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 Hamilton, Labeo rohita Hamilton and Cirrhinus mrigala Hamilton, 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 Hamilton), rohu (Labeo rohita Hamilton) and mrigal (Cirrhinus mrigala Hamilton) and exotic carps such as silver carp (Hypophthalmichthys molitrix Valenciennes), grass carp (Ctenopharyngodon idellus Valenciennes), common carp (Cyprinus carpio Linnaeus) 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, 2012a, b) 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, Hennequay 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, infiltrated 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
**Table 1**

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

<table>
<thead>
<tr>
<th>Characters</th>
<th>Range</th>
<th>Mean Values</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>9.74-11.74</td>
<td>10.74</td>
<td>1.41</td>
</tr>
<tr>
<td>WS</td>
<td>3.10-5.03</td>
<td>4.07</td>
<td>2.12</td>
</tr>
<tr>
<td>LPC</td>
<td>4.58-8.38</td>
<td>6.48</td>
<td>2.68</td>
</tr>
<tr>
<td>WPC</td>
<td>1.04-3.04</td>
<td>2.04</td>
<td>1.41</td>
</tr>
<tr>
<td>Ratio: LS/WS</td>
<td></td>
<td>2.63</td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>18-20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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
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**

<table>
<thead>
<tr>
<th>Type host</th>
<th>Labeo rohita (Ham.) vern. Rohu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type locality</td>
<td>Nanoki pond, Patiala</td>
</tr>
<tr>
<td>Pathogenicity</td>
<td>Highly pathogenic</td>
</tr>
<tr>
<td>Type specimen</td>
<td>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</td>
</tr>
<tr>
<td>Site of infection</td>
<td>Gills (Intrafilamental vascular type)</td>
</tr>
<tr>
<td>Prevalence of infection</td>
<td>36.67% (11/30)</td>
</tr>
<tr>
<td>Etymology</td>
<td>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.</td>
</tr>
</tbody>
</table>
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 baciailla*; *T. andhræae* Qadri (1962) from gills of *L. fimбриatus*; *T. rodgi* 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. citharinii* 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. zahrahea* Szekely et al. (2009) from gills of *Barbonymus goniomonotus*; *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. zahraea*, *T. rodgi* and *T. anilae*. But larger spore size in *T. rohitae*, polar capsules occupying less than half of spore body cavity in *T. jiroveci*, *T. rodgi* and *T. zahraea*; 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 *Cirrhinus 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 dilution 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 *Mxobolus koi*, on common resulted in the fusion of neighboring plasmodia. According to Current and Janouy (1978) location of the plasmodia formed by *Hennegeuya exilis* in the gill filament was intrafilamental. Other species located in the gill filament were *Mxobolus nanokiensis* Kaur et al. (2013), *M. salinus* Adrianho et al. (2009) and *M. pavlovskii* Molnar (1979) infecting Labeo rohita, Salminus brasiliensis and Cyprinus carpio respectively.

**REFERENCES**


15. Hemananda T, Mohil N, Bandyopadhyay PK, Mitra AK. Thelohanellus imphalensis sp. nov. (Myxozoa) infecting gills of a major carp Labeo rohita Hamilton 1822 from Thoubal, Manipur, India. Protistol, 2010, 6, 280-283
34. Longshaw M, Flear PA, Feist SW. Descriptions, development and pathogenicity of Myxozoa (Myxozoa: Myxobolidae) parasites of juvenile cyprinids (Pisces: Cyprinidae). J. Fish Dis, 2005, 28, 489-508
37. Martins ML, Souza VN. Henneguya piaractus n. sp.
(Myxozoa: Myxobolidae), a gill parasite of \textit{Piaractus mesopotamicus} Holmberg, 1887 (Osteichthyes: Characidae), in Brazil. \textit{Rev Bras Biol}, 1997, 57, 239-245
40. \textit{Mukhopadhuy D, Haldar DP. Thelohanellus endodermitus} sp. n. A new \textit{Myxozoa} (Myxozoa: Bivalvulida) from the major carp, \textit{Labeo rohita} (Hamilton-Buchan) in a sewage farm in West Bengal, India. \textit{Environ. Ecol}, 2004, 22, 139-142
41. \textit{Qadri SS. New myxosporidian from the Indian fresh water fish, Labeo fimbriatus} II. \textit{Thelohanellus andhrae} sp. n. Z. \textit{Parasitenk}, 1962, 21, 517-520
42. \textit{Rukyan A. Histopathological changes in gills of common carp (Cyprinus carpio L.) infected with the myxosporean parasite Myxobolus koi} Kudo, 1920. \textit{Asian Fishery Sci, 1990, 3, 337-341}
44. \textit{Southwell T, Prashad B. Notes from the Bengal fisheries laboratory, No. 5 Parasites of Indian fishes with a note on the carcinoma in the climbing perch. Records of Indian Museum (Calcutta), 1918, 15, 341-355}
47. \textit{Yokoyama H, Inoue D, Kumamaru A, Wakabayashi H. Myxobolus koi} (Myxozoa: Myxosporea) forms large-and small-type “cysts” in the gills of common carp. \textit{Fish Pathol, 1997, 32 (4), 211-217}