



## *Myxobolus leafa* sp. nov. (Myxozoa: Bivalbulida) from the gill filament of *Labeo bata* (Hamilton) from Manipur, India

Hindistan (Manipur)'daki *Labeo bata* Balığının Solungaç Filamentinden Yeni Bir Tür *Myxobolus leafa* sp. nov. (Myxozoa: Bivalbulida)

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### ABSTRACT

**Objective:** The aim of this study was to investigate the gill filaments of *Labeo bata* in India parasitologically and to describe the new Myxozoa parasite species, *Myxobolus leafa* sp. nov., which was determined.

**Methods:** Fish were collected alive from fish farms from Manipur (India). Sporogonic plasmodia were removed with sterile forceps and observed under phase contrast microscope. For permanent preparations, air dried smears were stained with Giemsa after fixation in acetone free absolute methanol.

**Results:** Mature spores of the new species appear as leaf-like structures in valvular or frontal view, tapering at both ends. The anterior end of the spore is broader than the posterior end. Spores measure 15.3-11.8  $\mu\text{m}$  ( $16.91 \pm 1.16$ ) in length and 13.6-15.3  $\mu\text{m}$  ( $14.15 \pm 0.67$ ) in breadth.

**Conclusion:** The new gill parasite species, *Myxobolus leafa* sp. nov., was described from fish in India. The prevalence of the parasite was 3.3% and two stages (cyst and spore) of the parasite existed in its life cycle. (*Türkiye Parazitol Derg* 2013; 37: 40-3)

**Key Words:** Myxozoa, parasite, fish, *Myxobolus leafa* sp. nov., India

**Received:** 01.10.2012

**Accepted:** 07.12.2012

### ÖZET

**Amaç:** Bu çalışmanın amacı Hindistan'daki *Labeo bata* balık türünün solungaç filamentlerini parazitolojik açıdan araştırmak ve tespit edilen yeni Myzoa parazit türünü *Myxobolus leafa* sp. nov. tanımlamaktır.

**Yöntemler:** Balıklar Manipur'daki (Hindistan) balık çiftliklerinden canlı olarak elde edilmiştir. Sporogonik plazmodyumlar steril penslerle çıkarılarak faz kontrast mikroskop altında incelenmiştir. Daimi preparasyonlar için, havayla kurumuş yayma preparatlar saf metanolden arındırılmış etanolde tespit edildikten sonra Giemsa'yla boyanmıştır.

**Bulgular:** Tespit edilen yeni parazit türünün olgun sporları önden bakıldığında yaprak benzeridir ve her iki uca doğru daralır. Sporların ön ucu arka uçtan daha geniştir. Sporların uzunluğu 15.3-11.8  $\mu\text{m}$  ( $16.91 \pm 1.16$ ), genişliği 13.6-15.3  $\mu\text{m}$  ( $14.15 \pm 0.67$ )'dir.

**Sonuç:** Hindistan'daki balıklardan yeni bir solungaç paraziti *Myxobolus leafae* sp. nov. tanımlanmıştır. Parazitin yaygınlığı %3.3'tür ve yaşam döngüsünde 2 safha (kist ve spor) mevcuttur. (*Türkiye Parazitol Derg* 2013; 37: 40-3)

**Anahtar Sözcükler:** Myxozoa, parazit, balık, *Myxobolus leafa* sp. nov., Hindistan

**Geliş Tarihi:** 01.10.2012

**Kabul Tarihi:** 07.12.2012

## INTRODUCTION

Numerous descriptions of myxosporean species of fish have been made from different geographic areas (1, 2). Myxozoa Bütschli, 1882 include more than 1200 valid species commonly found in fish (2, 3). Among them, *Myxobolus* Bütschli, 1882, with 744 species described, is the largest genus of the family Myxobolidae and has been reported as an important pathogen in freshwater fish (3).

Manipur, located in the Himalayan range, has a rich fish faunal diversity. However, the survival of many indigenous fish species has been hampered with the introduction of many new exotic fish species. This has also given rise to an increase in parasites including myxozoans. Many of these myxosporidians have pathogenic effects on the fish, causing serious damage to various body parts and resulting in heavy economic loss to the fish farmers and also threatening the biodiversity of indigenous fish species.

During a parasitological survey, examinations were conducted on various host fish species, which revealed the presence of a new myxozoan species of the genus *Myxobolus* from the gill filaments of *Labeo bata* (Hamilton) collected from a fish farm of Langmeidong, Manipur, India.

