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Cardiac myxosporiosis of pearl-spot, *Etroplus suratensis* (Bloch), due to *Myxobolus etropli* sp. nov.

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Abstract

A new myxosporean, *Myxobolus etropli* sp. nov., was found to infect the bulbus arteriosus of *Etroplus suratensis* (Bloch) from brackishwater lagoons of Muttukkadu, Chennai coast, India. A survey from May 1993 to October 1994 revealed a prevalence rate of 33.7% of this parasite. Macroscopic discoloured foci/cysts were seen in the bulbus arteriosus of the fish. The parasite showed strict host and site specificity. Histopathology showed that the infection was restricted to the bulbus. This is the first report of a myxosporean from *E. suratensis*.

Introduction

The chromids, comprising the pearl-spots (Cichlidae), are common brackishwater fish of the tropics. Although *Etroplus suratensis* (Bloch), one of the three species recorded in India, is of particular importance as a food fish, both in capture fisheries and aquaculture, relatively little is known about its parasites and their potential to cause disease. During an 18 month survey of the parasites of commercially important fish at Muttukkadu Lagoon, Chennai, a new species of myxosporean was recorded from the bulbus arteriosus of *E. suratensis*. Previously, there has been only a single report of a myxosporean from *Etroplus*, this being the description of *Ceratomyxa etroplusi* from the gall bladder of *E. maculatus* (Rajendran & Janardanan 1992). The present study describes *Myxobolus etropli* sp. nov., providing information for the first time concerning spore morphometrics, location within host and seasonal prevalence within the host population. Gross and microscopic pathology of the infection and the histological evidence of infiltration of spores to other organs are also provided.

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Materials and methods

Adult and juvenile specimens of *E. suratensis* were collected at 2 week intervals from the Muttukkadu Lagoon of the Chennai coast from May 1993 to October 1994. Live fish were brought to the laboratory and immediately examined for parasites. All the internal organs were carefully examined to study the distribution of parasites within the host. Myxosporean cysts were carefully removed from the affected tissues, placed on glass slides in physiological saline, ruptured with fine needles, and observed under a phase-contrast microscope. Smears were air-dried, fixed in methanol and stained with Giemsa. Schaudinn's fluid-fixed smears were stained with Heidenhain's iron haematoxylin, and counter-stained with eosin (Mohr 1981). Polar filament extrusion was induced with 1–2% KOH, saturated aqueous urea and H_2O_2. Fresh spores were treated with Lugol's iodine for detection of an iodinophilous vacuole. The India ink technique was used to detect a mucus envelope (Lom & Vavra 1963). Drawings of spores were made on fresh/stained materials with the aid of a camera lucida. Measurements were taken from fresh materials with an ocular micrometer according to the criteria formulated for myxosporeans by Lom & Arthur (1989). Photomicrographs of fresh and stained materials were taken using a WILD MPS 46 microcamera fitted to a Leitz Laborlax S microscope. Both infected and uninfected hearts were fixed in 10% neutral buffered formalin (NBF) or Bouin's fluid and subjected to routine histology.

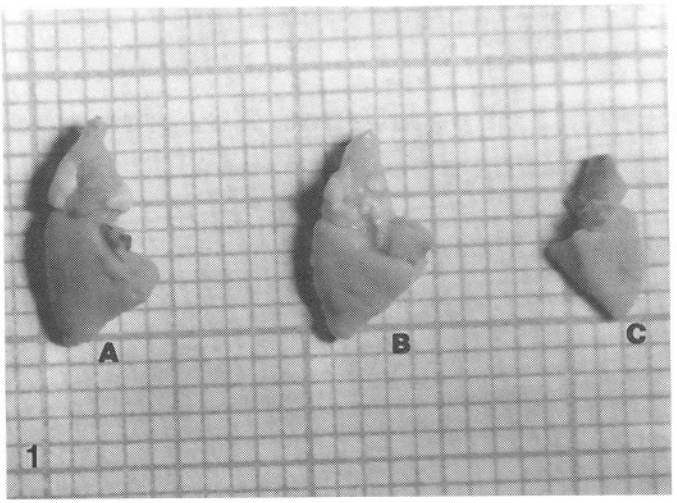


Figure 1 Heavily infected hearts of *E. suratensis* showing discoloured, elevated myxosporean cysts on the bulbus arteriosus (A & B); uninfected heart showing normal bulbus (C) (length of each smallest square is 1 mm).

