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STUDIES ON FISH PARASITIC MYXOZOANS OF MAJOR CARPS IN MEERUT DISTRICT

ABSTRACT

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Supervisor
Dr. H.S. Singh
Professor

Submitted by:
Anupma
M.phil.

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ABSTRACT

STUDIES ON FISH PARASITIC MYXOZOANS OF MAJOR CARPS IN MEERUT DISTRICT

The thesis entitled 'Studies on fish parasitic Myxozoans of Major carps in Meerut district' includes 217 pages. The work contains description of seven species belonging to the genus *Myxobolus* Bustschli, 1882 which were obtained from the carp fishes *viz.*, *Myxobolus* species- *M. bhadrensis* (Seenappa and Manohar, 1981) Szekely *et al.*, 2015; *M. calbasui* Chakravarty, 1939; *M. catlae* (Chakravarty, 1943) Szekely *et al.*, 2015; *M. haldari* Gupta and Khera, 1989; *M. hosadurgensis* Seenappa and Manohar, 1981; *M. kalavatiae* Szekely *et al.*, 2015; and *M. saranae* (Gupta and Khera, 1990) Kaur *et al.*, 2013. They are described here on the basis of spore morphology, morphometric and molecular analysis. All the species included in this thesis has been already described hence redescribed herein. Except these seven *Myxobolus* species, two more species were also obtained during the course of present work which are described in the appendix of this thesis. Out of these two species, one is *Henneguya namae* Haldar *et al.*, 1983, from the genus *Henneguya* Thélohan, 1892, collected from the host *Chanda nama* and other is *Myxobolus sophorae* Jayasri, 1982 collected from the host *Puntius sophore.* These two myxosporean species were recovered from the host fishes except carp fishes. Since, the protocol of molecular analyses was standardized on these species hence both of these are given in the appendix of this thesis.

The thesis is divided into following chapters-

1. Introduction
2. Review of literature
3. Material and Methods
4. Observation and Remarks
5. General discussion
6. Summary
7. References
8. Appendix

All the microphotographs and figures given in the thesis were original, captured and drawn by the investigator. The thesis is elucidated with the help of 25 figures, 19 plates

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and 33 tables. The summary (Chapter 6) and a list of references (Chapter 7) concerned with the work are given at the end of the thesis. Data of two myxosporean species is provided in the appendix (Chapter8). The digitized photographs, histological slides and C. D. are deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut, U. P. India.

Chapter 1. Introduction

Introduction provides information related to the importance of host fish major carps, general morphology of myxospores, classification of phylum Myxozoa, life cycle of myxosporean parasites, disease caused by myxozoan parasites, their phylogenetic position based on the molecular data and aim of the study.

Chapter 2. Review of literature

Information about the history of myxozoan parasites of foreign lands and from India is provided in this chapter. This chapter includes morphological and molecular history of myxozoan parasites respectively. Some earlier and pioneer contributions made from foreign lands are also given. Special attention to the myxozoan fauna of India is provided.

Chapter 3. Materials and Methods

This chapter includes the methodologies which were used in the present investigation. It is divided into following sections- Sample collection, Histological examination, Morphometric analyses and Molecular analyses of parasites. Host fishes were collected from different location of Meerut district and Bairaj, Bijnor. Total 226 fishes belonging to five genera and seven species, were examined during the present work, to check the presences of myxozoans parasites. Various body parts of host fishes were examined under dissecting microscope and slides were prepared from these tissues. These slides were observed with Olympus CH30 fitted with AxioCam ERc 5s for the presence of myxosporean parasites and photographs from fresh specimens of myxospores were taken. Tissue samples of infected organs were preserved in 4% Formalin for histological examination and photographs of histological section were taken with the help of Nikon ECLIPSE Ts2. Measurements of myxospores were taken following the guidelines of Lom and Arthur (1989). Molecular examination of specimens of myxospores was done and partial 18s rDNA sequences were generated for phylogenetic analysis. These sequences were corrected manually using MEGA 6 software and aligned with other reference

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sequences retrieved from GenBank using Clustal W. The data set was tested for best-fit model of nucleotide substitution and model parameters using Akaike Information Criterion. MEGA 6 software was employed for ML phylogenetic reconstructions.

