

Two new species of *Myxobolus* (Myxosporea, Bivalvulida) from the Indian major carp *Labeo rohita* (Ham.)

Harpreet Kaur and Ranjeet Singh

Department of Zoology, Punjabi University, Patiala, India

Summary

During a survey on fish parasites in Wetlands of Punjab, India, two new myxosporean species, *Myxobolus sushmii* sp. nov. and *Myxobolus punjabii* sp. nov. were found infecting the Indian freshwater fish *Labeo rohita* (Ham.). Spores of *M. sushmii* sp. nov., a parasite infecting the eye ball, are measuring $9.0\text{--}10.2$ (9.6 ± 0.84) \times $7.3\text{--}9.3$ (8.3 ± 1.41) μm in size. The spores have ovoidal to sub-spherical shape with flat anterior end between the openings of two polar capsules and rounded posterior end. Spore valves are thick with 6–8 parietal folds arranged around the margin of spore. Two polar capsules are distinctly unequal, broad and pyriform in shape with pointed anterior end. Larger one is $4.1\text{--}5.3$ (4.70 ± 0.84) \times $2.9\text{--}3.5$ (3.2 ± 0.42) μm and smaller one is $2.9\text{--}3.4$ (3.10 ± 0.35) \times $1.0\text{--}3.0$ (2.0 ± 1) μm in size. Prominent U-shaped intercapsular process is present. *M. punjabii* sp. nov. infect the caudal fin. The sub-spherical spores are measuring $8.1\text{--}9.7$ (8.9 ± 3.1) \times $6.4\text{--}7.5$ (6.9 ± 0.77) μm in size. Shell valves are without parietal folds. Two polar capsules are distinctly unequal. Larger one measures $3.4\text{--}5.4$ (4.4 ± 1.41) \times $2\text{--}3.4$ (2.7 ± 0.98) μm ; smaller one is $2.8\text{--}3.6$ (3.2 ± 0.56) \times $1.2\text{--}2.2$ (1.7 ± 0.7) μm in size. They are tear shaped with pointed anterior end and broad posterior one. Both are placed parallel to each other. Intercapsular process is absent.

Key words: freshwater fishes, Harike Wetland, India, Kanjali Wetland, *Labeo rohita*, myxozoans, *Myxobolus*

Introduction

Myxozoans are economically important group of microscopic metazoan parasites, which cause disease in a large variety of commercially important fishes. They have been also reported in platyhelminthes, reptiles, amphibians, mammals and were also

detected in fecal sample of human beings (Boreham et al., 1998). As many as 104 species of the genus *Myxobolus* have been reported from Indian fishes (Kalavati and Nandi, 2007), however, myxozoan parasites infecting freshwater fishes of North India are studied insufficiently. The genus *Myxobolus* combines the most common myxosporeans para-

sitizing fishes. Eiras with co-authors (2005) listed approximately 744 species of *Myxobolus* all over the world. In the present study, two new species belonging to the genus *Myxobolus* Bütschli isolated from eye ball and caudal fin of *Labeo rohita* (Ham.) caught in Wetland, Punjab were described in accordance with the guidelines by Lom and Arthur (1989).

Material and methods

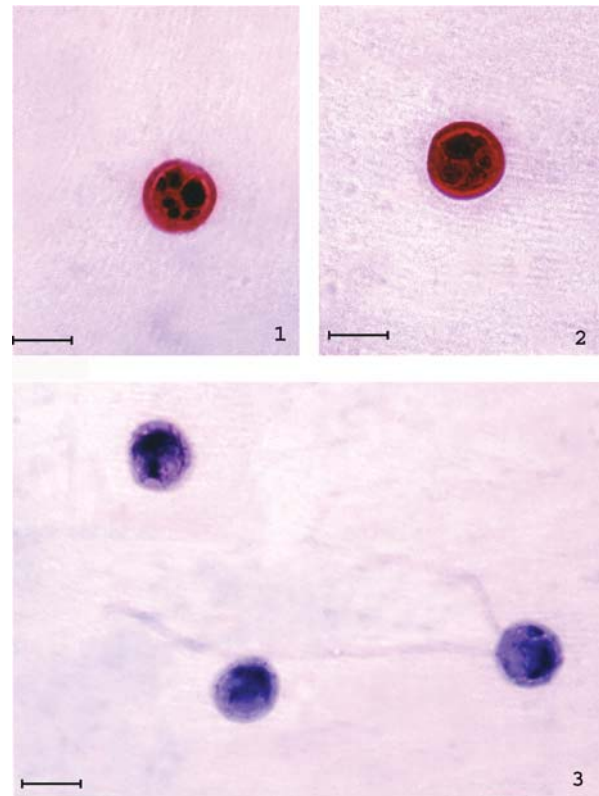
Infected eye and caudal fins were smeared on the clean slides in a drop of 0.98% NaCl solution, covered with cover slip and examined for the presence of spores. To observe iodophilous vacuole the smears were immersed in Lugol's iodine solution. To stimulate the filament extrusion the smears were treated with 8% KOH. For permanent preparation, the smears were fixed in Bouins fixative and stained with Ziehl-Neelsen and Iron-haematoxylin. Drawings were made from stained material with the aid of camera lucida. 15-20 spores were measured with the aid of a calibrated ocular micrometer. All measurements are given in microns (μm) as range values followed by mean \pm SD in parentheses.

