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RESEARCH ARTICLE

DESCRIPTION AND MOLECULAR PHYLOGENY OF AN EXOTIC MYXOZOAN, *MYXOBOLUS ARCTICUS* (PUGACHEV AND KHOKHLOV, 1979) IN KIDNEY OF *CLARIAS BATRACHUS* FROM RIVER GOMTI, LUCKNOW, INDIA

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ABSTRACT

The *Myxobolus arcticus* Pugachev and Khokhlov, 1979 (P.- Myxozoa; C.-Myxosporea; O.- Bivalvulida; F.-Myxobolidae) is a protozoan parasite residing in brain, nerves and spinal cord of the salmons (F.-Salmonidae). Its principal fish hosts are sockeye salmon - *Oncorhynchus nerka,* masu salmon - *O. masou* and Arctic char *Salvelinus alpinus* found in North Pacific coast of Far East Asia and North America. This is the first report revealing two new facts; first that *Myxobolus arcticus* was in the kidney (a new site of infection) and, second the host is a freshwater native catfish *Clarias batrachus* (O.- Siluriformes; F.- Clariidae), from river Gomti at Lucknow, Uttar Pradesh, India. The present article deals with morphological, morphometric and molecular description of this parasite *Myxobolus arcticus.* Further the morphometric parameters and small sub unit ribosomal gene (SSu rDNA) sequences of mature spores (trophozoites) were compared to demonstrate the morphological and genetic similarities between geographically distant isolates of *M. arcticus*. Sequence analysis of present *M. arcticus* (accession number KF662475) revealed that it has 98% sequence similarity with *M. arcticus* from *O. nerka* of Canada (accession number JN003829) and *O. masou* of Japan (NCBI accession number JN003830). Based on the maximum parsimony and maximum likelihood inferences, it is confirmed that present *M. arcticus* is conspecific to *M. arcticus* from Japan and Canada.

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INTRODUCTION

The freshwater catfish *Clarias batrachus* (Family-Claridae) is an important native food fish of India. For good production, health of fish should be excellent but *C. batrachus* is highly prone to parasitic infections particularly protozoans which are responsible for high mortality in eggs, fries & fingerlings. Among protozoans, the Myxozoans encompass more than 1,300 known species mainly as parasites of fish and of few amphibians and reptiles. Out of these more than 450 species belong to the genus *Myxobolus* (Lom & Dykova, 1992). The *Myxobolus arcticus* Pugachev and Khokhlov, 1979 is a freshwater myxozoan which infects central nerve tissues of salmonid fishes in the North Pacific coasts of Far East Asia and North America. There is an interesting story related to the origin of the *M. arcticus* Pugachev and Khokhlov, 1979. Shulman (1988) in a report of myxosporeans of the USSR, found that some spores of *M. neurobius* isolated from the brain of Pacific salmon are pyriform, with a distinctly narrow anterior end, in contradiction to the original description by Schuberg and Schro¨der (1905). Pugachev and Khokhlov (1979) erected the species *M. arcticus* for these spores and also provided a supplemental diagnosis for *M. neurobius* in the USSR, clearly separating the two species. Although differences between these species were clarified, it took years for this Russian-language manuscript to be recognized by English speaking scientists. As a result, there are several reports of *M. arcticus* described as "*M. neurobius*" with pyriform spores infecting the brain of O*ncorhynchus* spp. from the Pacific Northwest (Bailey & Margolis, 1987; Quinn *et al*., 1987). Presently records of these infections are considered to belong *M. arcticus* (Urawa and Nagasawa, 1988; Kent *et al*.,

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1994; Awakura *et al*., 1995; McDonald and Margolis, 1995). The principal host of *M. arcticus* is masu salmon *Oncorhynchus masou* in Japan and *O. nerka* in Canada and Alaska (Awakura *et al*., 1995; McDonald & Margolis 1995, Moles & Jensen 2000). The *M. arcticus* is being used as a biological tag to identify the stock origins of salmon in open seas and has been associated with reduced swimming performance in host fish (Moles *et al*., 1990; Margolis, 1998; Moles & Heifetz, 1998).