Chapter 4. Observation and Remarks

This chapter provides information about the result obtained in this work. All of the species included in this thesis were already described and redescribed in the present work. Out of these seven species, molecular data for only three species *M. bhadrensis*, *M. catlae* and *M. kalavatiae* was available at NCBI and rest four species were identified on the morphological basis only with the help of available literature. Redescription is supplemented with appropriate remarks and molecular data. This chapter is divided into following sections-

Section 4.1. Myxosporean parasite *M. bhadrensis* (Seenappa and Manohar,1981) Szekely *et al.*, 2015; was collected from the kidney of *L. rohita*. Detailed comparison of morphological and available molecular data of this species, confirmed the identification of this species as *M. bhadrensis*. However, morphological comparison of this species exhibits minor variations in certain morphological features and measurements from original description given by the Seenappa and Manohar, 1981. Reasons for these variations are discussed in detail. Partial 18S rDNA sequences comprised of 913 bp and 910 bp (accession numbers MN994379 and MN994417).

Section 4.2. Identification of spores of *M. calbasui* Chakravarty, 1939 was done on the basis of morphological and morphometric characters only, because no molecular data is available for this species. In the present study, spores of *M. calbasui* were collected from the two piscine hosts, intestine of *C. mrigala* and liver of *C. reba*. Both the specimens collected in this study, show minor variations in certain morphological characters and morphometric, from it's original description. Spores of *M. calbasui* are large in size and rounded-oval in shape with pointed anterior end and round posterior end. In original description, number of polar filament coils was not given as length of the polar filament was given. But filamental coils are seen in both the polar capsules of both the specimens of present study. Minor variation is also seen in the number of polar filament coils of both the specimens of this study. Partial 18S rDNA sequences of *M. calbasui* isolates comprised of 1577 bp, 1570bp and 1577 bp and 1570 bp (accession numbers MT012462, MT012463 and MT009485, MT012423). Molecular analysis of these two samples

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confirmed that these two specimens are same. Molecular data also validates the identification of *M. calbasui*.

Section 4.3. Spores of *M. catlae* (Chakravarty, 1943) Szekely *et al.*, 2015 were identified on the basis of morphological and molecular data. Spores of *M. catlae* were collected from the gills of two piscine hosts *C. mrigala* and *C. reba*. Due to the large, elongate spores, very long polar capsules which occupies most of the spore cavity and large number of polar filament coils, this species is different from most of the known *Myxobolus* species. The spores of some *Myxobolus* spp. obtained from Indian fresh water fishes show morphological similarity with spores of *M. catlae*. Analyses of the 18S rDNA sequences comprise of 1579 bp, 1575 bp and 1581 bp, 1576 bp (accession numbers MT002743, MT003644 and MT002747, MT002748) of the present specimens with available molecular data confirmed it's identification as *M. catlae* (Chakravarty, 1943) Szekely *et al.*, 2015.

Section 4.4. In the present work, specimen of *M. haldari* Gupta and Khera,1989 were also recovered from the kidney of *L. rohita* and were identified on the basis of morphometric data only, because no molecular data was available for this species in the GenBank. For this species, 18S rDNA sequences were generated first time in India comprised of 1005 bp and 1002 bp (accession numbers MT002391 and MT002392). Detailed analyses of morphological, morphometric and molecular data of this species differentiate it from *M. bhadrensis* which was also recovered from the kidney of same host fish, *L. rohita*, during the course of present study. Minor variations were seen in the morphological features and measurements from the original description. In present specimen, filament coils are seen in the polar capsules while number of filament coils is not provided in original description. However, lengths of polar filaments are provided, reasons of the variations are discussed in details. Clustering of *M. haldari* at a separate branch in the tree (Fig. 14) also validate it's identification as a different species.

Section 4.5. Spores of *M. hosadurgensis* Seenappa and Manohar, 1981 were recovered from the kidney of *C. mrigala* and identification was done on the basis of morphometric data only because no molecular data is available for this species in the GenBank. 18S rDNA sequences were first time generated for this species, comprising of 1658 bp and 1650 bp (accession numbers MT002915 and MT002924). Minor variations were seen in the present study, from original description. Spores of *M. hosadurgensis* are long, ovoid

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and ellipsoidal in shape. Polar filament coils are visible in the present specimens but in the original description number of coils is not given. Host is same but site of infection is different from original description in the present study. Comparison of *M. hosadurgensis*, with other *Myxobolus* spp. with unequal polar capsules shows clear morphological differentiation. Newly generated 18S rDNA sequences of the present specimen did not resemble with other available 18S rDNA sequences of myxosporean hence validate the morphological identification of *M. hosadurgensis* and also supplemented it's morphological data.

