Protistology 6 (2), 126–130 (2009)

Protistology

A new myxosporean species, *Myxobolus eirasi* sp. nov. and a known species *M. venkateshi* Seenappa and Manohar, 1981 from the Indian major carp fish *Cirrhina mrigala* (Ham.)

Harpreet Kaur and Ranjeet Singh

Department of Zoology, Punjabi University, Patiala, India

Summary

During a survey on parasites infecting fishes in Wetlands of Punjab, India, a new myxosporean species, *Myxobolus eirasi* sp. nov., and one known species *Myxobolus venkateshi* Seenappa and Manohar, 1981 were found in the Indian major carp *Cirrhina mrigala* (Ham.). The parasites invade host's fins and gills. Spores of *M. eirasi* sp. nov., are pyriform in frontal view; with broader anterior end and narrower bluntly rounded posterior end. Polar capsules are two, equal and oval in shape. Polar filaments are ribbon shaped, forming 3-4 turns. The spores are distinctly different from other species of *Myxobolus* with similar shape of spores in having two large darkly stained bodies on the lateral margins of the sporoplasm just beneath the polar capsule. Furthermore in the described species a thick band–like structure is present. It goes along the lower margin of the sporoplasm joining the two darkly stained bodies on either side. Intercapsular process is absent.

Key words: Myxosporea, *Myxobolus,* freshwater fishes, Wetland, Punjab

Introduction

Myxobolus spp. are the most common myxosporean parasites in fishes. Eiras et al., 2005 gave a synopsis of 744 species of the genus *Myxobolus* including those infecting amphibians. In the past little attention has been paid to the myxosporean parasites infecting freshwater fishes in the Wetlands of Punjab. In the present paper we described a new species *Myxobolus eirasi* sp. nov. and reported occurrence of a known species *Myxobolus venkateshi* Seenappa and Manohar 1981. Both species infect the fins and gills of *Cirrhina mrigala* (Ham.). The species description is prepared in accordance with the guidelines by Lom and Arthur (1989) .

Material and methods

Fishes collected from the Ropar and Kanjali Wetlands were brought to the laboratory and examined for myxozoan infection. Plasmodia when found were removed and teased on slides. The preparations were covered with cover slips and examined for the presence of myxospores. Fresh spores were treated with 8% KOH solution for the extrusion of polar filaments. For permanent preparation, air-dried smears were stained with Ziehl-Neelsen and with Iron-haematoxylin. Drawings were made from stained material with the aid of camera lucida. Measurements of spores (based on 15-20 fresh spores treated with Lugol's Iodine

solution) were performed with the aid of a calibrated ocular micrometer. All measurements are given in μm as range values followed by mean ± SD in parentheses.

Results

Myxobolus eirasi sp. nov. (Figs 1, 2, 6, 7)

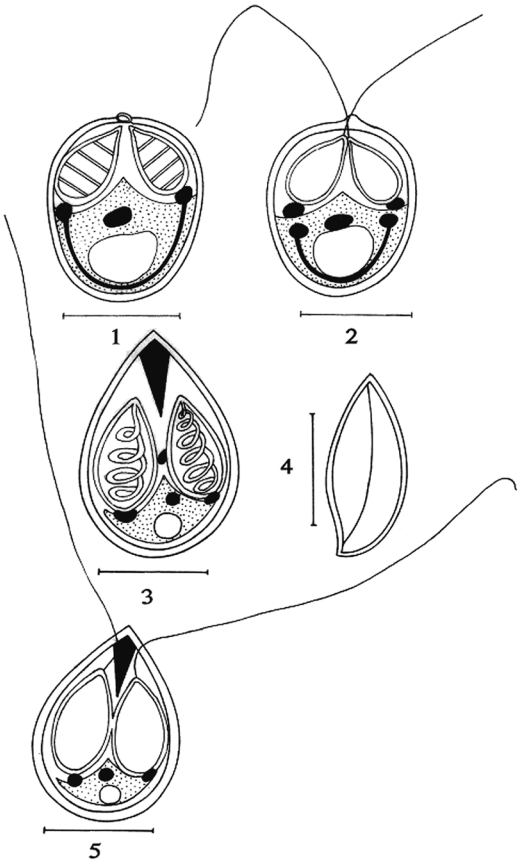
Plasmodia. Milky-white, small, oval to spherical, 2-3 in number on each caudal fin, measuring 0.07-0.1 × 0.09-0.3mm in size, approximately 40-50 spores present per plasmodium.

