TWO NEW MYXOSPOREAN PARASITES (MYXOZOA: MYXOBOLIDAE) FROM THE FRESHWATER FISH *PUNTIUS TICTO PUNCTATUS* DAY IN KERALA, INDIA

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ABSTRACT

Two new species of Myxobolus, *Myxobolus puntiusi* sp. nov. from the heart wall, and *Myxobolus ticto* sp. nov. from the gills, muscles, intestine and liver of the two-spotted barb, Puntius ticto punctatus Day are described.

KEYWORDS

Myxobolus puntiusi sp. nov., *Myxobolus ticto* sp. nov., Myxosporea, parasites, *Puntius ticto punctatus.*

Kerala with its vast expanse of water bodies has a rich freshwater fish fauna. The two-spot barb, *Puntius ticto punctatus*, is a common freshwater fish found in paddy fields, ponds, streams and rivers of Kerala. During the course of an explorative study on the myxosporean fauna of freshwater fishes in the Malappuram district of Kerala, we came across two species belonging to the genus *Myxobolus* from *P. ticto punctatus*. The histozoic genus *Myxobolus* was established by Bütschli (1882) with *M. muelleri* from *Leuciscus cephalus* as its type. Subsequently, more than 760 species have been added to this genus from several parts of the world. Of these, more than 100 species are restricted to the Indian subcontinent.

Detailed observations proved that the two species are distinctly different from the known species and, are therefore, reported here as *Myxobolus puntiusi* sp. nov. and *Myxobolus ticto* sp. nov.

MATERIALS AND METHODS

Fifty-nine *Puntius ticto punctatus* were collected from paddy fields, ponds, streams and rivers of Malappuram district of Kerala during a 10 month period, from March to December 2005. The collected fishes were brought live to the laboratory, and examined immediately for myxosporean infections or maintained live in cement cisterns. The fishes were killed by cervical rupture and examined under a stereozoom dissecting microscope for any externally visible myxosporeans. Internal organs were dissected out and placed into separate petridishes containing 0.75% saline and examined under the microscope. Cyst-like plasmodia were carefully isolated, placed in a few drops of saline taken on a slide, ruptured with fine needles, a cover glass was placed over, and observed under phase-contrast objectives of 'Zeiss Axioskop 2 Plus' microscope to study the spores and developmental stages. Contents of gall bladder and urinary bladder were observed without adding saline. Fresh spores were treated with Lugol's iodine for detecting iodinophilous vacuole. India Ink technique, after Lom & Vavra (1963), was used to reveal any mucous envelopes around the spores. Polar filament extrusion was made using saturated urea solution. For permanent preparations, air-dried smears of spores and development stages were fixed in methanol and stained with Giemsa's stain; Schaudinn's fluid-fixed smears were stained with Heidenhain's iron haematoxylin and counter stained with eosin (Mohr, 1981).

Measurements were taken from fresh materials; on the average 20 spores were measured following the guidelines given by Lom & Arthur (1989). Measurements are in micrometres (µm); mean values are in parentheses. Sketches were made with a prism type camera lucida.

MYXOBOLUS PUNTIUSI SP.NOV.

(Fig. 1)

Material examined

Type: iii.2005–xii.2005, Vellimuttam, Malappuram, Kerala, India. Deposited in the parasite collections, Parasitology Laboratory, Department of Zoology, University of Calicut, Kerala, India (No. Z/Par/M (M) 01). Host: *Puntius ticto punctatus* Day, Site of infection: Heart wall.

Etymology

Named after the generic name of the type-host, *P. ticto punctatus*

Description

Cyst-like plasmodia: Round or oval, milky-white, with thin walls, found attached to heart wall; measured 249.6-296.4 × 109.2-140.4 (269.1 × 120.9), contained fully formed spores.

Spores: Ellipsoid to ovoid with broad anterior end in valvular view; lenticular in sutural view; measured 13.5-16.5 × 10.5-12.0 (15 × 11.02). Spore valves smooth, symmetrical more thickened toward posterior end. Sutural line distinct, with 12 sutural folds, distributed around spore margin. Intercapsular ridge prominent. Polar capsules two, pyriform, slightly unequal. Larger capsules measured 6.75-7.5 × 3-4.5 (7.08 × 3.97) and smaller 5.25-6.75 × 3-4.5 (5.7 x 3.67). Polar filaments with 7-9 coils in larger capsules and 5-7 coils in smaller capsules. Extruded filaments unequal, uniformly thick, 48-78 (55.7) and 42-57 (49.5) in length. Sporoplasm finely granular, filling almost the entire extracapsular space and contained two nuclei.

Prevalence: Eleven of 59 (18.6%) fishes examined were infected.