Section 4.6. Spores of *M. kalavatiae* Szekely *et al.*, 2015 were collected from the gills of *L. rohita* and identified on the basis of morphological and molecular data. Spores are ovoidal, elongated in frontal and sutural view. Polar capsules are two and equal sized. Filament coils are 5 in both the capsules. Size of specimens of present study are larger than the original description and filament coils are seen in the polar capsules of present specimen while no polar filament coils were seen in the original description. Host fish is also different from original description in the present study hence a new host record is found for this species. Partial 18S rDNA sequences comprises of 1950 bp and 1957 bp (accession numbers MN994420 and MN994422).

Section 4.7. Specimens of *M. saranae* (Gupta and Khera, 1990) Kaur *et al.*, 2013 were collected from the kidney of *L. bata* and identified on the basis of morphometric data only because no molecular data was available for this species in the GenBank. 18S rDNA sequences were first time generated for this species in India and comprise of 934 bp and 930 bp (accession numbers MT002911 and MT002914). Host fish, *L. bata* was collected from the Bairaj, Bijnor. 18S rDNA sequences generated from the present specimen did not resemble with other available 18S rDNA sequences of myxosporean hence validate the morphological identification of this species. Gupta and Khera (1990) first time described this species from the gills of host fishes, *Puntius sarana* and *Labeo calbasu.* However, Kaur *et al.*, (2013) described it from caudal fins of *L. rohita*. In the present study this species is recovered from the kidney of *L. bata*, that is a new site of infection and new host is found for this species.

Chapter 5. General discussion

It includes significance of results of the present work. This chapter include a general discussion on the current status of Myxozoan parasites, morphological characters of

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myxozoans used for species characterization, molecular characters used for inferring phylogenetic relationships, current status of genus *Myxobolus*, current status of *Myxobolus* species from carps and their host specificity, phylogenetic analyses of *Myxobolus* species collected in the present work and phylogenetic analyses of *Myxobolus* species collected in the present work using ML method.

Chapter 8. Appendix

Section 8.1. *Henneguya namae* Haldar *et al.*, 1983 was collected from the gills of host fish, *Chanda nama*. This specimen was identified as, *Henneguya* species on the basis of detailed examination of generic characters of Genus *Henneguya* which are as follow- Spores are round, oval or fusiform, with valves from which caudal appendages (single or double) emerges from posterior pole. Spores with two polar capsules. First time *H. namae* Halder *et al.*, 1983 was reported from *Ambassis nama* (= *Chanda nama*) at Krishnagar, West Bengal, India. Minor variations were seen in the certain morphological features and measurements of various parts. Parasitic cysts were small, oval, rounded and were found attached to the gill arch and gill filaments. Cyst were found full of spores. Spores are large, elongated in outline, with posterior end is tapering and used to prolong into tail. The tail is bifurcated and divaricated. Polar capsules two in number and equipped with filament. In smaller capsule the number of coils of polar filament is 6-7, but in larger capsule number of coils ranges 8-9. Number of coiling of polar filaments, seen in the present specimen is less as compared to that observed by Halder *et al.*, (1983). Earlier this species was described on the basis of morphological features only but in the present study redescription is supplemented with molecular data also. Newly generated 18S rDNA sequences of *H. namae* (accession numbers MN218392 and MN218393) also validate the status of this morphologically identified species. In India, for this species molecular data is generated first time.

Section 8.2. *Myxobolus sophorae* Jayasri, 1982 was obtained from the kidney of *Puntius sophore* and redescribed in the present study. Specimens of host fish *Puntius sophore* were collected from the Sotiganj Fish market, Meerut, during the present work. This species was identified on the basis of morphological resemblance only because no molecular data is available for this species in the GenBank and original description of this species also shows lack of details. Spores of *M. sophorae* are ovo-rounded shaped in frontal view. Anterior and posterior both ends are blunt and rounded in frontal view.

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Anterior end of spores is narrower than the posterior end. Polar capsules are two in number, slightly unequal in size and equipped with polar filaments. Number of filamental coils seen is 5-6 in both the polar capsules but number of coils have not given in the text and have not even drawn in line drawings as originally described by the Jayasri (1982). Molecular data (accession numbers MN595207 and MN595208) is first time generated for this species and this data in addition of morphological details, supplemented and validates the status of this species.

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