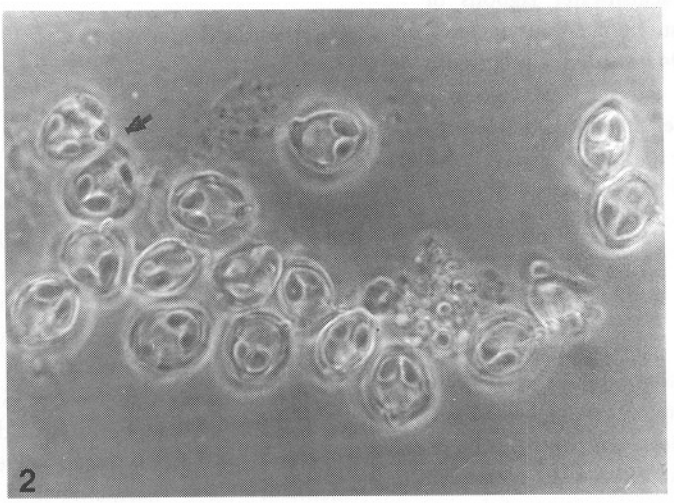


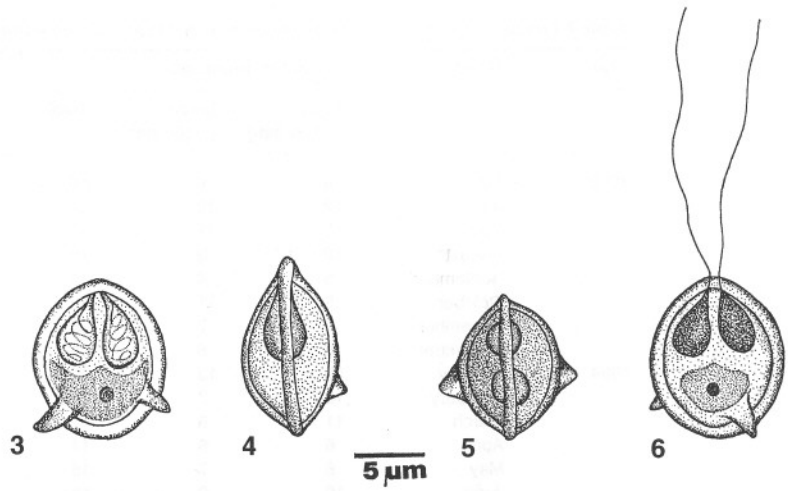
Figure 2 Spores and developing stages of *Myxobolus etropli* sp. nov. A disporous pansporoblast showing valvular nucleus (arrow) (×850).

Results

Myxobolus etropli sp. nov.

Vegetative stages/plasmodia were round to oval or irregular in shape, and milky-white to light yellow in colour. Cysts of different sizes, ranging from 0.1 to 2.0 mm were found attached to the wall of the bulbus (Fig. 1). Cysts were opaque, enclosed by a thick host tissue envelope; upon rupture they released developing and mature spores (Fig. 2). Pansporoblasts were disporoblastic (Fig. 2). Developing plasmodia in transverse section revealed an outer sporulating region containing early stages and an inner region with fully developed spores.

Spores (Figs 2, 3–6) are oval with slightly pointed anterior ends in the valvular view and with short wing-like outgrowths at the posterior end, and are lenticular in sutural and polar views. Spores measure 10.2–14.5 (12.2) × 8.5–11.9 (10.5) μm in size. Shell valves are smooth and symmetrical. The suture is prominent, being straight to slightly curved. Spore valves possess short, wing-like thickening/outgrowths at the posterior end. There are two polar capsules, equal in size, pyriform and opening separately, and they measure 3.8–6.0 (5.0) × 1.7–3.4 (2.4) μm. The intercapsular process is absent. Each polar capsule encloses four to five coils of polar filament. Extruded polar filaments are equal



Figures 3–6 Drawings of spores of *Myxobolus etropli* sp. nov. (**3**) Spore in valvular view; (**4**) spore in sutural view; (**5**) spore in capsular view; (**6**) spore with extruded polar filaments.

in length. Sporoplasm fills the extracapsular space containing one or two sporoplasmic nuclei. The iodinophilous vacuole is absent. A few aberrant spores with three polar capsules were also observed.

