, respectively. Singh and Kaur (2012c) also studied the biodiversity of the myxozoan parasites in these wetlands. They recorded 36% infection in carp fishes with various genera: *Myxobolus, Thelohanellus, Triangula* and *Neothelohanellus*.

Spores of the genus *Thelohanellus* are histozoic, characterized in having smooth shell. Spores 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 contain two sporoplasmic nuclei.

During the present study, two already known species, i.e. *T. batae* Lalitha Kumari, 1969, *T. mrigalae* Tripathi, 1952 and one new species, *T. thalli* sp. nov. has been reported of which complete description is given. Spores up to generic level were identified with the help of the key given by Kaur and Singh (2012a). The descriptions have been prepared in accordance to the guidelines by Lom and Arthur (1989).

Material and methods

Fishes collected from Harike and Kanjali wetlands were brought to the laboratory and examined for myxozoan infections. Plasmodia were removed, placed on clean slides and examined with the light microscope under 100× oil objective (Magnus inclined Trinocular microscope MLX-Tr) 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 of stained material were made with the aid of camera lucida. Spores were measured with calibrated ocular micrometer. All measurements are presented in μm as range values followed by mean ± SD (standard deviation) 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

Thelohanellus batae Lalitha Kumari, 1969

Plasmodia. Small, located within the wall lining of the duodenum and on the pectoral, 2–3 in number and measure 0.2–0.3 mm and 0.7–0.9 mm in diameter, respectively. Each plasmodium contains 12–13 spores.

Spores were histozoic, measure 8.9×3.2 μm (measurements based on 8–10 spores in frontal view), pyriform to egg shaped in valvular view having pointed anterior end and rounded posterior end (Figs 1a, 1b, 3a, 3b) (Table 1). Shell valves were thin, smooth, symmetrical and measuring 0.2 μm in thickness. Parietal folds absent. Polar capsule was elongately pyriform, measuring 4.5×1.7 μm, occupying nearly half of the spore body cavity. Polar filament form 4–6 coils arranged perpendicular to the polar capsule axis which extrude at the anterior tip of the spore (Figs 2a, 2b). Polar filament thin, thread-like measuring 30 μm in length. Sporoplasm occupies the whole extracapsular space behind the polar capsule and contain a sporoplasmic nucleus measuring 1.5 μm in diameter. An iodinophillous vacuole was absent.

Remarks. The present observations (LS/WS: 2.7) on *T. batae* Lalitha Kumari, 1969 are in conformity with the original description (LS/WS: 1.9) except some variations in the size of the spore and the polar capsule. Spore and polar capsule are smaller in size in the present species. Earlier, this parasite was recorded from the gill filaments of *Labeo bata*. In a new host *Wallago attu*, two new sites of infection (wall of duodenum and pectoral fin), and a new locality (Harike wetland) are recorded for this parasite (Table 2).

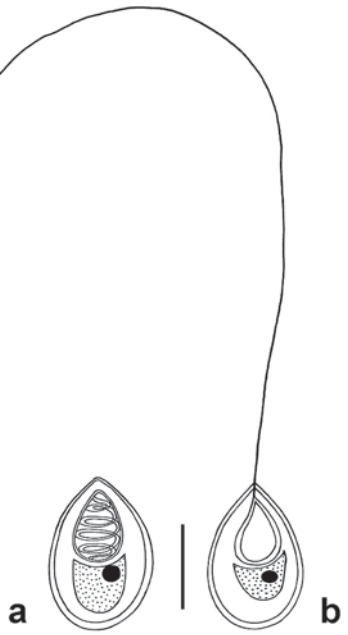

Fig. 1. Line drawing (Camera Lucida) of *T. batae* Lalitha Kumari, 1969 spores. a – Spore stained with Ziehl-Neelsen (valvular view); b – spore stained with Iron-haematoxylin; the polar filament is extruded. Scale bar: 0.005 mm.

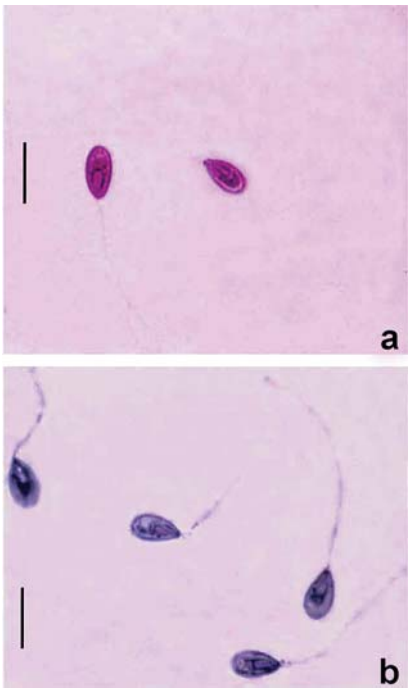

Fig. 2. Micrographs of *T. batae* Lalitha Kumari, 1969 spores. a – Spore stained with Ziehl-Neelsen; b – spores stained with Iron-haematoxylin; the polar filament is extruded. Scale bars: 10 μm.