Results

MYXOBOLUS SUSHMII SP. NOV. (FIGS 1-3, 8-10).

Plasmodia. Creamy- white, large, oval to spherical in shape plasmodia are measuring 2×3 mm in size, 2-3 in number in each eye ball. 60- 65 spores are present per plasmodium.

Spores. The spores are histozoic, ovoidal to sub-spherical in shape measuring $9.0-10.2$ (9.6 ± 0.84) \times $7.3-9.3$ (8.3 ± 1.41) μm in size and lenticular in lateral view. The anterior end is flat between the openings of two polar capsules and posterior end is broad and rounded. Spore valves are thick, symmetrical, measuring 1.0 μm in thickness, with 6-8 parietal folds arranged all around the margin of spore. Two polar capsules are distinctly unequal. Larger one is measuring $4.1-5.3$ (4.70 ± 0.84) \times $2.9-3.5$ (3.20 ± 0.42) μm and smaller one is $2.9-3.4$ (3.10 ± 0.35) \times $1.0-3.0$ (2.0 ± 1) μm in size. Both are broadly pyriform with pointed anterior end and rounded posterior one. The capsules open independently. Both polar capsules are situated parallel to each other in spore cavity. Larger one occupies half of the spore body; the smaller one – one third of it. Large prominent U- shaped intercapsular process is present. In



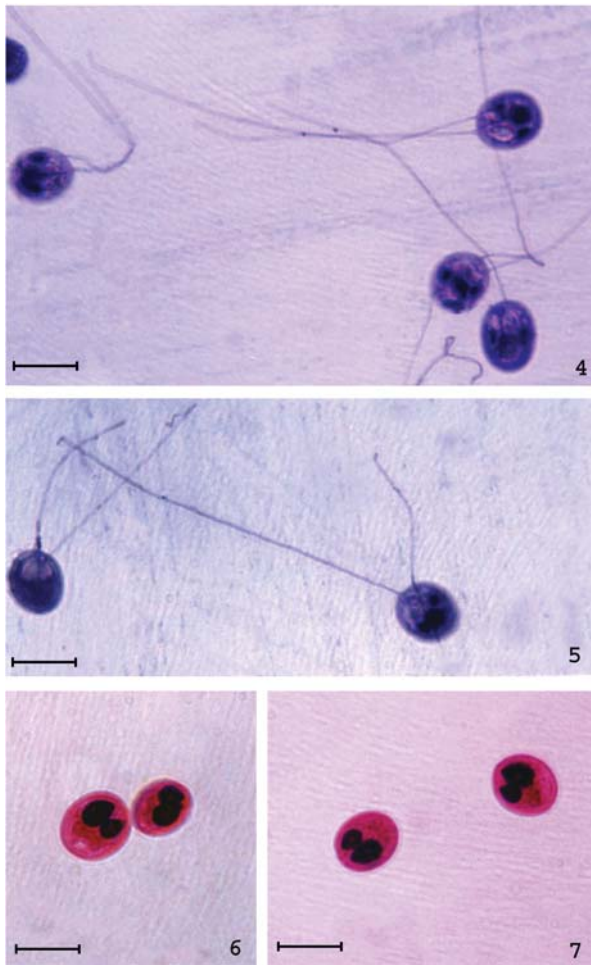
Figs 1-3. Photomicrographs of *M. sushmii* sp. nov.: 1, 2 - spores stained in Ziehl-Neelsen; 3 - spores with the polar filaments extruded through the independent openings stained with Iron-haematoxylin. Scale bars = $10 \mu\text{m}$.