## METHODS

Host fish of the genus *Labeo bata* (Hamilton) were collected alive from fish farms of Langmeidong, brought to the laboratory and examined immediately. Sporogonic plasmodia, when found, were carefully removed with sterile forceps, smeared on clean grease free slides with drops of 0.5% NaCl solution, covered with cover slips and sealed with bee wax for examination under the oil immersion lens of the Olympus CH2 phase contrast microscope. Some of the fresh smears were treated with various concentrations (2-10%) of KOH solution for the extrusion of polar filaments. The Indian ink method of was employed for observing the mucous envelope of spores (4). For permanent preparations, air dried smears were stained with Giemsa after fixation in acetone free absolute methanol (5). Measurements (based on twenty fresh spores treated with Lugol's iodine) were done with the aid of a calibrated ocular micrometer. All measurements are presented in  $\mu\text{m}$  as mean $\pm$ SD followed in parentheses by the range.

Drawings were made on fresh or stained material with the aid of a mirror type camera lucida and the Corel Draw 10.0 computer programme.

To describe the morphology of the parasite, several abbreviations have been used, as follows.

## RESULTS

### *Myxobolus leafa* sp. nov. (Figures 1a-j, Tables 1, 2)

Phylum: Myxozoa

Class: Myxosporea

Order: Bivalvulida

Family: Myxobolidae

Genus: Myxobolus

Species: *leafa*

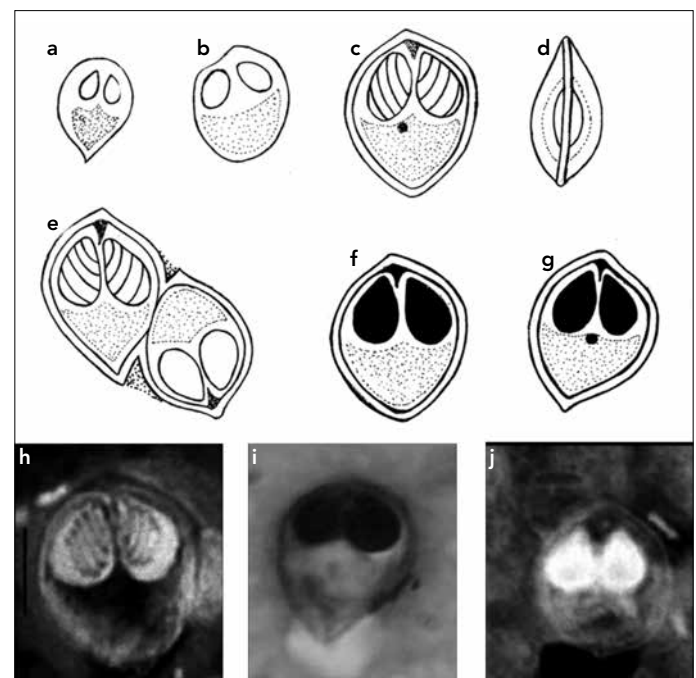
## Diagnosis

**Cyst:** Numerous yellowish white, spherical cysts or plasmodia are found attached to the gill filaments of the host, measuring 1.4-1.6 mm in diameter. Cysts contain mostly mature spores. A few developmental stages are also observed.

**Spore:** Mature spores appear as leaf-like structures in the valvular or frontal view, tapering at both ends. The anterior end of the spore is broader than the posterior end. The spore measures 15.3-11.8  $\mu\text{m}$  (16.91 $\pm$ 1.168) in length and 13.6-15.3  $\mu\text{m}$

**Table 1.** Measurements of 20 fresh/stained spores of *Myxobolus leafa* sp. nov.

Characters	Range	Mean	SD	SE	CV (%)
LS	15.3-18.7	16.91	1.168	0.261	6.907
BS	13.6-15.3	14.152	0.673	0.150	4.755
LPC	5.95-8.5	6.67	0.724	0.162	10.854
BPC	5.1-5.95	5.31	0.368	0.082	6.23
<b>Spore Index</b>					
LS:	BS=1:	0.836			
LPC:	BPC=1:	0.796			
LS:	LPC=1:	0.394			
BS:	BPC=1:	0.375			
LS: length of the spore, BS: breadth of the spore, LPC: length of the polar capsule, BPC: breadth of the polar capsule, LPF: length of the polar filament, DIV: diameter of iodophilous vacuole					



**Figure 1.** (a-g) Camera lucida drawings of different stages of matured spores of *Myxobolus leafa* sp. nov. (h-j) Photomicrographs of matured fixed spores in valvular view (Giemsa stained) of *Myxobolus leafa* sp. nov. scale bar - 6.67  $\mu\text{m}$ . (a, b) Fresh spore-developmental stages. (c) Fresh spore in valvular view. (d) Fresh spores in sutural view. (e) Fresh spore in valvular view-Lugol's iodine. (f, g) Fixed spores in valvular view-Giemsa stained