THELOHANELLUS MRIGALAE TRIPATHI, 1952

Plasmodia. Small, white to pale yellow, rounded, located on the caudal fin, 2–5 in number and measuring 0.7–0.9 mm in diameter. Each plasmodium contains 8–10 spores.

Spores were histozoic, measuring 11.6× 8.3 μm (measurements based on 12-13 spores in frontal view), elongately oval in valvular view having rounded blunt with a knob-like projection at the tip and broad rounded posterior end (Figs 5a, 5b, 6) (Table 3). Shell valves were thick, smooth, symmetrical and measuring 0.62 μm in thickness. They were stained dark blue with Iron-haematoxylin throughout the length of spore (Figs 4a, 4b, 5a, 5b). Parietal folds absent. Polar capsule was broadly pyriform to ovoid in shape, measuring 5.0×3.0 μm, having a distinct neck and broad rounded posterior end. It was placed anteriorly occupying nearly half of the spore body cavity. Polar filament form 6–7 coils arranged obliquely to the polar capsule axis. It

Table 1. Measurements (μm) of *Thelohanellus batae* Lalitha Kumari, 1969.

Character	Range	Mean value	SD
LS	8.5–9.3	8.9	0.56
WS	3.0–3.4	3.2	0.28
LPC	4.2–4.8	4.5	0.42
WPC	1.6–1.8	1.7	0.14
LS/WS		2.7	
NC		4–6	
Parietal folds		absent	

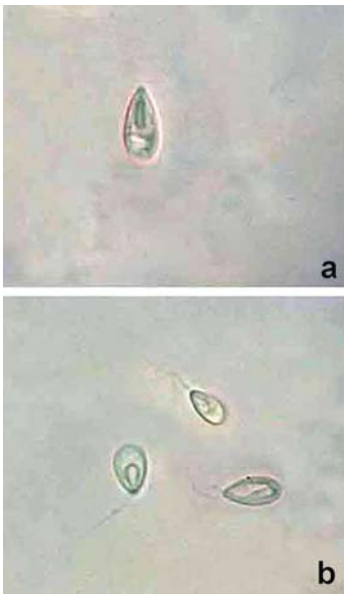

Fig. 3. Micrographs of *T. batae* Lalitha Kumari, 1969 spores. a , b – fresh spores. ×1000.

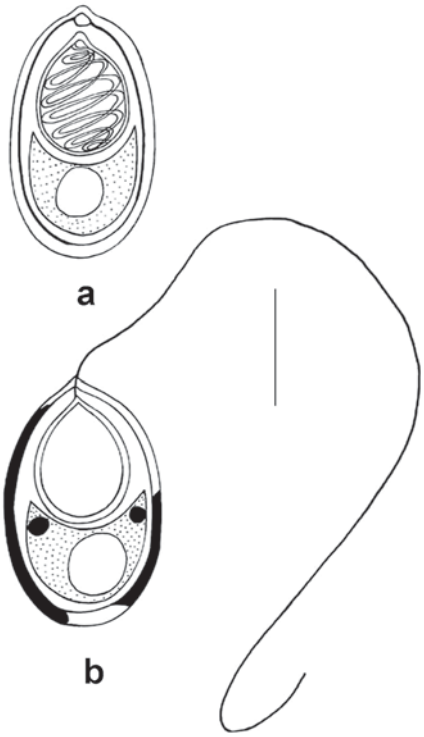

Fig. 4. Line drawing (Camera Lucida) of *T. mrigalae* Tripathi, 1952 spores. a – Spore stained with Ziehl-Neelsen (valvular view); b – spore stained with Iron-haematoxylin; the polar filament is extruded. Scale bar: 0.005 mm.