larger capsule polar filament is coiled into 4-6 turns compared to 3-4 turns in smaller one. The coils are aligned obliquely to the longitudinal axis of the capsule. The extruded polar filaments are unequal in length and in thickness, the thick one is measuring $48.3 \mu\text{m}$ and the thin one is $30 \mu\text{m}$ in length. Two capsulogenic nuclei measuring $0.86-1.46$ (1.16 ± 0.42) μm in diameter, are present just beneath each polar capsule. Sporoplasm is agranular, homogenous, hemispherical and contains two sporoplasmic nuclei measuring $0.83 \mu\text{m}$ in diameter. Iodinophilous vacuole is absent.

MYXOBOLUS PUNJABII SP. NOV. (FIGS 4-7, 11-13).

Plasmodium. White, small, rounded plasmodia measuring 0.08×1.0 mm in size, 4-5 in number are found attached to each caudal fin. 30-35 spores are present per plasmodium.

Spores. The spores are histozoic and measuring $8.1-9.7$ (8.9 ± 1.31) \times $6.4-7.5$ (6.9 ± 0.77) μm in size. They are sub-spherical in shape with rounded



Figs 4-7. Photomicrographs of *M. punjabii* sp. nov.: 4, 5- spores with the polar filaments extruded through the independent openings stained with Iron-haematoxylin; 6, 7- spores stained in Ziehl-Neelsen. Scale bars = 10µm.

anterior and posterior ends. In sutural view, spores are lenticular and shell valves are protruded towards outside at anterior end and pointed slightly curved at posterior end. Shell valves are thick, smooth, symmetrical, measuring 1.16 µm in thickness. Two polar capsules are distinctly unequal, tear shaped with sharp pointed tip at the anterior end and broad posterior end. Larger polar capsule measures 3.4-5.4 (4.4 ± 1.41) × 2-3.4 (2.7 ± 0.98) µm; smaller one is 2.8-3.6 (3.2 ± 0.56) × 1.2-2.2 (1.7 ± 0.7) µm in size. The polar capsules are situated parallel to each other. The larger one is coiled into 6-7 turns while the smaller one – into 4-5 turns, which aligned perpendicular to the axis of polar capsule. Polar filaments are thin, unequal in length when extruded. They are open independently and measuring 57-59.6 (58.3 ± 0.18) and 13-15 (14 ± 1.41) µm, respectively. Intercapsular

process is absent. Two capsulogenic nuclei are present beneath each polar capsules, measuring 1.50 µm in diameter. Sporoplasm is agranular and homogenous occupying all extracapsular space behind the polar capsules. Iodinophilous vacuole is large and measuring 2.19-4.13 (3.16 ± 1.37) µm in diameter. Two sporoplasmic nuclei are measuring 1.50 µm in diameter.