Remarks

The present myxosporean from the heart wall of *Puntius ticto punctatus* has ellipsoid to ovoid spores without any posterior processes and two pyriform polar capsules at the anterior end. These characters are significant enough for inclusion of this species under the genus *Myxobolus* Bütschli, 1882 of the family

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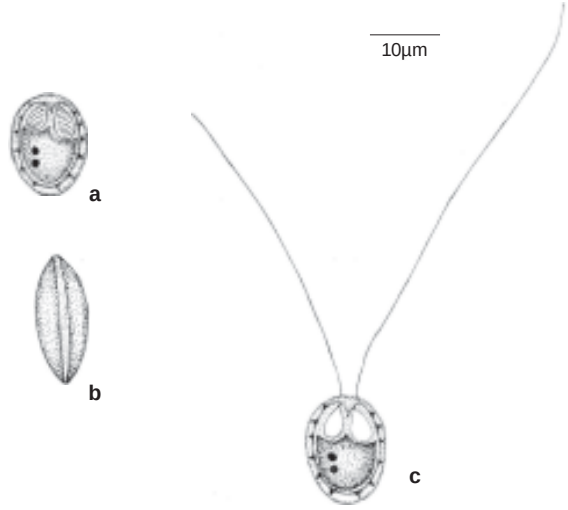

Figure 1. **Myxobolus puntiusi** ***sp. nov.*** ***a - Spore - valvular view; b - Spore - sutural view; c - Spore with extruded polar filaments***

Myxobolidae Thelohan, 1892.

Myxobolus puntiusi sp. nov. closely resembles *M. buri* Egusa, 1985 from the brain of *Seriola quinqueradiata*, *M. karuni* Masoumian *et al.*, 1994 from primary gill filaments of *Barbus grypus* and *M. bulbocordis* Masoumian *et al.*, 1996 from the heart of *B. sharpeyi* in spore shape, and in the presence of sutural folds and intercapsular ridge. *M. buri* and *M. bulbocordis* are different from the present form in spore dimensions and number of sutural folds. Further, equal polar capsules of *M. buri* and *M. bulbocordis* make them distinctly different. *M. karuni* also differs in the nature of intercapsular ridge and in having equal polar capsules. In spore dimensions, the present form comes close to *M. margitae* Molnar, 2000 infecting the gill filaments of *Alburnus alburnus*. But it differs in having unequal polar capsules, and sutural folds. Besides, the site of infection is different. A comparison of characters of the present species with that of the five related species is presented in Table 1. In view of the differences stated above, this species is considered as distinct and named *Myxobolus puntiusi* sp. nov., after the generic name of the host.

Myxobolus ticto sp. nov.

(Fig. 2)

Material examined

Type: iii.2005–xii.2005, Vellimuttam, Malappuram, Kerala, India. Deposited in the parasite collections, Parasitology Laboratory, Department of Zoology, University of Calicut Kerala, India (No. Z/Par/M (M) 02). Host: *Puntius ticto punctatus* Day. Site of infection: Gills, muscles, intestine and liver

Etymology

Named after the species name of the type-host, *P. ticto punctatus*

Description

Cyst-like Plasmodia: Round or irregular in outline, milky-white, found in the gills, muscles, intestine and liver; measured 223-452×215-405 (317×284). Most plasmodia contained fully formed spores.

Spores: Oval to pyriform with moderately pointed anterior end in valvular view and lenticular in sutural view; measured 12.75-15×7.75-9 (14.55×7.8). Spore valves symmetrical, smooth, meet along a distinctly raised sutural ridge, more thickened toward posterior end; sutural folds 6-8, in posterior one-third of spore. Polar capsules two, pyriform, equal; measured 4.7-7.5×2.25-3.0 (6.63×2.92). Each capsule enclosed 6-8 coils of polar filament; extruded filaments equal, uniformly thick, 42-52.5 (43.87) long. Sporoplasm finely granular, contained two nuclei.

Prevalence: Twenty of 59 (29.5%) fishes examined were infected.

Table 1. Comparative characters of **Myxobolus puntiusi** ***sp. nov. and of the four related species.***

Species	LS	BS	LPC	BPC	NC	Inter-capsular ridge	No.of sutural folds	Site of infection	Hosts	References
Myxobolus buri Egusa,1985	9.2-11.8 (10.6)	7.9-10.2 (9.2)	3.9-5.4 (4.5)	3.2-3.9 (3.4)	3	Small	9	Brain	*Seriola quinqueradiata*	Egusa (1985)
Myxobolus karuni Masoumian *et al.*,1994	13-14.9 (14.1)	9.7-10.4 (10.2)	6.5-7.5 (6.2)	5.2-6.3 (5.8)	10-11	Long	4	Gills	*Barbus grypus**	Masoumian *et al.* (1994)
Myxobolus bulbocordis Masoumian *et al.*,1996	17.3-19.6 (19)	13.8-15.5 (15.3)	8.1-9.2 (8.4)	2.8-3.2 (3)	8-9	Distinct	9	Heart	*Barbus sharpeyi***	Masoumian *et al.* (1996)
Myxobolus margitae Molnar, 2000	13-14 (13.7)	9.5-10 (9.7)	4.5-5.5 (5)	3-4.5 (3.97)	7-8	Large	-	Gill filaments	*Alburnus alburnus*	Molnar (2000)
Myxobolus puntiusi sp. nov.	13.5-16.5 (15)	10.5-12 (11.02)	Larger 6.75-7.5 (7.08)	3-4.5 (3.67)	7-9	Prominent	12	Heart wall	*Puntius ticto punctatus*	
			Smaller 5.25-6.75 (5.7)		5-7					

