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Species

Thelohanellus dykovi sp. nov. (Myxozoa: Bivalvulidae), a pathogenic gill parasite in cultured Indian major carp, *Labeo rohita* (Hamilton 1822) in Punjab (India)

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ABSTRACT

The plasmodia of a new myxosporean, *Thelohanellus dykovi* sp. nov. were found infecting gills of cultured Indian major carp, *Labeo rohita* (Hamilton, 1822). The infection rate was found to be 36.67% (out of 30 fishes examined 11 were infected) in a rural fish pond, Nanoki located in Patiala district, Punjab. Each gill filament contained one large size (0.8-1mm) plasmodia. Spores of *T.dykovi* sp. nov. were morphologically unique in having tapering anterior end and rounded posterior end measuring 10.74x4.07µm in size. Shell valves were thin, 0.50µm in thickness, smooth and symmetrical. Parietal folds were absent. Polar capsule single, elongated, pyriform, measuring 6.48x2.04µm with a distinct tubular neck and occupied one third of the total spore body cavity. Polar filament with 18-20 coils arranged perpendicular to the polar capsule axis. Sporoplasm finely granular, cup shaped, binucleate and iodinophilous vacuole absent. The present species has been proposed as new to science on the basis of its peculiar shape and morphometrics of the spores. Histological sections indicated that plasmodia were present throughout the length of the gill filament and caused complete destruction of the respiratory epithelium.

Keywords: aquaculture, histopathology, gills, Indian major carp, *Labeo rohita*, *Thelohanellus*

1. INTRODUCTION

Fisheries have played an important role in food and nutrition all over the world. India ranks third among the world's freshwater fish producers with Indian major carps viz. *Catla catla* Ham., *Labeo rohita* Ham. and *Cirrhinus mrigala* Ham. being the most preferred cultured species (FAO 2003). Parasites and diseases are the most serious limiting factors in aquaculture because fishes are usually cultured in high density in a restricted water body, where fish pathogens can easily be transmitted. Polyculture practices employed in the farmland cause overcrowding resulting in disease outbreak and mortality. In Punjab, polyculture species consists of Indian major carps: Catla (*Catla catla* Ham.), rohu (*Labeo rohita* Ham.) and mrigal (*Cirrhinus cirrhosus* Ham.) and exotic carps such as silver carp (*Hypophthalmichthys molitrix* Valen.), grass carp (*Ctenopharyngodon idellus* Valen.), common carp (*Cyprinus carpio* Linn.) and bighead carp (*Aristichthys nobilis* Rich.).

Myxozoans are one of the economically important group of microscopic metazoan parasites infecting freshwater fishes harvested for food. These parasites are host-specific and tissue-specific, and are known to cause serious disease in both wild and cultured fishes. Myxosporidians are emerging as one of the most important group of parasites infecting fishes in wetlands of Punjab Kaur and Singh (2008, 2008/2009, 2009, 2010a, 2010b, 2010/2011, 2011a,b,c,d,e,f, 2012b) causing serious threat to fish health. Histopathological analysis of tissues is also an important approach for detection of myxozoanosis. Kaur et al. (2013) have studied pathogenic myxosporean parasite causing haemorrhagic gill disease in cultured Indian major carp fish, *Labeo rohita* (Hamilton, 1822) in Punjab. Due to pathogenic potentials of some species they can adversely affect growth, reproduction and involve epizooties being able to cause the death of the host Longshaw et al. (2005). Economic losses caused by these parasites in aquaculture have been well documented Lom and Dykova (2006).

The genus *Thelohanellus* Kudo, 1933 is the sixth most speciose genus after *Myxobolus* Butschli, 1882*, Myxidium* Butschli, 1882, *Henneguya* Thelohan, 1892, *Ceratomyxa* Thelohan, 1892 and *Chloromyxum* Mingazinni, 1890. This genus includes a total of 108 nominal species worldwide and 40 species from India (Zhang et al., 2013). In the present study, a new species, *T. dykovi* sp. nov. infecting gill filaments of the Indian major carp, *Labeo rohita* has been described.