Although genus *Myxobolus* Butschli, 1882 has been widely studied in India and several species like *Myxobolus bivacuolatus*; *M. clarii*; *M. clariae sp. nov*; *M. koumingensis*; *M. kwangtungensis*; *M. tripathi* ; *M. saraswatii*; *M. utlouensis* sp. nov.; *M. shaochingensis*; *M. magurii* and *M. leqingensis* have been reported from *C. batrachus* in past but there is no report of *M. arcticus* occurrence in India (Narasimhamurti & Kalavati, 1986; Abidi, 2002; Abidi *et al*., 2015(b), Chakravarty, 1943; Hemananda *et al*., 2009; Chen & Ma, 1998; Kalavati *et al*, 1981; Gupta & Saraswati, 1993; Eiras *et al*., 2005; Sarkar, 1993; Wu *et al*., 1998). Hogge and his coworkers established that morphologically similar species can be differentiated using molecular data (Hogge *et al*., 2004). The small subunit ribosomal gene (SSU rDNA) is commonly used for molecular systematics in the Myxosporea for elucidating relationships because it is highly variable among very closely related species (Kent *et al*., 2001; Ferguson *et al*., 2008). Therefore to confirm the identity of present *Myxobolus* species, sequence analysis of its SSU rDNA gene was done and resultant sequence (NCBI Accn. no. KF662475) was compared with the sequences of same species from distant hosts and other closely related species to ascertain its position and similarity to them through construction of molecular phylogenic tree using maximum parsimony and maximum likelihood techniques. The present report is the first record of *Myxobolus arcticus* Pugachev and Khokhlov, 1979 from India.

MATERIALS AND METHODS

Morphology and Morphometry

Collection of Desi magur, *Clarias batrachus* was done from river Gomti (latitude 26°52'24.27"N and longitude 80°54'55.77"E) around Lucknow (Uttar Pradesh, India). Screening of total 105 (one hundred and five*)* apparently healthy fish was done for isolation of parasites. Squash preparations of all the internal organs and gills were made and examined through a Nikon E600 microscope with 100X objective (plus immersion oil) for the presence of myxosporans. It was observed that both kidneys are filled with numerous minute spores of *Myxobolus* sp. but cysts were not seen. Spores in fresh wet mount were treated with 8-10 % KOH solution for extrusion of polar filaments. For permanent preparations, air-dried, methanol fixed smears were stained with Geimsa. Drawings were made from stained material with the help of Camera Lucida. Morphometry of fresh spores (n=50) was done with the help of software NIS-E-Br. All measurements were taken in micrometers (µm). For statistical analysis "Statistical Mean" and "Standard Deviation (SD)" are calculated from the raw data using Microsoft Excel. Morphometric data is presented as mean± SD (range).

DNA isolation and PCR amplification

DNA was isolated from spores through phenol: chloroform method (Sambrook *et al*., 1989) and used as template DNA for PCR reactions. For amplification of DNA, specific primers (Mcer1F - CCCGTCGCTACTACCGAGT & Mcer1R - GATCCTTCCGCAGGTTCAC) were selected from the 18 SSU rDNA sequences through NCBI, designed with the help of software 'Primer3' and were synthesized by Sigma-Aldrich. The standard reaction volume was 50 µl containing 1x PCR buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 2.0U taq polymerase, 0.25 µM of each primer and 100ng of the DNA template. PCR amplification was performed using Eppendorf Master Cycler ep Gradient S, Germany. PCR conditions were as follows: initial denaturation at 95°C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 1 min.; annealing at 52°C for 30 seconds; extension at 72°C for 2 min. and final extension at 72°C for 1 min. to amplify the product. Amplicons were excised from the agarose gel electrophoresis.