**Table 2.** Morphometric comparison of *Myxobolus leafa* sp. nov. to closely related species

Characters	<i>M. drijagini</i>	<i>M. iucundus</i>	<i>M. hendricksoni</i>	<i>M. magnaspherus</i>	<i>M. mokhayeri</i>	<i>Myxobolus leafa</i> sp. nov.
LP	12.5	13	13.1	18	14.4-16.6 (15.8)	15.3-18.7, (16.19±1.13)
BS	9.5	10.7	12.3	20	11.6-13.3 (12.5)	13.6-15.3 (14.15±0.67)
LPC	Unequal L: 6, S: 3.35	8	-	10	Unequal L: 7.7- 7.9 (7.5), S: 5.8 -7.5 (6.6)	5.95-8.5, (6.67±0.72)
BPC	Unequal, L: 3.8, S: 2.35	3.6	-	6	L: 4.1-4.9 (4.6), S: 3.3-4.1 (3.6)	5.1-5.95 (5.31±0.36)
Host	<i>Hypophthalmichthys molitrix</i>	<i>Galaxia maculata</i>	<i>Pimephales promelas</i>	<i>Lepomis gibbosus</i>	<i>Capoeta trutta</i>	<i>Labeo bata</i>
Site of infection	Subcutaneous tissue	Skin	Brain	Kidney	Fin	Gill
Reference	(6)	(7)	(8)	(9)	(10)	Present study
LPC: length of the polar capsule, BPC: breadth of the polar capsule, BS: breadth of the spore						

(14.15±0.67) in breadth. On sutural view the spore is pyriform in shape with a distinct, slightly curved sutural line without a ridge or fold. Two shell valves are symmetrical, smooth, thin and uniform with no parietal fold. The posterior part of the spore is surrounded by a mucus envelope.

Two equal polar capsules are oval to pyriform in shape with a broadly pointed anterior end and rounded posterior end. Each polar capsule measures 5.95-8.5 µm (6.6±0.72) in length and 5.1-5.95 µm (5.31±0.36) in breadth. Inside the polar capsule, polar filaments coil obliquely forming 4-5 turns. Polar capsules converge slightly at the anterior part. An intercapsular ridge is present as an outgrowth of the inner wall of the spore at the anterior half of the spore cavity. The extracapsular region is occupied by a double crescent shaped sporoplasm. An oödinophilous vacuole is absent but a tiny sporoplasmic nucleus is present at the upper part of the sporoplasm.

In many cases, the mucus envelope is very prominent while in some spores, the mucus envelope remains in restricted areas at the posterior most part of the spore. In some other cases, the posterior end is prominently pointed-like the tip of a leaf, while in some spores it is bluntly pointed. In most cases the anterior portion is wider than the posterior portion.

#### Taxonomic Summary:

Type Host: *Labeo bata* (Hamilton)

Type Locality: Langmeidong, Thoubal Dist.[24° 27'30.47" N; 93°55'15" E]

Site of Infection: Gill

Prevalence: 7/210 (3.3%)

Type material: Slides containing holotype (MU/P/My) and paratype (MU/P/My<sub>a-d</sub>) has been deposited in the collection of Parasitology Section, Life Sciences Department, Manipur University, Canchipur, Imphal, India.)

Number of specimen measured: 30

Etymology: This species was named as per structural resemblance of the parasite to a leaf.

#### DISCUSSION

Due to the presence of two equal pyriform to oval polar capsules with intercapsular appendage, the present myxozoan species is placed under the genus *Myxobolus* Bütschli, 1882 and group I of Tripathi's grouping of the genus *Myxobolus* (3). When the present species is compared with other closely related species of the genus *Myxobolus*, it resembles *M. drijagini* Akmerov, 1954 (6) reported from the subcutaneous tissue of *Hypophthalmichthys molitrix* from Amur River, Russia [LP-12.5, BS-9.5, LLPC-6, LSPC-3.35, BLPC-3.8, BSPC-2.35]; *M. iucundus* Hine, 1977 (7) reported from the skin of *Galaxias maculata* from New Zealand [LP-13, BS-10.7, LPC-8, BPC-3.6]; *M. hendricksoni* Mitchell et al. (8), reported from the brain of *Pimephales promelas* from USA [LP-13.1, BS-12.3]; *M. magnaspherus* Cone et al. (9), reported from the kidney of *Lepomis gibbosus* [LP-18, BS-20, LPC-10, BPC-6] and *M. mokhayeri* Baska et al. (10), reported from the fin of *Capoeta trutta* [LS-15.8, BS-12.5, LLPC-7.5, BLPC-4.6, LSPC-6.6, BSPC-3.6]. However, *M. drijagini* have a smaller spore size, unequal polar capsule and anteriorly more expanded spore than the present species. *M. iucundus* have a smaller spore size, and longer and narrower capsule than the present species. *M. hendricksoni* also have a smaller spore size than the present species. Moreover, its ovoid or spheroid spore differs from the leaf-like spore of the present species. *M. magnaspherus* have larger and broader spores than the present species. *M. mokhayeri* have an unequal sized polar capsule whereas the present species have equal sized polar capsules.

#### CONCLUSION

Considering all these differences, we propose the *Myxobolus* species we have obtained from Manipur as new to science and name it as *Myxobolus leafa* sp. nov.

#### Conflict of Interest

No conflict of interest was declared by the authors.

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