Spores. The spores are histozoic, pyriform, with broader anterior end and narrower bluntly rounded posterior end, measuring 8.4-8.8 (8.6 ± 0.15) × 6.5-6.9 (6.7 ± 0.15) μm in size. Spore valves are smooth, symmetrical measuring 0.8 μm in thickness. Parietal folds are absent. Two anteriorly situated polar capsules are equal, oval in shape and measuring 3.1- 3.3 (3.2 ± 0.1) × 1.4-1.75 (1.57 ± 0.12) μm in size each. They have a distinct neck at the anterior end. Both polar capsules converge closely anterioly but diverge apart posteriorly. The polar filaments are ribbon shaped, forming 3-4 turns in each polar capsule. The polar filaments are equal when extruded, thread-like and measuring 25-26 (25.5 ± 0.7) μm. They are always crossing at the tip of the spore. A small knob-like structure bearing a common pore is present at the anterior tip of the spore. Intercapsular process is absent. Sporoplasm is agranular, homogenous with a nucleus measuring 1.65-1.7 (1.67 ± 0.02) μm in diameter. Two large capsulogenic nuclei are present on the lateral outer margin of sporoplasm. Two large darkly stained bodies are present on the lateral margins of sporoplasm just beneath each polar capsule. A thick band- like structure originates from one of the darkly stained body to meet the second similar body located just beneath the other polar capsule. The band- like structure runs along the lower margin of the sporoplasm in the posterior end of the spore shell. Iodinophilous vacuole is large, measuring 3.03-3.3 (3.16 ± 0.16) μm in diameter (Fig.7).

Myxobolus venkateshi Seenappa and Manohar, 1981 (Figs 3-5, 8, 9)

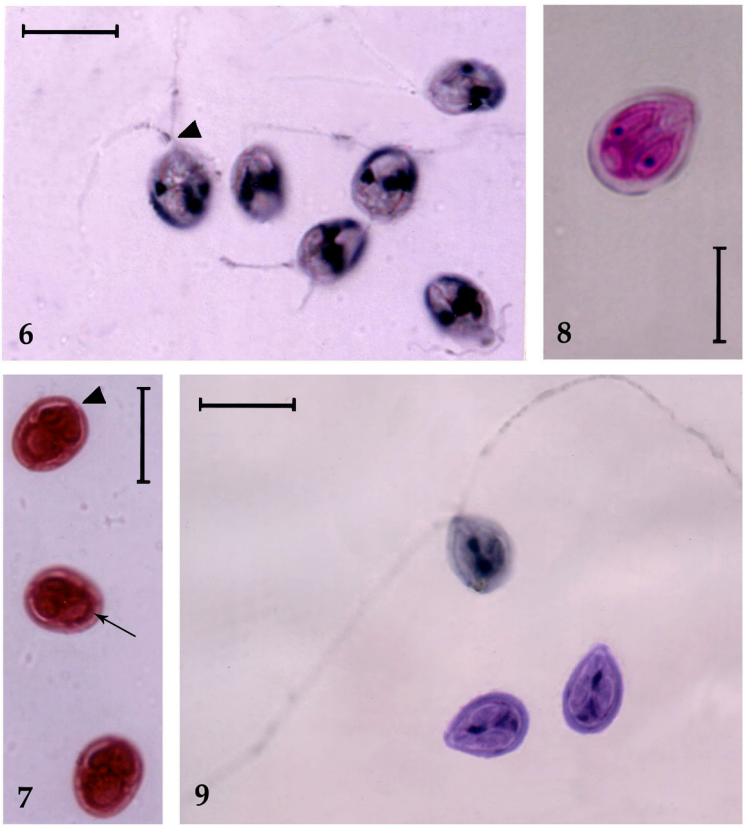
Plasmodia. Creamish-white, small, microscopic, found attached to the mucous membrane around gill lamellae, 30-35 spores present per plasmodium.