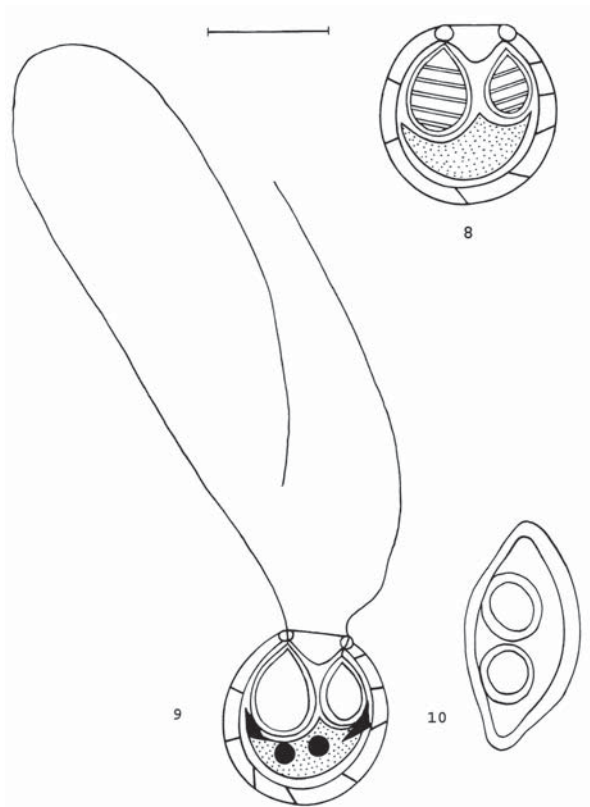
Discussion

Myxobolus sushmii sp. nov. While comparing *M. sushmii* sp. nov. with *M. maggauddi* Bajpai et al., 1981 from branchial filaments of *Trichogaster fasciatus*; *M. chakravartyi* Haldar et al., 1983 from internal musculature of *Catla catla*; *M. bliccae* Donec and Taoziyakova (in Shulman, 1984) from gills of *Abramis sapa* and *Blicca bjoerkna*; *M. njinei* Fomena et al., 1985 from gill arch and connective tissue of *Barbus camptacanthus*; *M. mahendrae* Sarkar, 1986 from gill arch of *Catla catla*; *M. punjabensis* Gupta and Khera, 1989 from fins and gills of *Labeo dyocheilus*; *M. bilongi* Fomena et al., 1994 from gills and fins of *Labeo* sp.; *M. labeosus* Sarkar, 1995 from mesentery associated with spleen of *L. fimbriatus*, it was found that present species differed from all of the above mentioned species in the morphometric characteristics (Table 1).

Spores of *M. sushmii* sp. nov. are ovoidal to sub-spherical in shape having anterior end flattened between two opening of the polar capsule and rounded posterior end. Its shape is different from oval spores of *M. maggauddi*; bowl-shaped spores of *M. punjabensis* and oval to egg-shaped but obliquely truncated (anteriolaterally) spores of *M. labeosus*. The present species have 6-8 parietal folds arranged all around the margins of spore in contrast to 5-6 in *M. mahendrae* restricted to the posterior end of shell valves and its absence in *M. chakravartyi*, *M. bilongi*, *M. bliccae* and *M. njinei*. The shape of the spores of the present species *M. sushmii* sp. nov. closely resemble to that of *M. mahendrae* and *M. chakravartyi*, however, differed in having a well developed U- shaped intercapsular process.

In light of above differences from closely related species the present myxozoan was considered as a new species.

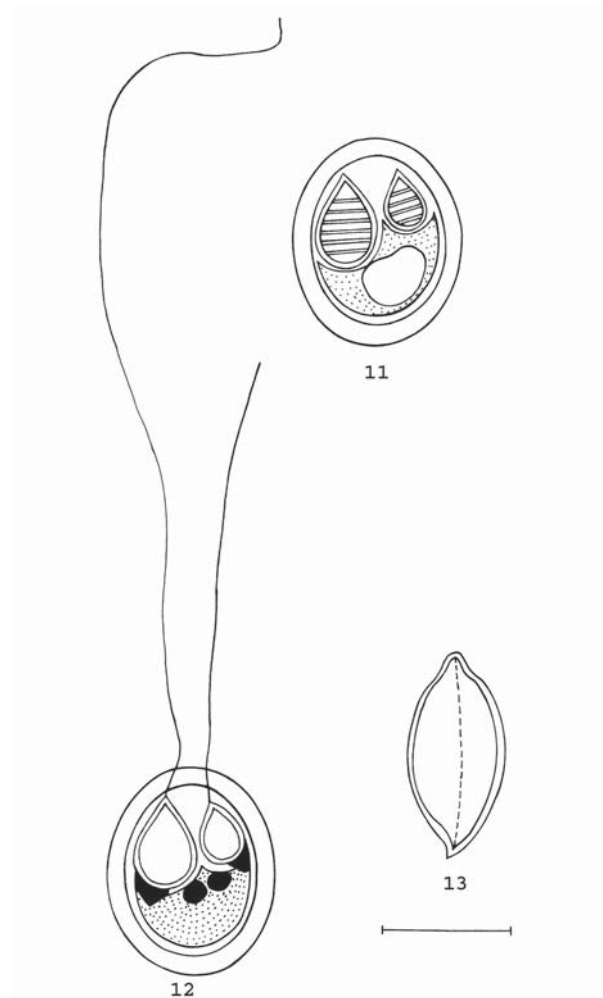
Myxobolus punjabii sp. nov. The shape and size of spores of the described species was compared with the spores of *M. amurensis* Akhmerov, 1960 from fins and gut of *Cyprinus carpio haematopterus*; *M. sphaerocapsularis* Shulman, 1962 from muscles of *Acheilognathus chankaensis*; *M. diversicapsularis*



Figs 8-10. Camera lucida drawing of the spores of *M. sushmii* sp. nov.: 8 - mature spore stained in Ziehl-Neelsen (valvular view); 9 - spore with extruded polar filaments; 10 - spore in sutural view. Scale bar = 5µm.