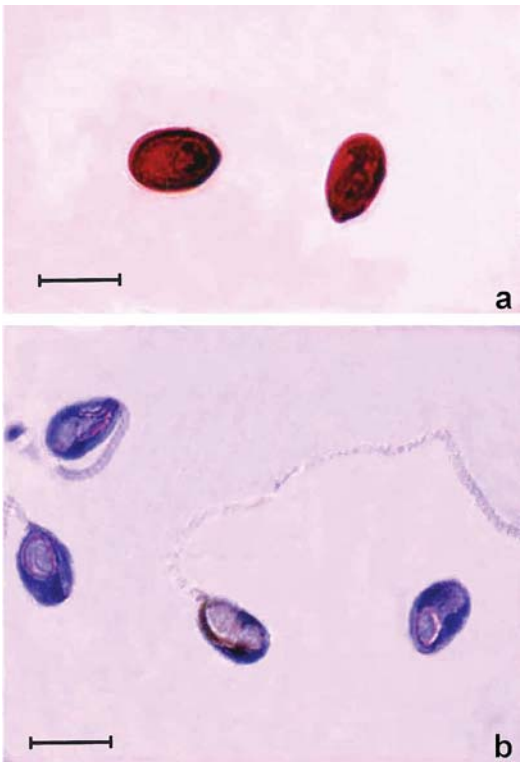

Fig. 5. Micrographs of *T. mrigalae* Tripathi, 1952 spores. a – Spore stained with Ziehl-Neelsen; b – spores stained with Iron-haematoxylin; the polar filament is extruded. Scale bars: 10 μm.

was thick, thread-like and measuring 28 μm in length after eversion (Fig. 5a, b and Fig. 6). Capsulogenic nucleus was absent. Sporoplasm occupies whole of the extracapsular space behind the polar capsule and contain a nucleus measuring 0.16 μm in diameter. An iodinophillous vacuole was present measuring 2.9–3.1 (3.0±0.14) μm in diameter.

Remarks. The present observations (LS/WS: 1.3) on *T. mrigalae* Tripathi, 1952 are in conformity with the original description (LS/WS: 1.7) except some variations in the size of the spore. Earlier, this parasite was recorded from skin on the head of *Cirrhina mrigala*. A new host – *Catla catla*, a new site of infection – caudal fin and a new locality – Kanjali Wetland are recorded for this parasite (Table 4).

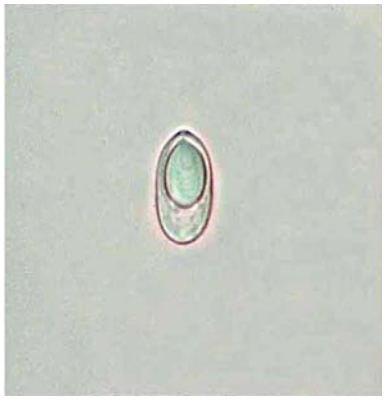

Fig. 6. Micrographs of *T. mrigalae* Tripathi, 1952 fresh spore. ×1000.

Table 2. Comparison of *Thelohanellus batae* in original description (Lalitha Kumari, 1969) and in present study (measurements are in μm).

Species	Host	Site of infection	Locality	Spore	Polar capsule
T. batae (present study)	*Wallago attu*	wall of duodenum, pectoral fin	Harike wetland, Punjab (India)	8.9×3.2	4.5×1.7
T. batae Lalitha Kumari, 1969	*Labeo bata*	gill filaments	Andhra Pradesh (India)	12.3×6.2	7.7×3.0

Table 3. Measurements (μm) of *Thelohanellus mrigalae* Tripathi, 1952.

Character	Range	Mean value	SD
LS	11.2–12.0	11.6	0.56
WS	7.9–8.7	8.3	0.56
LPC	4.0–6.0	5.0	1.4
WPC	2.9–3.0	3.0	0.14
LS/WS		1.3	
NC		6-7	
Parietal Folds		absent	

Table 5. Measurements (μm) of *Thelohanellus thaili* sp. nov.

Character	Range	Mean value	SD
LS	11.27–12.07	11.67	0.56
WS	7.02–7.42	7.22	0.28
LPC	6.9–7.7	7.3	0.56
WPC	4.1–4.7	4.4	0.42
LS/WS		1.6	
NC		4-5	
Parietal Folds		absent	

THELOHANELLUS THAILI SP. NOV.

Plasmodia. Minute and present around gill lamellae. Each plasmodium contains 10–11 spores.

Spores were histozoic, measuring 11.67× 7.22 μm (measurements based on 4–7 spores in frontal view), pyriform in valvular view, tapering towards the anterior end with bluntly pointed tip and broad rounded posterior end (Table 5). Shell valves were thick, smooth, symmetrical and measuring 0.51 μm in thickness. Parietal folds absent (Figs 7a, 7b). Polar capsule was flask-shaped, long-necked, measuring 7.3×4.4 μm in size. It was placed eccentrically in the spore body cavity (aligned to one side of the inner wall of the spore) occupying nearly three-quarters of the spore body cavity (Fig. 9). Polar filament contain 4–5 coils arranged obliquely to the polar capsule axis, thread-like, measuring 58.3 μm and extrude through a distinct pore at the anterior end of the spore (Figs 8a, 8b). One capsulogenic nucleus measuring 1.03–1.63 (1.33±0.42) μm in diameter was present. Sporoplasm occupies the whole of the extracapsular space behind the polar capsule. Two sporoplasmic nuclei are equal in size, measuring 1.35 μm in diameter. An iodinophillous vacuole was absent.