Spores. The spores are histozoic, pyriform in valvular view measuring 9-11 (10 ± 1) × 5.9-7.5 (6.7 ± 0.8) μm in size and 3.8 μm in thickness,



Figs 1-5. Spores of two investigated species of *Myxobolus*. 1, 2 – Camera lucida drawings of the spore of *Myxobolus eirasi* sp. nov.: 1 – mature spore stained with Ziehl-Neelsen (valvular view); 2 – spore with extruded polar filaments. 3-5 – camera lucida drawings of spores of *Myxobolus venkateshi* (Seenappa and Manohar, 1981): 3 – valvular view of spore stained with Ziehl-Neelsen; 4 – spore in sutural view; 5 – spore with extruded polar filaments. Scale bars = 0.005 mm.

pointed anteriorly and broad rounded posteriorly. Spores are lenticular in lateral view, sutural line indistinct. Shell valves are thick, symmetrical, smooth, measuring 1.0 μm in thickness, without any parietal folds. The polar capsules are two, equal in size measuring 3.9-5.7 (4.8 ± 0.90) × 1-2.6 (1.8 ± 0.80) μm lying parallel to spore axis, situated in the middle of the spore body. The polar filaments make 5-6 coils situated perpendicular to the longitudinal axis of each polar capsule. A very large intercapsular appendix is present at the anterior end. Sporoplasm is composed of homogenous material containing one sporoplasmic nucleus and two capsulogenic nuclei beneath each polar capsule. Iodinophilous vacuole is observed in few spores.



Figs 6-9. Micrographs of stained preparations of investigated species of *Myxobolus*. 6 – Micrographs of the spores of *M. eirasi* with polar filaments extruded through the common pore (◄), the preparation was stained with Iron haematoxylin; 7 – spores with unextruded polar filaments, the following features are indicated: common pore at the anterior end (◄) and thick band-like structure in sporoplasm (←), the preparation was stained with Ziehl Neelsen. 8, 9 – Micrographs of unextruded spores of *M. venkateshi* stained with Ziehl Neelsen (8) and spores with extruded polar filaments (9), the preparations was stained with Iron-haematoxylin. Scale bars = 10 μm.

Discussion

The described species *M. eirasi* sp. nov. was compared with *M. calbasui* Chakravarty, 1939 from gall-baldder of *Labeo calbasu, L. rohita; M. mrigalae* Chakravarty,1939 from scales of *C. mrigala; M. catlae* Chakravarty,1943 from gills of *Catla catla*; *M. indicus* Tripathi, 1952 from muscle, liver and intestinal wall of *Cirrhina mrigala*; *M. crucifilus* (synonyms *Gyrospora crucifilus* Qadri,1962) reported from gills of *Labeo fimbriatus*; *M. impressus* Miroshnichenko,1980 from fins and gills of *Barbus barbus*; *M. carnaticus* Seenappa and Manohar,1980a from inner base of hemibranchs of *C. mrigala*; *M. vanivilasae* Seenappa and Manohar,1980b from below scale, muscles, integument of *C. mrigala*; *M. hosadurgensis* Seenappa and Manohar,1981 from gills and muscle of *C. mrigala*; *M. shettii* Seenappa and Manohar,1981 from gills of *C. mrigala*; and *M. vedavatiensis* Seenappa and Manohar,1981 from gills of *C. mrigala*; *M. yogendrai* (Tripathi, 1952) Landsberg and Lom,1991; *M. indirae* (Kundu,1985) Landsberg and Lom, 1991 from head cartilage and tail fin of *C. mrigala*; *M. nodulointestinalis* Masoumian et al., 1996 from intestine of *Barbus sharpeyi*; from under scales of *C. mrigala* and *M. orissae* Haldar et al., 1996 from gills of *C. mrigala*