2. MATERIAL AND METHODS

Fish specimens were procured from Nanoki pond in the district Patiala (Punjab) for a period of six month i.e November 2013 to April 2013 freezed in ice-box and were brought to the laboratory for further investigation. Infection rate was highest in the month of March having a pH 8.26, water temperature 24.8° and DO 11.56 mg/dm3. The following organs were carefully examined: gills, liver, intestine, stomach, kidneys, gall bladder, scales and fins. Plasmodia were removed, placed on microscopic slides and examined in the light microscope under 100X oil objective (Magnus inclined Trinocular microscope MLX-Tr) for the presence of myxospores. The spores were treated with 8% KOH solution to evert the polar filaments. For permanent preparations, air dried smears were stained with Ziehl-Neelsen, Giemsa and Iron-haematoxylin. Complete description of the species was prepared according to the guidelines of Lom and Arthur (1989). For histopathological studies infected gills were cut into small pieces and fixed in Bouin's and Carnoy's fixatives, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax, sectioned at 4-6µm and stained with Luna's method (Figure 1).

3. RESULTS AND DISCUSSION

3.1. Description of spore

(Measurements based on 10-12 spores in frontal view); (Table 1)

Spores histozoic, measuring 10.74x4.07µm, elongated pyriform in valvular view, having tapering anterior end with slight bent in the anterior half of the spore and rounded posterior end. Shell valves thin, 0.50µm in thickness, smooth and symmetrical. Parietal folds absent. Polar capsule single, measuring 6.48x2.04µm in size, elongated, pear shaped with distinct tubular neck and occupies one third of the total spore body cavity. Polar filament form 18-20 coils, arranged perpendicular to the polar capsule axis. Polar filament thread-like measuring 51µm in length, when extruded. Sporoplasm occupies whole of the extracapsular space behind the polar capsule and contain two sporoplasmic nuclei measuring 1.17-1.33 µm in diameter. Iodinophilous vacuole is absent.

3.1.1. Histopathological findings

In the present study, the histological sections of gills of *Labeo rohita* Ham. infected with *T. dykovi* sp. nov. indicated that the plasmodia are intrafilamental vascular type (FV). Each plasmodia was bounded by a thin membrane and its lumen was filled with large number of spores, infilterated epithelial cells and macrophages. Plasmodia are multilocular occupying whole of the length of the gill filament (100%) thereby causing total destruction of its supporting elements i.e. extracartilaginous matrix (chondrocytes) and blood supply i.e. central venous sinus. In the

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Table 1

Measurements (µm) and ratio of *T. dykovi* sp. nov.

Characters	Range	Mean Values	SD
LS	9.74-11.74	10.74	1.41
WS	3.10-5.03	4.07	2.12
LPC	4.58-8.38	6.48	2.68
WPC	1.04-3.04	2.04	1.41
Ratio: LS/WS		2.63	
NC		18-20	
Parietal Folds		Absent	