Slukhai (in Shulman, 1966) from gills of *Rutilus rutilus*; *M. rachmani* Allamuratov, 1966 from kidneys of *Alburnoides tenniatus*; *M. musseliusae* Yakovchuk, 1979 from gills of *Cyprinus carpio*; *M. alburni* Donec, 1984 from fins of *Alburnus alburnus*; *M. gobiorum* Donec, 1984 from fins of *Gobio gobio*; *M. drjagini* (Akhmerov, 1954) Landsberg and Lom, 1991 from skin of *Hypophchthysthalmi* and *M. dossoui* Sakiti et al., 1991 from gill arch cartilage of *Tilapia zillii*. The present species differed in the morphometric characteristics as well as in the shape of polar capsules from all the above mentioned species (Table 2).

The spores of *M. punjabii* sp. nov. are characterized by sub-spherical shape, thick and smooth shell valves without any parietal folds. Furthermore, the polar capsules are unequal, tear shaped with very sharp pointed anterior and broad rounded posterior ends, situated parallel to each other, containing 6-7 and 4-5 turns of the polar filaments. Both polar filaments are unequal and extrude independently through anterior end of spore. Therefore, in



Figs 11-13. Camera lucida drawing of the spores of *M. punjabii* sp. nov.: 11, 12 - spores stained in Ziehl-Neelsen (valvular view); 13 - spore in sutural view. Scale bar = 5µm.

this respect, they are closer to *M. gobiorum*, *M. musseliusae* and *M. dossoui* but differ from the above mentioned species in absence of intercapsular process. The latter is very large in *M. dossoui*, medium-sized in *M. gobiorum* and *M. musseliusae*. In light of such differences we hereby propose this species as a new one.

TAXONOMIC SUMMARY OF *M. SUSHMII* SP. NOV.

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

Type locality: Harike Wetland Punjab, India.

Type specimens: Paratypes are spores stained in Ziehl-Neelsen and Iron-haematoxylin deposited in the museum of Department of Zoology, Punjabi University, Patiala. No. LR/Q/ZN/17.01.2009 and LR/Q/IH/ 17.01.2009.

Table 1. Comparative description of the present *Myxobolus sushmii* sp. nov. with morphologically similar species.

Species	Host	Location	Locality	Spore (µm)	Polar capsule (µm)
<i>M. sushmii</i> sp. nov. (present study)	<i>Labeo rohita</i>	eye ball	Harike Wetland, Punjab (India)	9.0-10.2 (9.6) x 7.3-9.3 (8.3)	4.1-5.3 (4.70) x 2.9-3.5 (3.20) and 2.9-3.4 (3.10) x 1.0-3.0 (2.0)
<i>M. maggauidi</i> Bajpai et al., 1981	<i>Trichogaster fasciatus</i>	branchial filament	West- Bengal (India)	10.8-11.7 (11.2) x 8.3-10.0 (9.2)	3.3-5.0 (4.0) x 2.5-3.3 (2.6) and 2.5-3.3 (2.6) x 1.7-2.5 (1.8)
<i>M. chakravarty</i> Haldar et al., 1983	<i>Catla catla</i>	internal-musculature	West- Bengal (India)	12.1-14.3 (12.3) x 7.7-10.5 (8.8)	5.5-6.6 (5.6) x 3.3-5 (4.3) and 4.4-5 (4.5) x 2.2-4.4 (3.2)
<i>M. bliccae</i> Donec and Taoziyakova (in Shulman, 1984)	<i>Blicca bjoerkna</i> ; <i>Abramis sapa</i>	gills	Ukraine	10.3-14 x 8.2-11.8	5.5-7.9 x 3-4.5
<i>M. njinei</i> Fomena et al., 1985	<i>Barbus</i> sp.	gill arch connective tissue	Cameroon	14-20 x 11.5-18.5	6.5-9 x 3.5-5.5
<i>M. mahendrae</i> Sarkar, 1986	<i>Catla catla</i>	gill arch epithelium	India	11.5-14.0 (12.7) x 9.8-10.5 (10.4)	6.3-7.3 (7.0) x 3.5-4.2 (3.7)
<i>M. punjabensis</i> Gupta and Khera, 1989	<i>Labeo dyocheilus</i>	gills, fins	Ludhiana and Harike Wetland, Punjab (India)	10-12 (10.86) x 10-11 (10.64)	5-6 (5.72) x 4-5 (4.4) and 4-5 (4.72) x 3-4 (3.52)
<i>M. bilongi</i> Fomena et al., 1994	<i>Labeo</i> sp.	gills, fins	Cameroon	14-17 x 11.5-14.5	6.5-8 x 4-6 and 4-6.5 x 3-4
<i>M. labeosus</i> Sarkar, 1995	<i>Labeo fimbriatus</i>	mesentery associated with spleen	West -Bengal (India)	8.0-10.0 (9.25) x 6.5-9.0 (7.62)	6.0-6.5 (6.15) x 2.0-4.0 (2.72) and 3.0-4.5 (4.02) x 1.8-2.5 (2.33)