Prevalence and seasonal pattern

One hundred and two out of 303 (33.7%) fish (body length, 42–177 mm; body weight, 5.5–312.8 g) were infected with this parasite. Both sexually mature and immature fish were infected. Thirty-one out of 166 (18.7%) small fish (body length <100 mm) and 71 of 137 (51.8%) large fish (body length >100 mm) were found to be infected (Table 1). Small fish which appeared uninfected on routine examination were found to contain the cysts when examined microscopically. Monthly prevalence rate is also given in Table 1. Maximum prevalence was recorded in September 1994, and the minimum in May 1993 and 1994.

Host and site specificity

The parasite was found restricted to *E. suratensis*; all other fish collected from the same habitat were uninfected. The parasite also showed strict specificity with regard to the site of infection, being found only in the bulbus arteriosus. In a few fish, squash preparations of kidney and liver revealed scattered spores, but no cysts were observed in histological sections from these organs.

Pathology

Parasitized fish did not show any external signs of infection. Upon dissection, the infected bulbus showed elevated discoloured foci (Fig. 1). In heavily infected fish, the entire bulbus was covered with large, diffuse cysts. In these cases, the bulbus became grossly enlarged and distorted; pericardial adhesion to the visceral organs was also seen.

Histological sections of the heart showed that infection was restricted to the bulbus arteriosus (Fig. 7). No infection could be seen in the atrium or ventricle of the heart. Both the adventitial and medial layers of the bulbus were infected. Infection of the outer adventitial layer was characterized by proliferating plasmodia which were not limited by a cyst wall or host response. In certain areas, occasional cysts were found encapsulated and the connective tissue layer appeared hyperplastic (Fig. 8). Plasmodia in the medial layer were characterized by large, multilocular cysts apparently metastasizing throughout the tissue layers. The parasite replaced the elastic fibres and smooth muscle of the medial layer in most of the infected areas. Necrosis of the elastic fibres was evident. In heavy infection, the wall of the bulbus contained nothing but numerous cysts and only a few strands of functional tissue.

Spores were found infiltrating across the inner layers of the bulbus into the lumen (Figs 9 & 10). Aggregations of spores and developing pansporoblasts were found in the lumen of the bulbus (Fig. 11).

Discussion

Few reports of cardiac myxosporiosis in freshwater or marine teleosts have been reported. Sindermann

Table 1 Prevalence rate of *Myxobolus etropli* sp. nov. in *Etroplus suratensis* from Muttukkadu, Chennai, from May 1993 to October 1994

Year	Month	No. of fish examined			No. of fish infected			
		Small (<100 mm)	Large (>100 mm)	Total	Small (<100 mm)	Large (>100 mm)	Total	Prevalence (%)
1993	May	4	6	10	0	0	0	0
	June	12	10	22	2	6	8	36.4
	July	15	11	26	5	9	14	53.9
	August	10	9	19	3	5	8	42.1
	September	6	4	10	2	2	4	40.0
	October	9	11	20	0	7	7	35.0
	November	9	7	16	2	3	5	31.3
	December	8	6	14	3	4	7	50.0
1994	January	19	13	32	5	7	12	37.5
	February	16	7	23	2	4	6	26.1
	March	11	6	17	0	2	2	11.8
	April	6	8	14	1	2	3	21.4
	May	8	7	15	0	1	1	6.7
	June	10	8	18	1	2	3	16.7
	July	4	5	9	0	4	4	44.4
	August	5	7	12	2	3	5	41.6
	September	6	6	12	1	6	7	58.3
	October	8	6	14	2	4	6	42.9