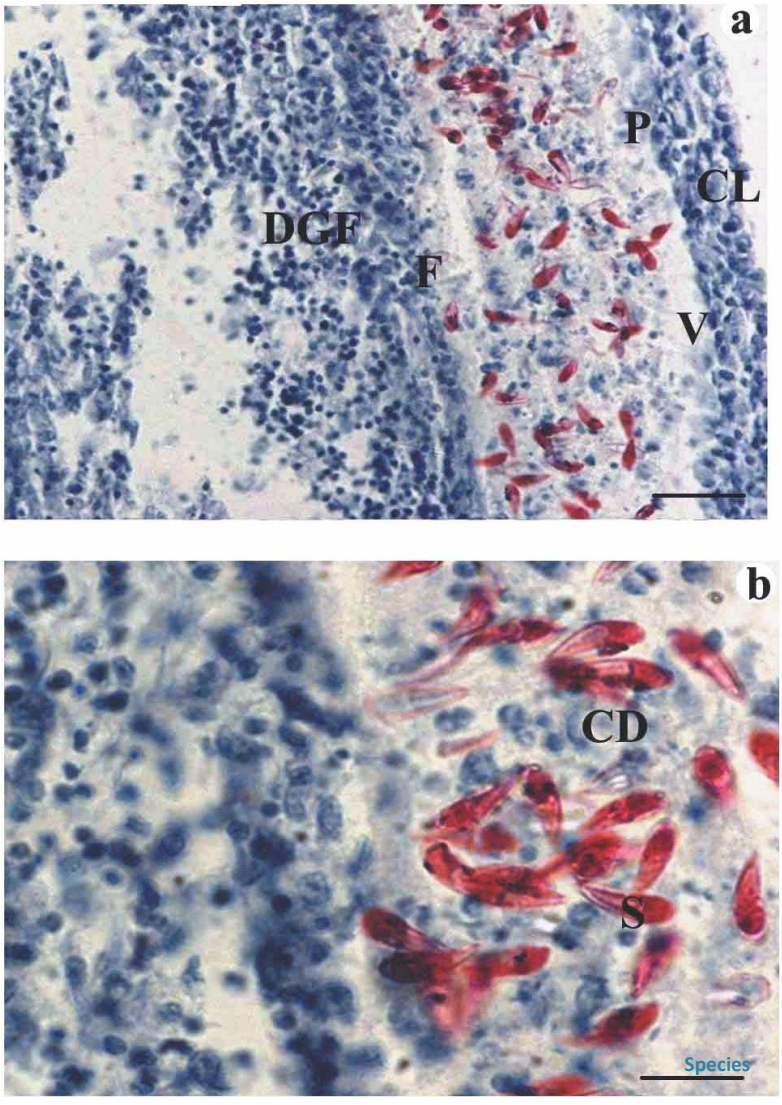

Figure 1

Histopathological changes in gill filament of *Labeo rohita* (Ham):

a. Sagittal section of gill filament of *Labeo rohita* showing intrafilamental type (FV) plasmodium of *Thelohanellus dykovi* sp. nov. occupying the entire filament causing degenerated gill filament (DGF) (400x)

b. Showing plasmodium filled with spores (S) along with cellular debris (CD) results in the total loss of respiratory surface (1000x)

LUNAS METHOD; scale bar: 0.01mm

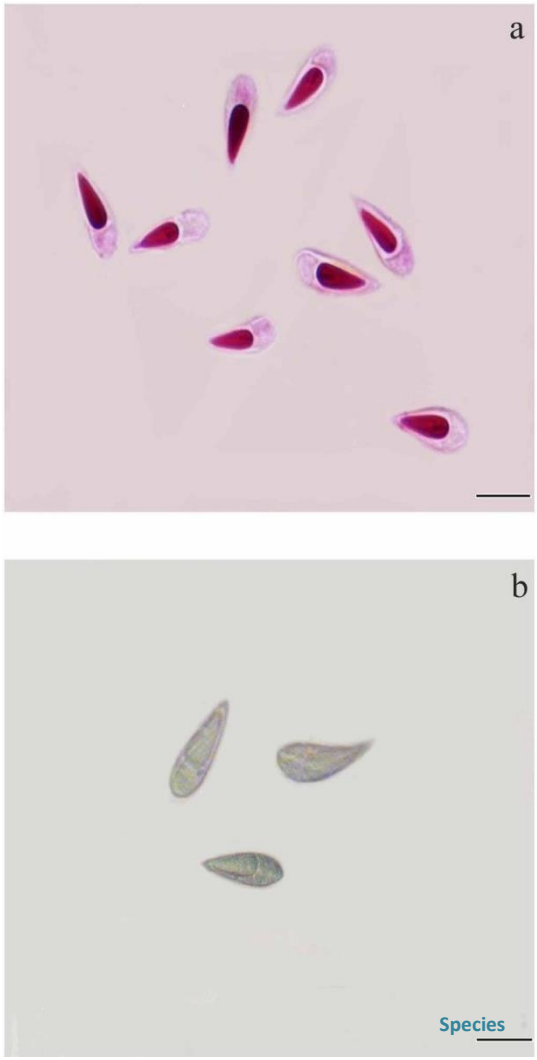

Figure 2

Micrographs of spores of *Thelohanellus dykovi* sp. nov. :
a- spore stained in Ziehl-Neelsen, **b-** fresh spores
Scale bar: 0.01mm

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Table 2

Comparative description of *Thelohanellus dykovi* sp. nov. with morphologically similar species
(Measurements are in micrometer)