DNA Sequencing and Analysis

For DNA sequencing, Sanger's dideoxy chain termination method was followed (Sanger *et al*., 1977). The alignment of sequences was done with the help of Clustal W and Mega 5 (Tamura *et al*., 2011). The other analogous sequences available in "GenBank" were searched by 'BLAST' for comparison and verification of the present sequence. Comparison of sequences was done on the basis of multiple hosts and sites of infection of *M. arcticus*.

Molecular Phylogeny

To determine the phylogenetic position of *M. arcticus* (from *C. batrachus*) in relation to other geographically distant conspecific parasites and closely related species; sequences of *M. arcticus* infecting masu salmon *Oncorhynchus masou* from Japan (accn. no. JN003830) and Sockeye salmon *O. nerka* from Canada (accn. no. JN003829), sequences of *M. cerebralis* random sample (accn. no. U96493) & *M. cerebralis* clone (accn. no. AY479924), along with two freshwater and two marine myxosporean out groups i.e. *Thelohanellus wuhanensis* (accn. no. JQ690370) & *T. kitauei* (accn. no. JQ690367) and *Zschokkella nova* (accn. no. DQ377690) & *Z. parasiluri* (accn. no. DQ377689) were downloaded from GenBank. Phylogenetic analysis was conducted using software MEGA5. The methods used for construction of phylogenetic tree are Maximum Parsimony and Maximum Likelihood with bootstrap value of 500.

RESULTS AND DISCUSSION

Prevalence

Out of 105 (one hundred & five) *C. batrachus*, only 4 fish had infection of *Myxobolus* sp. in kidneys. The parasite was identified as *Myxobolus arcticus* on the basis of morphological features and morphometry of the mature spores (Trophozoites). Thus prevalence of *M. articus* infection in *C. batrachus* is 3.80%.

Taxonomic Summary

Phylum - Myxozoa
Class - Myxosporea
Order - Bivalvulida
Family - Myxobolidae
Genus - *Myxobolus*
Species – *arcticus*

Description

The spores are histozoic. Mature spores (Trophozoites) are pyriform, narrowing down towards anterior side with sharp tips, having two elongated polar capsules with about 5-8 no. of filament coils, connected with the suture towards the anterior end of the spore; sporoplasm with one round nucleus and one or more vacuoles (Fig.-1 and 2). The length of spores was 13.47 ± 0.62 (12 - 14.1); width was 8.60 ±0.41 (7.8 - 9.8) and thickness was 6.40 ± 0.53 (5.30 - 6.99). The length and width of polar capsule was 7.68 ± 0.74 (6.43 to 8.94) and 2.97 ± 0.21 (2.36 - 3.33) respectively (Fig. - 3).

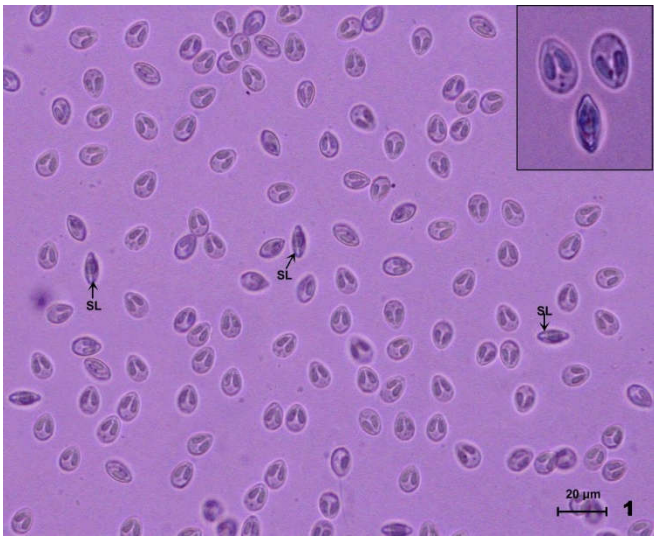

Fig. 1. Mature Spores of *Myxobolus arcticus.* SL- Sutural line. Insert showing enlarged Frontal and Sutural (Lateral) view of Spores