The spores of the described species differ in size (8.6 × 6.7) from the spores of *M. calbasui* (12.4-15 × 8.9); *M. mrigalae* (7.2-8.2); *M. catlae* (14.5-16.5 × 6.1); *M. indicus* (9.5-10.8 × 7.5-8.2); *M. crucifilus* (9-10 × 8-8.5); *M. impressus* (10.5-13.7 × 9.2-11); *M. hosadurgensis* (10.5 × 6.3); *M. vedavatiensis* (13.8 × 9.2); *M. yogendrai* (9-9.5 × 7.2); *M. indirae* (12.6-9.6); *M. nodulointestinalis* (12.6 × 8.1) and *M. orissae* (15.7 × 6.8). Intercapsular process is absent in *M. eirasi* sp.nov. whereas it is present in *M. carnaticus, M. vanivilasae* and *M. shettii*. The spore shape of present species is rather similar to that of *M. crucifilus; M. nodulointestinalis* and *M. impressus*.

Spores in *M. eirasi* sp. nov. are pyriform with broad anterior end and bluntly rounded posterior end. Its shape is different from that of *M. crucifilus* and *M. impressus*. In the latter species the spores are pyriform with more narrower and bluntly curved posterior end. It is also different from *M. nodulointestinalis* in which spores are elongated and ovoid in shape. Polar capsules are equal, smaller in size 3.1-3.3 (3.2 ± 0.1) × 1.4-1.75 (1.57 ± 0.12), pyriform in shape with distinct neck as compared to circular ones in *M. crucifilus* and pyriform ones without neck in *M. nodulointestinalis*. The intercapsular process is lacking in *M. eirasi* sp. nov. as in *M. crucifillus*, however, it is present in *M. impressus* and *M. nodulointestinalis*.

In *M. eirasi* sp. nov. the polar filament measure 25-26 (25.5 ± 0.7) μm when extruded. It is short, thin and thread-like. It is characterized with uniform thickness all along its length unlike that in *M. crucifilus* (42-43 μm). In the latter species three fourth of its length is very thick, broad, symmetrical while its anterior one fourth abruptly becomes narrow and terminates in fine undulating process. The species described bears a band-like structure in its sporoplasm. This structure originates from one rounded body to join the second one lying laterally just beneath the other polar capsule. Thread-like structure has also been reported in the sporoplasm of *M. crucifilus*. However above mentioned structure in the latter species joins the posterior ends of each polar capsule directly (without any prominent bodies). Sporoplasmic thread is absent in *M. nodulointestinalis* and *M. impressus*. At the anterior end of the spore in these species, a knob-like structure bearing a pore is present through which both the polar filaments are extruded together after crossing each other.

The differences discussed above indicate unique characteristics of the isolated parasites and justify the erection of a new species *Myxobolus eirasi*.

Taxonomic summary

Type host: *Cirrhina mrigala* (Ham.).

Site of infection: caudal fins.

Type locality: Ropar and Kanjali Wetland, Punjab, India.

Type specimen: Paratypes are the spores stained in Ziehl Neelsen and Iron haematoxylin on slide no. CM/K/ZN/5/27/04/2008, CM/K/ IH/6/27/04/2008 and CM/K/IH/7/04/2008. Paratypes are deposited at the Departmental library of Punjabi University Patiala, Punjab (India). Pin Code-147002

Prevalence of infection 25/30 (83%)

Etymology: The new species *Myxobolus eirasi* has been named after an eminent worker in the field of Protozoology Dr. J.C Eiras (Departamento ae Zoologia e Antropolgia, Faculdade de Ciencias, Universidade do Porto, Portugal).