BPC - Breadth of polar capsule; BS - Breadth of spore; LPC - Length of polar capsule; LS - Length of spore; NC - Number of coils of polar filament.
*Junior synonym of *Puntius grypus*; **Junior synonym of *Puntius sharpeyi*

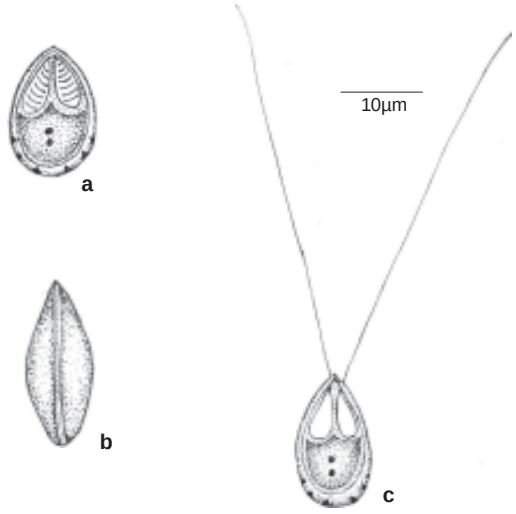

Figure 2.* Myxobolus ticto *sp. nov.
a - Spore - valvular view; b - Spore - sutural view; c - Spore with extruded polar filaments

Remarks

The present myxosporean from the gills, intestine, muscles and liver of *Puntius ticto punctatus* comes close to *M. cyprini* Doflein, 1898 from the intestine, liver, kidney, pancreas and muscles of *Cyprinus carpio*, *M. anisocapsularis* Schulman, 1962 from the gill lamellae of *Hemibarbus labeo*, *M. mahendrae* Sarkar, 1986 from the gill arch epithelium of *Catla catla* and *M. cognati* Cone *et al*., 1996 from the operculum and pectoral fins of *Cottus cognatus* in the size of spores and polar capsules and in the nature of spore valves. Absence of sutural folds in the spores of *M. cyprini* and *M. anisocapsularis* make them different from the present form which has 6-8 sutural folds in the posterior one-third of the spore. Besides, the overlapping polar capsules *of M. cyprini* and spore shape of *M. anisocapsularis* make them distinct. The other two species *M. mahendrae* and *M. cognati* are different in having uniformly thickened spore valves; in addition, *M. mahendrae* has truncated anterior end and *M. cognati* has the sutural folds which are distributed all around spore margin. The present *Myxobolus* deserves comparison with *M. exiguus* Thelohan, 1895 from the gills of *Abramis brama* and tissue of stomach and pyloric caeca of *Mugil capito* and *M. chelo* in spore shape. But it differs in spore dimensions and in absence of intercapsular ridge. A comparative study of characters of the present form and that of the above related species presented in Table 2 shows that the present form is distinctly different from the other five species. In view of the differences in morphology and morphometry, the species under discussion is considered as new and named *Myxobolus ticto* sp. nov., after the species name of the type-host.

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Table 2. Comparative characters of* Myxobolus ticto *sp.nov and of the five related species.

Species	LS	BS	LPC	BPC	NC	Number of sutural folds	Site of infection	Hosts	References
Myxobolus exiguus Thelohan,1895	8-12	6-9.3	4-7	2.5-2.7	-	3	Gills Tissue of stomach and pyloric caeca	*Abramis brama* *Mugil capito* and *M. chelo*	Thelohan (1895)
Myxobolus cyprini Doflein,1898	10-16	8-12	5.2-7	-	-	-	Intestine, liver, kidney, pancreas and muscles	*Cyprinus carpio*	Doflein (1898)
Myxobolus anisocapsularis Shulman,1962	15-15.5	7.7-8.4	Larger 6-8.5 Smaller 2.5-4	4 2.5-4	- -	- -	Gill lamellae	*Hemibarbus labeo*	Shulman (1962)
Myxobolus mahendrae Sarkar,1986	11.52-13.96 (12.7)	9.77-10.47 (10.37)	Larger 6.28-7.33 (6.98) Smaller 4.19-6.98 (5.44) 5.5-7.5 (6.6)	3.49-4.19 (3.73) 3.14-3.49 (3.42)	7-9 5-6	5-6	Gillarch	*Catla catla*	Sarkar (1986)
Myxobolus cognati Cone *et al.,* 1996	2-14 (13.3)	9.5-10.5 (10)	8.1-9.2 (8.4)	3 (3)	8-11	6-8	Operculum and pectoral fins	*Cottus cognatus*	Cone *et al.* (1996)
Myxobolus ticto sp. nov.	12.75-15 (14.55)	6.75-9 (7.8)	4.5-7.5 (6.63)	25-3 (2.92.)	6-8	6-8	Gills, muscles, intestine and liver	*Puntius ticto punctatus*	

BPC - Breadth of polar capsule; BS - Breadth of spore; LPC - Length of polar capsule; LS - Length of spore; NC - Number of coils of polar filament.

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