Sequence Analysis and Molecular Phylogeny

Further on finer comparison of present parasite *M. arcticus* with other *M. arcticus* species described by earlier workers (Urawa *et al*., 2009, 2011; Nagasawa *et al*., 1994), minute morphometric variations were observed in spore organelles shape and size. This inconsistency compelled us to do sequence analysis of SSu-rDNA gene of present *M. arcticus.* The resultant sequence was deposited in GenBank as *Myxobolus arcticus* (accn. no. KF662475). Comparison of this sequence with sequences of other geographically distant conspecific parasites and closely related species through BLAST displayed 98% similarity with *M. arcticus* infecting masu salmon *Oncorhynchus masou* from Japan (accn. no. JN003830) and Sockeye salmon *O. nerka* from Canada (accn. no. JN003829); while it showed 96% similarity with *M. cerebralis* (accn. no. AY479924) clone.

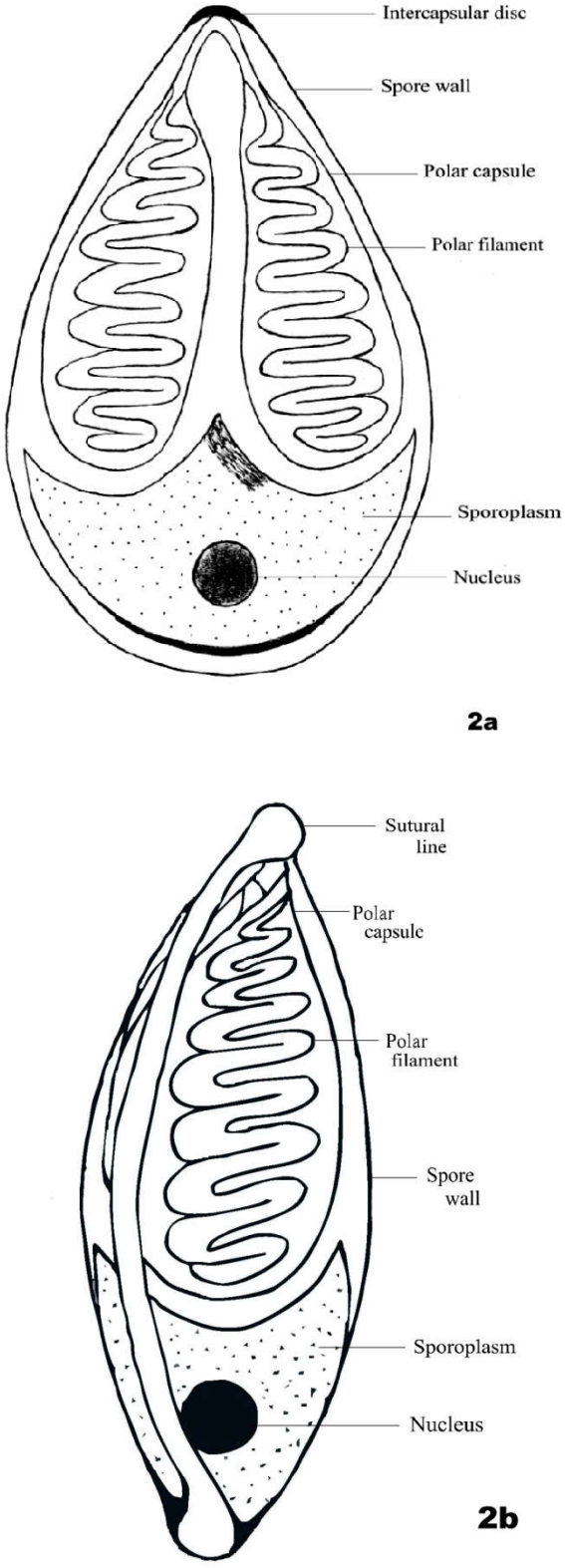

Fig. 2. Camera Lucida drawings of the mature spore of *M. arcticus* showing (A) Frontal and (B) Sutural view