Species	Host	Site of infection	Locality	Spore	Polar capsule
***Thelohanellus dykovi* sp. nov. present study**	*Labeo rohita*	Gills	Nanoki pond, Punjab (India)	10.74x4.07	6.48x2.04
***T. rohitae* Southwell and Prashad, 1918**	*L. rohita* and *L. bata*	Gills	West Bengal(India)	31.5x11.5	18x15.9
***T. gangeticus* Tripathi, 1952**	*Chela bacaila*	Muscles	West Bengal (India)	16.85-5.4	7.2x2.5
***T. andhrae* Qadri, 1962**	*L. fimbriatus*	Gills	Andra Pradesh (India)	12.85x5.0	7.0x2.25
***T. rodgii* Hagargi, 1979**	*L. calbasu*	Gills	West Bengal (India)	36.0x12.5	17.5x7.5
***T. jiroveci* Kundu and Haldar, 1981**	*L. bata*	Branchiae	Ranaghat, West Bengal (India)	16.3x6.8	7.3x4.1
***T. valeti* Fomena and Bouix, 1987**	*Barbus aspilus*	Operculum and Stomach wall	Africa	12.0x4.7	6.25x2.3
***T. orissae* Haldar *et al.*, 1997**	*Cirrhinus mrigala*	Gills	Orissa (India)	7.29x3.11	3.72x2.32
***T. citharini* Kostoingue *et al.*, 1999**	*Citharini citharus*	Heart tissue	Africa	11.1x6.1	6.6x3.2
***T. bifurcata* Basu and Haldar, 1999**	*L. rohita* x*Catla catla*	Gill lamellae	West Bengal (India)	34.89x9.21	23.3x6.6
***T. zahrahae* Szekely *et al.*, 2009**	*Barbonymus gonionotus*	Gills	Malaysia	23.8x9.0	9.9x6.3
***T. endodermitus* Mukhopadhyay and Haldar, 2004**	*L. rohita*	Under surface of scales	West Bengal (India)	13.66x5.35	7.14x3.0
***T. disporomorphus* Basu *et al.*, 2006**	*C. mrigala*	Tail fin	Halisahar West Bengal (India)	32.1 and 14.2x8.9 and 8.5	21.1 and 5.2x7.9 and 4.0
***T. anilae* Hemananda *et al.*, 2010**	*L. rohita*	Gills	Hingalganj West Bengal (India)	33.27 and 13.26x12.75 and 6.8	17.55 and 7.31x5.35 and 3.10

infected gill filament, the epithelial cells lining the secondary lamellae showed hypertrophy and hyperplasia resulting in accumulation of necrotic tissue thereby leading to total destruction of respiratory surface.

3.1.2. *T. dykovi* sp. nov. (Figures 2, 3)

Taxonomic characters

Type host : *Labeo rohita* (Ham.) vern. Rohu

Type locality : Nanoki Pond, Patiala

Pathogenicity : Highly pathogenic

Type specimen : Paratypes are spores stained in Ziehl-Neelsen and Giemsa, deposited in the museum of Department of Zoology, Punjabi University, Patiala, (India) Slide no. T/ZN/02.02.2012 and T/G/02.02.2012

Site of infection : Gills (Intrafilamental vascular type)

Prevalence of infection : 36.67% (11/30)

Etymology : The specific epithet *dykovi* has been given after the name of Professor Iva Dykova, an eminent worker in the field of Parasitology at Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic.

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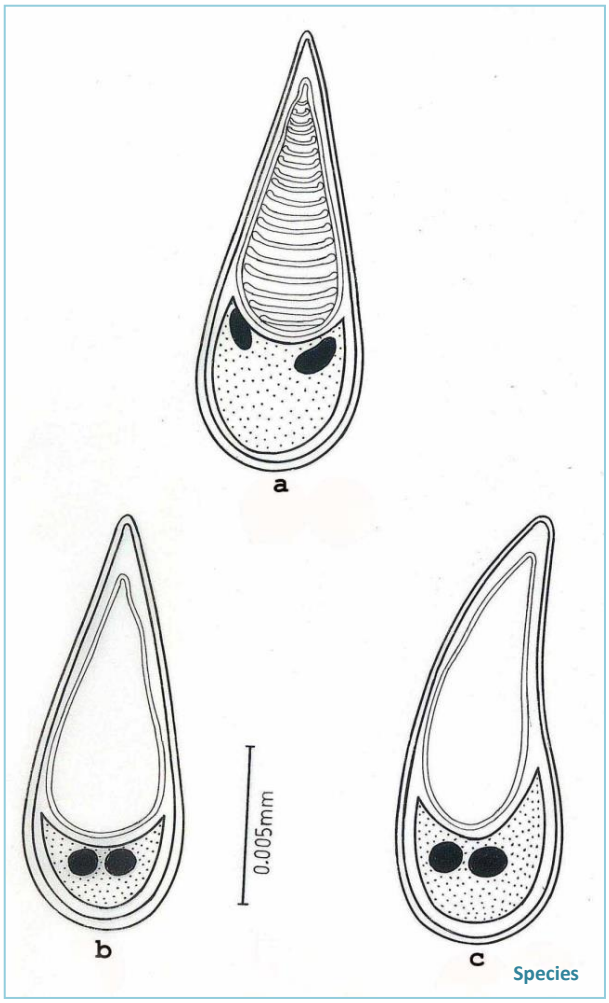