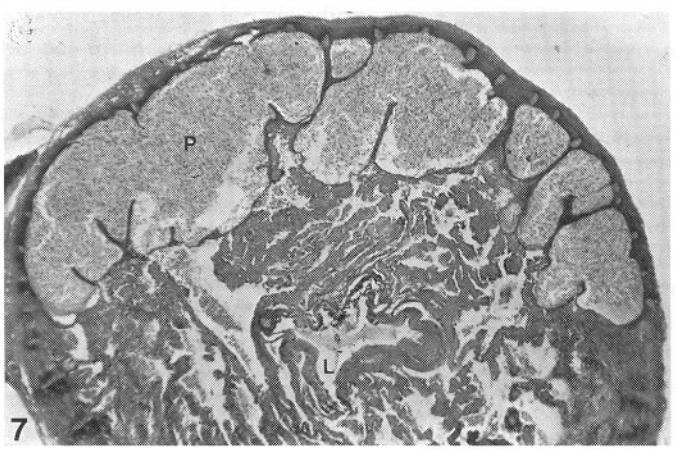


Figure 7 Histological section of a heavily infected heart showing proliferating plasmodia of *Myxobolus etropli* in the bulbus arteriosus (P) spreading through the adventitial and medial layers: L, lumen of the bulbus (H&E, ×30).

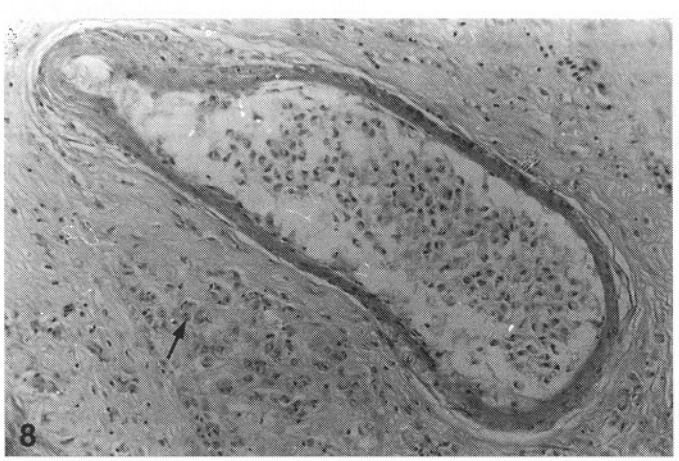


Figure 8 An encapsulated cyst of *Myxobolus etropli* in the adventitial layer of the bulbus surrounded by a hyperplastic tissue layer and proliferating infection not limited by the cyst wall. Arrow indicates free spore aggregations in the adventitial layer (H&E, ×190).

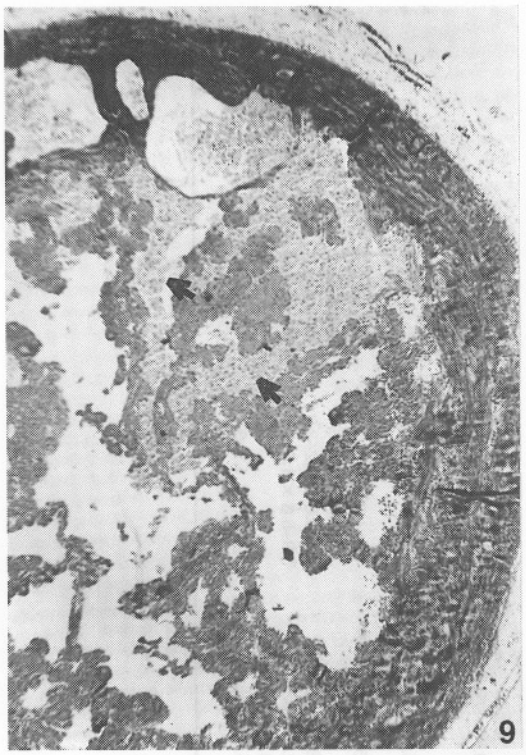


Figure 9 Sections of bulbus showing proliferating plasmodia and spore mass infiltrating across the tissue layers into the lumen (arrow) (H&E, ×38).

