Prevalence, Morphological and Molecular Characterization of Microsporidia in *Channa punctatus* in Lucknow

SUMMARY

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Swati

Research Scholar
Department of Zoology
Enrollment No- 600/19

Under the Supervision of

Prof. Kamal Jaiswal

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
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Department of Zoology
School of Life Sciences

Babasaheb Bhimrao Ambedkar University
(A Central University, NAAC Accreditation 'A^{++}' Grade)
VidyaVihar, Raibareilly Road, Lucknow-226025
Uttar Pradesh, India

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Summary

Microsporidia are ubiquitous distributed worldwide, smallest known primitive, eukaryotic cryptic, intracellular or intra-nuclear obligatory fungal related parasites, infecting a very broad range of hosts from unicellular paramecium to multicellular animals including humans. Initially they were considered as protozoan parasites but later based on molecular data and its phylogenetic analysis it was found that they are closely related to the fungi so presently they are placed under the ARM clade (Aphelida, Rozellida and Microsporidia) of the super-phylum 'Ophisthosporidia'. Presently 1,500 species in about 220 genera have been reported including over 160 species of microsporidia in 21 genera infecting both marine and freshwater fishes worldwide.

India stands third largest in fish producing country and second largest aquaculture fish producer in the world and being the second largest fish producing country in the world, India accounts for 7.56% of the global fish production. Fisheries sector contributes about 1.24% of the country's gross value added (GVA) with an annual production of 137 lakh tons in the year 2018-19. Fisheries sector provides employment to about 28 million people in India, with the annual growth of 10.87% since 2014-2015 and export earnings from fish production was of 46.662.85 crore during 2019-20.

Aquaculture plays an important role in global efforts to eliminate hunger and malnutrition by supplying fish and other aquatic products rich in protein, essential fatty acids, vitamins and minerals. Fishes are good source of protein, along with the protein they also provide micronutrients like iodine, zinc, magnesium, selenium, calcium and they contain omega 3 PUFA which suppress the risk of many diseases, cancer, inflammatory and autoimmune complications (Simopoulos, 2002). The production of fishes has been significantly hampered due to several parasitic infections and other

factors as predation, diffused pollution, acidification, siltation, coal mining etc. One of the main problems affecting fish and resulting in enormous losses for the fish business is parasitic infestation and illnesses. Approximately 80% of fish diseases are parasitic, particularly in warm water fish. The presence of parasites is not unique to fish, practically every living thing possesses at least one parasite or a variety of parasites. Infection in fishes is caused by a variety of parasites from various animal groups, including protozoa, helminthes (Trematodes, cestodes, nematodes, and acanthocephalans), Annelida (hirudinaria), and Arthropods (crustacea). Parasitic infestation leads to a general decline in fish growth and reproduction, a decline in fish flavor, and strange behavioral changes like sluggishness which makes them an easy target for predators (Sonu *et al.,* 2001).

Channa punctatus (Bloch, 1793) or *Ophiocephalus punctatus* or *Channa punctate* belongs to the superorder Acanthopterygii of class Actinopterygii is the Spotted Snakehead, carnivorous, cannibalistic, air-breathing fish species commonly found in India and its neighboring countries like Bangladesh, Pakistan, Nepal, Myanmar, and China. This fresh water food fish species is very delicious and nutritive with low fat content, having high market value. Beside the socio economic values, now a days this fish is used as an experimental model for various toxicological, geno-toxicological and mutagenic research activities. This fish is easy for transportation, sensitive for environmental pollutants, could maintain in the laboratory in less requirement, high survivality, easily available throughout the season, and present at affordable prices, so considered suitable for various research activities.

From the past twelve years the population of this fish decreased drastically and recently the IUCN has been listed this fish among the 66 near threatened (low risk) food fish species in India. Pollution, parasitic infestation, overexploitation, siltation, loss of

habitat, destructive fishing practices, introduction of alien species etc. are the major factors which are responsible for its population decay.

In the present study microsporidian infection was detected in *Channa punctatus* collected from the river Gomti, Lucknow through light and scanning electron microscopy and its prevalence, morphological and molecular identification was done. The present thesis is divided into six chapters. The first chapter of the thesis is about the introduction of the microsporidian parasite, host (*Channa punctatus*) and effect of microsporidia on aquaculture and also imbibed the background information. Second chapter reviewed different studies conducted about microsporidian infection in different host reported in different climatic conditions and various drug approaches to target microsporidiasis.