Differential diagnosis. The studied species was compared to 17 representatives of the genus *Thelohanellus* infecting fish (Table 6). It differs from all of them by morphometric characters.

Morphologically, spores of the present species resemble *T. batae, T. chelae, T. chrysopomati, T. coeli, T. potaili, T. wallagoi, T. chandannagarensis, T. anilae, T. imphalensis* and *T. niloticus*. It can be differentiated from all of the above mentioned species in having a flask-shaped polar capsule with a long neck opening to the exterior through a distinct pore. Furthermore, the polar capsule is eccentric and occupying nearly three-quarters of the spore body cavity in contrast to pyriform, terminal and oblique polar capsule in *T. batae*. The

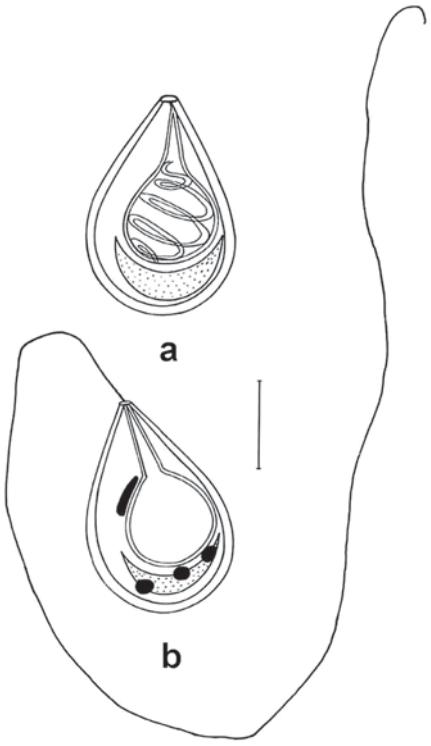

Fig. 7. Line drawing (Camera Lucida) of *T. thaili* sp. nov. a – Spore stained with Ziehl-Neelsen (valvular view); b – spore stained with Iron-haematoxylin; the polar filament is extruded. Scale bar: 0.005 mm.

Table 4. Comparison of *Thelohanellus mrigalae* in original description (Tripathi, 1952) and in present study (measurements are in μm).

Species	Host	Site of infection	Locality	Spore	Polar capsule
T. mrigalae (present study)	*Catla catla*	caudal fin	Kanjali wetland, Punjab (India)	11.6x8.3	5.0x3.0
T. mrigalae Tripathi,1952	*Cirrhina mrigala*	skin on the head	West Bengal (India)	10.8-12.0x6.3 7.2	5.4-7.2x3.6-5.0

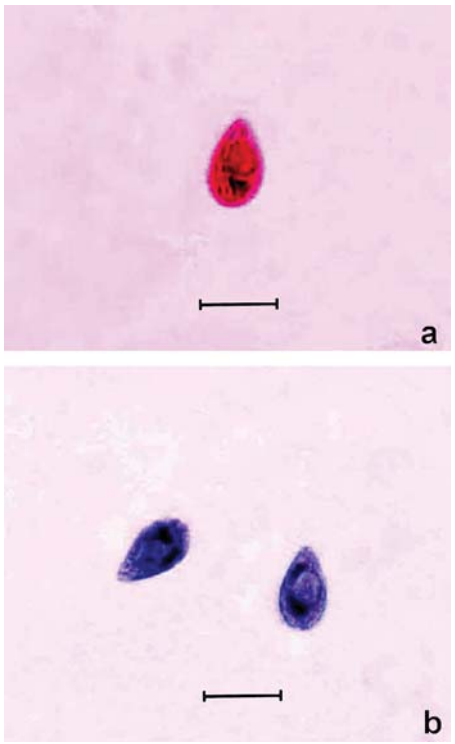

Fig. 8. Micrographs of *T. thaili* sp. nov. spores. a – Spore stained with Ziehl-Neelsen; b – spores stained with Iron-haematoxylin. Scale bars: 10 μm.

polar capsule is anteriorly placed and oval in *T. chandannagarensis*, terminal, oval or elongated in *T. chelae*. It is bifurcated in *T. imphalensis* and presence of pointed and anteriorly placed polar capsule in *T. niloticus* demarcates them from the spores of the present species in this study.