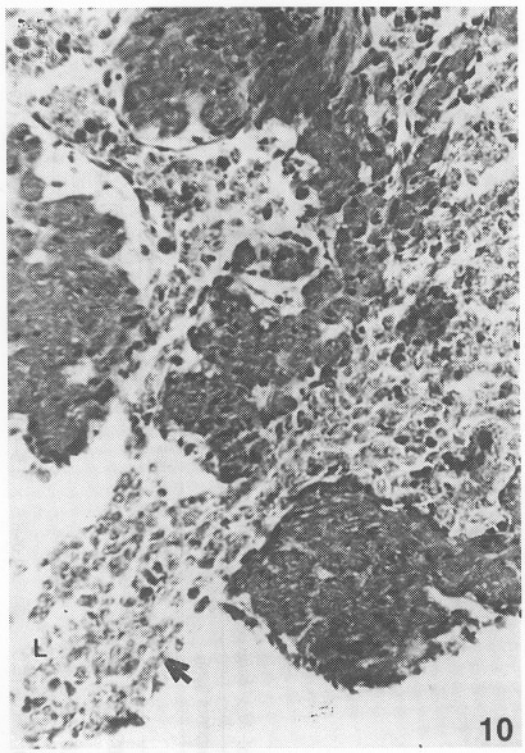


Figure 10 Spores and developing stages of *Myxobolus etropli* (arrow) penetrating into the lumen of the bulbus (H&E, ×238).

(1990) documented species of *Henneguya* infecting the bulbus arteriosus/heart of fish. Previously, only eight species of *Myxobolus* have been recorded from the heart of fish (Table 2). Of these, *M. cordis* (Keysselitz 1908), *M. bilineatum* (Bond 1938), *M. dogieli* (Bykhovsky & Bykhovskaya 1940), *M. sprostoni* (Shulman 1962) and *M. colossomatis* (Molnar & Bekesi 1993) resemble the present species on the basis of morphometry. The presence of a distinct capsule process at the posterior end, the absence of an intercapsular appendix, and the ratio of spore length to polar capsule length clearly demarcates the present species from these. In addition, its occurrence in a new host, and strict host and site specificity, justify its description as a new species, and the name *Myxobolus etropli* sp. nov. is proposed, after the generic name of the host.

Myxosporeans are characterized by a varying degree of host specificity, but they are tissue-specific parasites and always develop in a specific host tissue (Molnar 1994) and the present species is no exception. However, scattered infiltration of spores in kidney and liver was recorded occasionally. A similar phenomenon was seen in infections of *Henneguya otolithi* in the bulbus arteriosus of *Otolithus* species by Ganapati (1941), who suggested that diffusion of spores occurred after the rupture of cysts located near the lumen of the bulbus. The spores and developing pansporoblasts released into the bloodstream are carried to, and become lodged in, the kidney. The occurrence of spores in the lumen of the bulbus and histological evidence of spores penetrating into the lumen suggest a similar mechanism in the present case.

Prevalence rate of *M. etropli* was high in large fish, infection being easily detectable by visual observation of the bulbus. Histological examination is needed to detect very early infection, and hence the prevalence may be much higher than is evident. Prevalence of infection showed some evidence of seasonality, and similar patterns have been reported for other myxosporeans (Brummer-Korvenkontio, Tellervo Valtonen & Pugachev 1991; Cone 1994).

The organ specificity seen in this study has been reported for *Henneguya* sp. and *H. sebasta* infection in the bulbus arteriosus of *Pomatomus saltratus*

Table 2 Comparison of host species and spore morphometrics of *Myxobolus etropli* sp. nov. with other species of *Myxobolus* recorded from the heart of fish