The present observations on *Myxobolus venkateshi* Seenappa and Manohar, 1981 are in conformity with the original description as well as with the redescription by Gupta and Khera, 1991 except for some tiny variations in the size of spore, the size and position of polar capsules. The parasites were recorded from the gills of freshwater fishes, *Cirrhina mrigala* (Ham.) and *Labeo calbasu* (Ham.) of Kanjali and Ropar Wetlands of Punjab, India. Total percentage of infection was found to be 88 %(22/25).

References

Chakravarty M. 1939. Studies on myxosporidia from the fishes of Bengal, with a note on the myxosporidea infection in aquarium fishes. Arch. Protistenknd. 92, 169-178.

Chakravarty M. 1943. Studies on myxosporidia from the common food fishes of Bengal. Proc. Indian Acad. Sci. 18, 21-35.

Eiras J.C., Molnar K. and Lu Y.S. 2005. Synopsis of the species of *Myxobolus* Butschli, 1882 (Myxozoa: Myxosporea: Bivalvulida). Sys. Parasitol. 61, 1-46.

Gupta S. and Khera S. 1991. On some species of the genus *Myxobolus* (Myxozoa: Myxosporea) from freshwater fishes of India. Ind. J. Parasit. 15, 1, 35- 47.

Haldar D.P., Samal K.K. and Mukhopadhyaya D. 1996. Studies on protozoan parasites of fishes in Orissa: eight species of *Myxobolus* Butschli (Myxozoa: Bivalvulida). J. Bengal Nat. Hist. Soc. 16, 3-24.

Kundu T.K. 1985. *Myxosoma indirae* sp. n. (Myxozoa: Myxosomatidae) from the head cartilage, scale and tail fin of *Cirrhina mrigala*. Acta. Protozool. 24, 333-337.

Landsberg J.H. and Lom, J. 1991. Taxonomy of the genera of the *Myxobolus/Myxosoma* group (Myxobolidae Myxosporea), current listing of species and revision of synonyms. Syst. Parasitol. 18, 165-186.

Lom J. and Arthur J.R. 1989. A guideline for the preparation of species descriptions in Myxosporea. J. Fish Dis. 12, 151-156.

Masoumian M., Basaka F. and Molnar K. 1996. *Myxobolus nodulointestinalis* sp. n. (Myxosporea,

Myxobolidae), a parasite of the intestine of *Barbus sharpeyi*. Dis. Of Aq. Org. 24, 35- 39.

Miroshnichenko A. I. 1980. *Myxobolus impressus* n. sp., a new myxosporidian (Cnidsosporidia, Myxosporidia) from freshwater fish in the Crimea. Biologicheskie Nauki. 9, 38- 39.

Qadri S. S. 1962. New myxosporidia from Indian freshwater fish *Labeo fimbriatus* I. *Gyrospora crucifila* gen. n. sp. n. Z. Parasitkde. 21, 513- 516.

Seenappa D. and Manohar L. 1980a. Two new species of *Myxobolus* (Myxosporidia: Protozoa) parasitic on *Cirrhina mrigala* (Hamilton) and *Puntius curmuca* (Hamilton). Curr. Sci. 49, 204-206.

Seenappa D. and Manohar L. 1980b. *Myxobolus vanivilasae* n. sp. parasitic in *Cirrhina mrigala* (Ham.). Proc. Indian Acad. Sci. (Animal Science). 89, 485-491.

Seenappa D. and Manohar L. 1981. Five new species of *Myxobolus* (Myxosporea: Protozoa) parasitic in *Cirrhina mrigala* (Ham.) and *Labeo rohita* (Ham.) with a note of new host record for *M. curmucae* Seenappa and Manohar (1980). J. Protozool. 28, 358-360.

Tripathi Y. R. 1952. Studies on the parasite of Indian fishes. I. Protozoa. Myxosporidia together with a checklist of parasitic protozoa described from Indian fishes. Rec. Indian Mus. 50, 63-88.

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