Figure 3

Line drawing (Camera Lucida) of spores of *Thelohanellus dykovi* sp. nov. : **a,b-** spore stained in Ziehl-Neelsen (valvular view), **c**- spore in side view
Scale bar: 0.05mm

3.1.3. Differential diagnosis

The present species of *Thelohanellus* was compared with *T. rohitae* Southwell and Prashad (1918) from gills of *Labeo bata* and *L. rohita*; *T. gangeticus* Tripathi (1952) from muscles of *Chela bacaila*; *T. andhrae* Qadri (1962) from gills of *L. fimbriatus*; *T. rodgii* Hagargi (1979) from gills of *L. calbasu; T. jiroveci* Kundu and Haldar (1981) from branchiae of *L. bata; T. valeti* Fomena and Boui (1987) from operculum and stomach wall of *Barbus aspilus***;** *T. citharini* Kostoingue et al. (1999) from heart tissue of *Citharinus citharus; T. bifurcata* Basu and Haldar (1999) from gill lamellae of *L. rohita* and *Catla catla* hybrid; *T. zahrahae* Szekely et al. (2009) from gills of *Barbonymus gonionotus***;** *T. anilae* Hemananda et al. (2010) from gills of *L. rohita; T. disporomorphus* Basu et al. (2006) from tail fin of *Cirrhinus mrigala; T. endodermitus* Mukhopadhyay and Haldar (2004) from under scales of *L. rohita; T. orissae* Haldar et al. (1997) from gills of *C. mrigala* but differ from all of the above species in morphological and morphometric characteristics (Table 2).

The spores of the present species are unique in having tapering anterior end and rounded posterior end. The anterior end of the polar capsule terminates into a distinct tubular neck and the posterior end is rounded in outline, occupying 1/3rd of the total spore body cavity. Morphologically the present species is comparable with the spores of *T. rohitae, T. jiroveci, T. valeti, T. zahrahae, T. rodgii* and *T. anilae***.** But larger spore size in *T. rohitae*; polar capsules occupying less than half of spore body cavity in *T. jiroveci, T. rodgii* and *T. zahrahae*; polar capsule blunt in *T. valeti***;** tear shaped polar capsules with sharply pointed anterior end in *T. anilae* differentiated all of them from the present species.

Histopathology of the myxozoan *Thelohanellus dykovi* sp. nov. infection in the gills of *L. rohita* were in conformity with the observations of Dey et al. (1988); Sanaullah and Ahmed (1980); Rukyani (1990); Azevedo et al. (2010) and Campos et al. (2011) according to them myxozoan gill filament infection cause alterations in the capillary network, hyperplasia of gill epithelium and structural disorganization of the secondary lamellae. The present study indicated that these alterations may partially compromise gill functions and therefore diminish the respiratory capacity and ionic exchange. Similar observations have been made by Awal et al. (2001) on the pathological changes in the gills of *Cirrhina mrigala* from Bangladesh having pathological changes like hypertrophy and hyperplasia with the presence of numerous inflammatory cells and accumulation of blood cells at the base of the secondary gill lamellae. Chavda et al. (2010) reported hemorrhagic condition with necrotic changes in epithelia and in connective tissues of gills in *Catla catla* infected with myxozoan parasite in central Gujarat region. Manrique et al. (2012) reported that intravascular plasmodia occupying secondary gill lamellae caused subepithelial edema leading to dilation of the sinusoids and lamellae.

In the present study, infected filament of the gill was completely distorted and lamellae were also disintegrated. Molnar et al. (2006); Martins and Sauza (1997); Martins et al. (1997); Haldar et al. (1983) also reported the presence of plasmodia in the gill filament as also in the present study. In *T. dykovi* sp. nov. development of plasmodia within the gill filament and its degenerative process has been found in accordance with the report of Lom and Dykova (1978). Yokoyama et al. (1997) reported infection caused by *Myxobolus koi*, on common resulted in the fusion of neighboring plasmodia. According to Current and Janouy (1978) location of the plasmodia formed by *Henneguya exilis* in the gill filament was intrafilamental. Other species located in the gill filament were *Myxobolus nanokiensis* Kaur et al. (2013), *M. salminus* Adriano et al. (2009) and *M. pavlovskii* Molnar (1979) infecting *Labeo rohita, Salminus brasiliensis* and *Cyprinus carpio* respectively.

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