No.	Species	Hosts	Site of infection	Measurements (μm)		References
				Spore	Polar capsules	
1	*Myxobolus cordis*	*Barbus barbus* *B. barbus borysthenicus*	Gastric muscles, cardiac atrium, cordis and wall of aortic bulb	12.0 × 10.0	4.5	Keysselitz (1908)
2	*M. bilineatum*	*Fundulus heteroclitus*	Ventricle, kidney, urinary bladder, gall bladder and brain	10.0–12.0 × 9.0–10.0		Bond (1938)
3	*M. dogieli*	*Leuciscus idus*; *Pelecus cultratus*; *Tinca tinca*; *Blicca bjoerkna*; *Carassius auratus gibelio*; *Cyprinus carpio*; *C. carpio haematopterus* and *Phoxinus* sp.	Heart, muscles, kidney and gall bladder	9.0–16.0 × 8.0–15.0	4.0–6.5 × 3.5–4.0	Bykhovsky & Bykhovskaya (1940)
4	*Myxobolus* sp.	*Leuciscus leuciscus; L. idus; Aspius aspius; Pelecus cultratus* and *Alburnus alburnus*	Muscles and heart, spores in kidney, spleen and liver	12.0–20.7 × 10.0–16.2	6.8–10.8 × 4.2–5.0 5.4–9.0 × 3.6–5.0	Donec (1962)
5	*M. sprostoni*	*Parasilurus asotus* and *Silurus soldatovi*	Intestinal wall, mesentery and heart	11.0–13.0 × 10.0–11.7	5.5–7.5 × 3.5–4.0	Shulman (1962)
6	*M. paralintoni*	*Lepomis gibbosus*	Heart	9.5–11.5 × 9.0–11.5	4.0–4.3 × 2.0–2.5	Li & Desser (1985)
7	*M. heterofilamentatus*	*Clarias lazera*	Kidney, liver, spleen, heart and urinary bladder	7.4–9.2 × 6.0–8.0 9.2–10.8 × 6.2–7.5	3.1–4.1 × 2.0–3.0 4.7–6.0 × 2.2–2.9	Landsberg (1986)
8	*M. colossomatis*	*Colossoma macropomum*	Fins, gills and heart	11.4–12.2 × 6.6–7.2 10.3–10.9 × 7.2–8.5	5.8–6.6 × 1.8–2.5	Molnar & Bekesi (1993)
9	*M. etropli* sp. nov.	*Etroplus suratensis*	Bulbus arteriosus, spores in kidney and liver	10.2–14.5 × 8.5–11.9	3.8–6.0 × 1.7–3.4	This paper

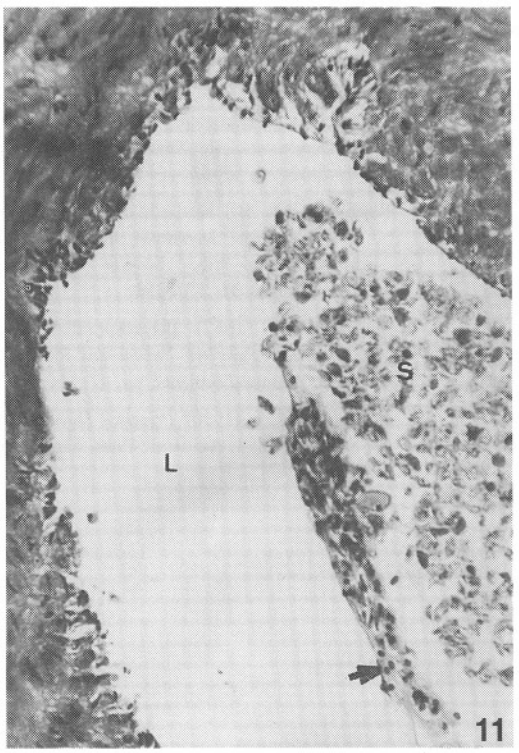


Figure 11 Section of bulbus showing the lumen (L) filled with blood cells (arrow), spores and pansporoblasts (S) (H&E, ×38).

and *Sebastus parci*, respectively (Meyers, Sawyer & MacLean 1977; Heckman & Jensen 1978). According to Molnar (1994), the organ specificity of myxosporeans depends on the tissue types that occur in the given organ. Heckman & Jensen (1978) found unencapsulated spore masses metastasized throughout the bulbus, causing necrosis, hyperplasia and hypertrophy of the connective tissue and smooth muscle, but they did not observe any encapsulation of the myxosporean by the host. The present species showed a similar pattern of pathological manifestations in the bulbus arteriosus of *E. suratensis*, except for encapsulation by the host. However, well developed fibrous encapsulation of parasites was reported by Meyers *et al.* (1977).

Proliferative infection in the muscular layers reduced the functional tissue of the bulbus, but as in many other myxosporean infections, no strong host tissue response was observed in the present study. As suggested by Mitchell, Seymour & Gamble (1985) for *Myxobolus hendriksoni*, the absence of a strong inflammatory response to the spores and plasmodia within the tissue may explain how fish are able to sustain heavy infections. Although mortality and/or any visible abnormal behaviour were not observed in the infected feral population of *E. suratensis*, further study is warranted to determine the impact of this parasite under culture conditions.

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