Further present species *M. arcticus* is placed in a closely related histozoic clade of *M. arcticus* species isolated from different distant hosts and sites of infection and inter-relationships are ascertained through molecular phylogeny

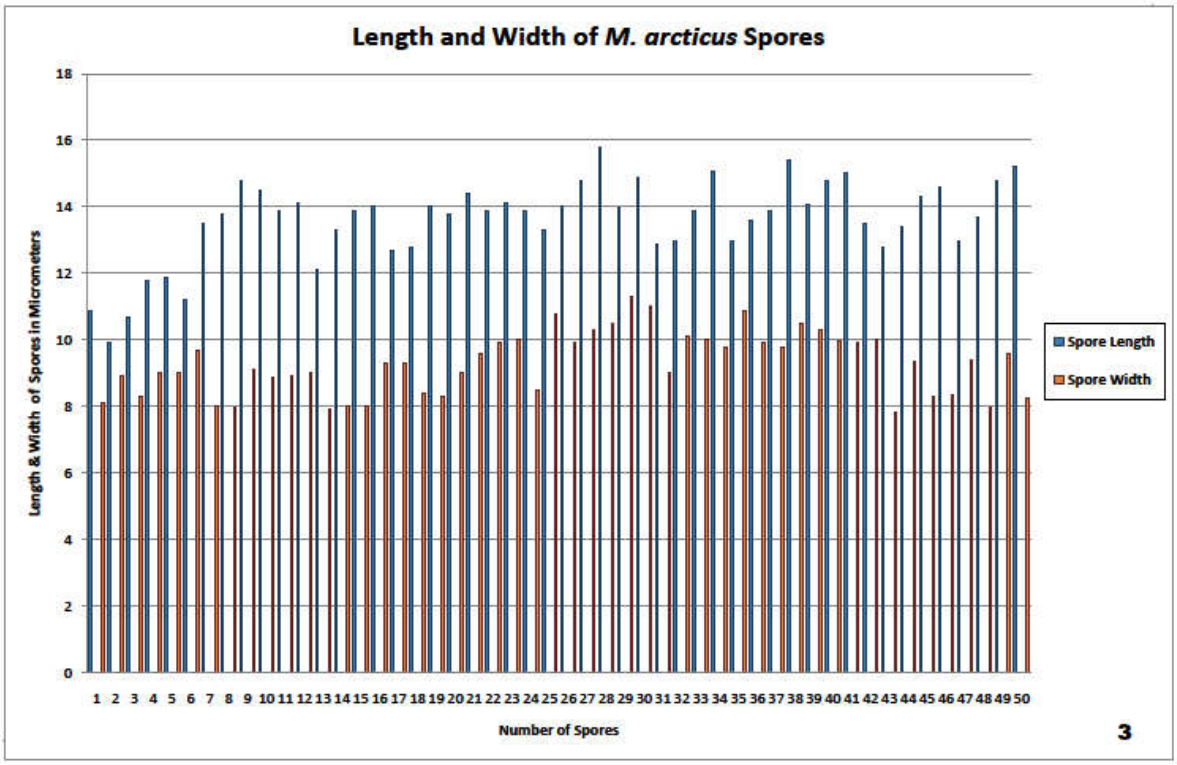

Fig.3. Histogram showing Length and Width of *M. arcticus* Spores

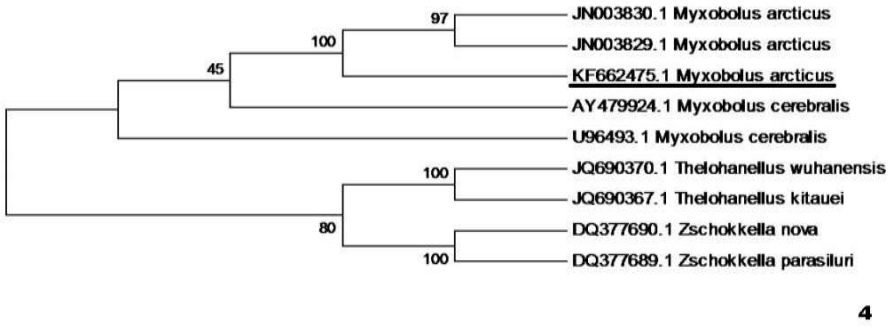

Fig. 4. Maximum Parsimony tree of the small subunit ribosomal DNA sequence of *M. arcticus* and other selected myxozoan species. Bootstrap confidence values on the nodes of branches. GenBank Accession numbers given before the species name

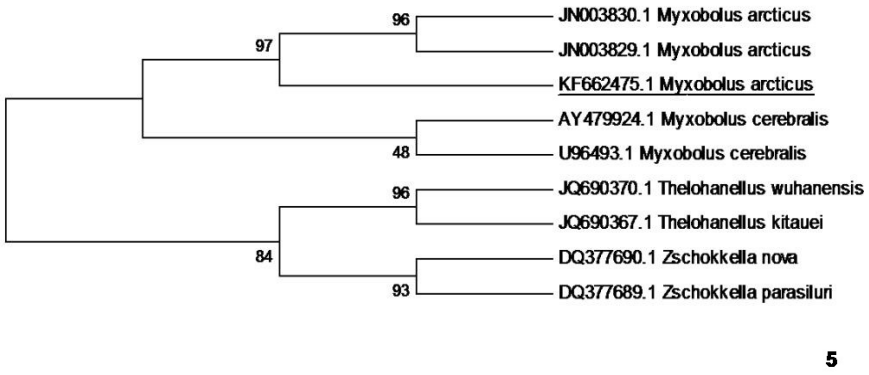

Fig. 5. Maximum Likelihood tree of the SSU rDNA sequence of *M. arcticus* and other selected myxozoan species. Bootstrap confidence levels on the nodes of branches. GenBank Accession numbers before the species name