Site of infection: Eye ball.

Prevalence of Infection: 8/28 (28.5%).

Etymology: New species “sushmii” has been named in honor of Dr. Sushma Gupta, an alumnus of Punjab University, Chandigarh (India) who has significant contribution towards the study of myxozoan parasites of freshwater fishes of North India.

TAXONOMIC SUMMARY OF *M. PUNJABII* SP. NOV.

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

Type locality: Kanjali Wetland Punjab, India.

Type specimens: Paratypes are spores stained in Ziehl-Neelsen and Iron-haematoxylin deposited in the museum of Department of Zoology, Punjabi University Patiala, India. No. LR/P/ZN/10.05.2009 and LR/P/IH/ 10.05.2009.

Site of infection: Caudal fin.

Prevalence of Infection: 5/15 (33.3%).

Etymology: New species has been named as *Myxobolus punjabii* after the name of the place “Punjabi University Patiala” where the present work was carried out.

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Table 2. Comparative description of the *Myxobolus punjabii* sp. nov. with morphologically similar species.

Species	Host	Location	Locality	Spore (µm)	Polar capsule (µm)
<i>M. punjabii</i> sp. nov. (present study)	<i>Labeo rohita</i>	caudal fin	Kanjali Wetland Punjab (India)	8.1-9.7 (8.9) x 6.4-7.5 (6.9)	3.4-5.4 (4.4) x 2-3.4 (2.7) and 2.8-3.6 (3.2) x 1.2-2.2 (1.7)
<i>M. amurensis</i> Akhmerov, 1960	<i>Cyprinus carpio haematopterus</i>	fins, gut	Amur basin	9-13.5 x 9-12.5	4.5-7 x 3.8-4.2
<i>M. sphaerocapsularis</i> Shulman, 1962	<i>Acheilognathus chankaensis</i>	muscles	China	17-18 x 11-12	7-8 x 5.6-6.5
<i>M. diversicapsularis</i> Slukhai (in Shulman 1966)	<i>Rutilus rutilus</i>	gills	Russia	8.5-13 x 8.5-12.5	3.8-5.5 x 2.2-3.7
<i>M. rachmani</i> Allamuratov, 1966	<i>Alburnoides tenniatus</i>	kidney	Central Asia	13.5-14.3 x 11.2-12	7.2-7.5 x 3.7-3.9
<i>M. musseliusae</i> Yakovchuk, 1979	<i>Cyprinus carpio</i>	gills	Russia	10.5-11.1 x 8.8-10	3.9-4
<i>M. alburni</i> Donec, 1984	<i>Alburnus alburnus</i>	fins	Russia	12-15.3 x 10-12	6.8-8.1 x 3-3.5
<i>M. dossoui</i> Sakiti et al., 1991	<i>Tilapia zillii</i>	gill arch cartilage	Benin	8.5-11 (9.9) x 8-10.5 (9.2)	4.5-6.5 (5.5) x 2.5-5 (3.1)
<i>M. drjagini</i> (Akhmerov, 1954) Landsberg and Lom, 1991	<i>Hypophchthysthalmi molitrix</i>	skin	Amur Basin	13-14 x 9-10	5.5-6 x 3-3.5
<i>M. gobiorum</i> Donec, 1984	<i>Gobio gobio</i>	fins	Ukraine	11-13 x 9-10	5.2-6.5 x 2.2-3

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Address for correspondence: Harpreet Kaur. Department of Zoology, Punjabi University, Patiala-147002, India; e-mail: harpreet_bimbira@yahoo.com