Spores of the species under consideration are pyriform, bluntly pointed at the anterior end with a distinct pore unlike anteriorly pointed spore of *T. wallagoi*, elongated pyriform spore in *T. anilae* and tear shaped spore of *T. niloticus*.

Furthermore, spores in the present species lack parietal folds and have shell valves equal and smooth unlike spores of *T. chrysopomati, T. potaili, T. chelae* and *T. coeli*.

In view of the above differences, it is concluded that the species under present study is really a new species. The name *Thelohanellus thaili* sp. nov. is proposed after the vernacular name, i.e. *thaili*, of the host fish, *Catla catla*.

Taxonomic summary.

Plasmodia. Minute and present around gill lamellae.

Spores. Spores are pyriform in valvular view. A single polar capsule is flask-shaped.

Type host. *Catla catla* (Ham.) vern. thail.

Type locality. Kanjali wetland, Punjab, India.

Fig. 9. Micrographs of *T. thaili* sp. nov. fresh spore. ×1000.

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. CC/ZN/22.05.2008 and CC/IH/22.05.2008.

Site of infection. Gill lamellae.

Prevalence of infection. 50% (10/20).

Clinical symptomatology. None.

Etymology. The specific epithet *thaili* has been given after the vernacular name of the host fish.

Acknowledgements

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Table 6. *Thelohanellus thaili* sp. nov. and morphologically similar species (measurements are in μm).

Species	Host	Site of infection	Locality	Spore	Polar capsule
T. thaili sp. nov. (present study)	*Labeo bata, Catla catla*	gill lamellae	Kanjali wetland, Punjab (India)	11.67×7.22	7.3×4.4
T. catlae Chakravarty and Basu (1948)	*Catla catla*	branchiae	West Bengal (India)	19.26–21.40×10.70–12.48	10.71–13.90×39.33–11.77
T. seni (Southwell and Prashad, 1918) Chakravarty and Basu,1948	*Catla catla*	branchiae	West Bengal (India)	12.48–14.94×8.56	6.42×4.52
T. calbasui Tripathi (1952)	*L. calbasu*	scales	West Bengal (India)	9.0-–10.8×7.2	5.4×3.4
T. boggoti Qadri (1962)	*L. boggot*	gills	Andhra Pradesh (India)	11.5×6.75	6.2×3.8
T. shortii Qadri (1967)	*L. fimbriatus*	fin	Andhra Pradesh (India)	12.53×6.91	7.07×4.21
T. batae Lalitha Kumari (1969)	*L. bata*	gill filaments	Andhra Pradesh (India)	12.3×6.2	7.7×3.0
T. chelae Lalitha Kumari (1969)	*Chela bacaila*	gall bladder	Andhra Pradesh (India)	9.8×5.5	4.9×2.8
T. chrysopomati Lalitha Kumari (1969)	*Barbus chrysopoma*	gill contents	Andhra Pradesh (India)	12.4×5.4	6.5×2.7
T. potaili Lalitha Kumari (1969)	*L. potail*	fin	Andhra Pradesh (India)	13.0×8.2	5.9×4.3
T. coeli Sarkar and Mazumdar (1983)	*Tachysurus tenuispinis*	gall bladder	West Bengal (India)	12.75×7.12	7.13×3.2
T. wallagoi Sarkar (1985)	*Wallago attu*	gall bladder	West Bengal (India)	9.25×4.85	5.47×2.71
T. assambai Fomena et al.,1994	*Labeo sp.*	–	Africa	10.5×6.0	7.5×2.7
T. chandannagarensis Basu and Haldar (2003)	*Catla catla*	gill filaments	West Bengal (India)	12.5×6.7	5.1×3.1
T. lagdoensis Fomena et al., 2007	*Citharinus citharus*	gut	Cameroon (Central Africa)	8.4×4.3	2.6×1.8
T. anilae Hemananda et al., 2010	*L. rohita*	gills	West Bengal (India)	Micro spore: 13.26×6.8 Macro spore: 33.27×12.75	Polar capsules of microspores: 7.31×3.10; Polar capsule of macro spore: 17.55×5.35
T. imphlaensis Hemananda et al., 2010/2011	*L. rohita*	gills	Imphal, Manipur (India)	20.4–22.1 (21.33)× 8.5–10.2 (9.43)	10.2–11.05 (10.79)× 3.4.0–4.25 (3.78)
T. niloticus Abdel Ghaffar et al., 2012	*L. niloticus*	gills	River Nile (Egypt)	23.3×13.4	11.7×4.7

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