Further, third chapter describes about the prevalence of microsporidian infection in *Channa punctatus* according to the season, age, sex, organ and collection sites wise. 578 fishes out of 842 (68.64%) were found to be infected with the microsporidian parasite and the parasite was reported from the kidney, liver and gills of the infected *Channa punctatus* collected from the river Gomti Lucknow. Overall seasonal prevalence data showed that prevalence of infection has increased in winter and decreased in summer and overall age wise prevalence data showed there is no significant difference in the prevalence between Fingerling to Young ($p>0.05$) and Young to adult ($p>0.05$) but the data is slightly significant ($p<0.05$) for the prevalence between fingerlings, to adult on applying two-way ANOVA. Sex wise prevalence data showed that males had slightly higher prevalence in comparison to the females. On observation of site wise prevalence it is found that Chhath Ghat have maximum prevalence of microsporidian infection followed by Ardonamau and Ghaila Ghat has the lowest prevalence of infection followed by Kudiya Ghat, respectively. As the Gomti river enters in Lucknow at Ghaila

Ghat, the fishes collected from this site had the lower prevalence of infection in comparison to the other site and this might be due to that at the entry point of Gomti river in Lucknow, there is not that much pollution as the pollution increases in the sites gradually and due to increased pollution in the Chhath Ghat the prevalence of microsporidian infection also increased.

Fourth chapter describes about the morphological identification of microsporidian parasitic spores infecting *Channa punctatus*. The identification of the parasite was done by light microscopy through wet mounting and Giemsa staining of the homogenized sample by observing different developmental stages of the parasite and hematoxylin and eosin staining of the tissues sections. Scanning electron microscopy of the homogenized sample was also performed for further confirmation of the parasite. Wet mounted smears showed oval to pyriform shaped shinning florescent green spores with slight Brownian motion having different developmental stages like meronts (uninucleated, binucleated, and multinucleated meronts with 3-8 nuclei) and sporophorous vesicles containing sporoblast and single spore were also observed in smears of different organs of *Channa punctatus*. Infection was found comparatively heavy in kidney, liver, gills, and gonads of symptomatic fish but the infection was also present in asymptomatic fishes. Wet mounted preparation of all organs showed the same pattern of developmental stages. On light microscopic analysis spores of two size were recorded i.e. 14.79± 0.27µm ×12.48±0.61µm (n=12) and 12.16 ±0.83 µm ×10.89±0.52µm (n=12). On Histological examination of H and E stained infected sections of gills showed extensive hypertrophy, hyperplasia and necrosis with curling of the secondary gill lamellae. Histology of muscle showed very minute infection by microsporidian parasite in *Channa punctatus* with no any significant histological changes. H and E stained section of kidney showed xenoma near the renal tubules. The histology of the

liver showed normal polyhedral hepatocytes with centrally placed nucleus (Figure 4.21), while the infected section showed the microsporidian spores with vacuolization, apoptosis with pyknotic hepatocytes and dilated blood sinusoids. Ultrastructural analysis through SEM further confirmed the microsporidian infection as fresh ovoid spores of two size range measuring 15.47±0.5µm ×13.09±0.3µm (n=12) and 12.54±0.42 µm ×10.62±0.7µm (n=12) (fig.7) have been identified, having a spongy outer surface.

In the fifth chapter molecular identification was done through DNA extraction, PCR amplification, sequencing and phylogenetic tree construction using maximum parsimony method with 1000 bootstraps and analysis of the obtained SSU rRNA sequence through homology search on NCBI by BLASTn and CLUSTAL-W. Sequences from the four closely related species of two genera were also aligned to show the variable sites using MEGA.11 version. The obtained 1360 bp long SSU rRNA sequence was submitted to the GenBank under the accession number OP537923. The sequence shows (98.98%) similarity with the *Ovipleistophora ovariae*, differing in 13 nucleotide base of SSU r-RNA sequence. Initially it was considered that *Ovipleistophora sp.* is a parasite that usually infects the ovary of the fish but later on some researchers also reported the infection in internal organs mostly liver and kidney, which also was revealed in the present study. Present study describes both the morphological and molecular charaterizaion of microsporidia which infects the *Channa punctatus* fish that were collecetd from the river Gomti, Lucknow.

The last chapter gives the insight about the significance and future prospects of the present study.