Species	Host& Place	Site of Infection	Spore			Polar Capsule		Reference
			Length (µm)	Width (µm)	Thickness (µm)	Length(µm)	Width(µm)	
Myxobolus arcticus	*Clarias batrachus* River Gomti,India	Kidney	13.47± 0.6 (12 -14.1)	8.60 ± 0.4 (7. 8 – 9.8)	6.40 ± 0.5 (5.3 – 6.99)	6. 70 ± 0.5 (5.79 - 7.7)	2.97 ± 0.2 (2.36- 3.33)	Present paper
M. arcticus	*Oncorhynchus nerka* , Canada	Medulla oblongata	14.2 (12.9 - 15.6)	8.6 (7.8 - 9.8)	-	8.1 (7.3 – 9.2)	3.4 (2.5 – 4.0)	Urawa et.al 2011
M. arcticus	*O. nerka* Ozernaya River, Kamchatka,Russia	Medulla oblongata / spinal cord	13.8 ± 0.6 (12.5 – 15.2)	8.6 ± 0.6 (7.6 – 9.8)	6.6 ± 0.4 (5.5 – 7.4)	8.1 ± 0.6 (7.0 – 9.2)	3.2 ± 0.2 (2.8 – 3.9)	Nagasawa et.al 1994
M. arcticus	*Salvelinus malma* Ozernaya River, Kamchatka, Russia	Medulla oblongata / spinal cord	14.4 ±0.6 (13.3 – 15.6)	10.0 ± 0.4 (9.4 – 10.9)	7.4 ± 0.3 (6.8 – 7.8)	8.6 ± 0.6 (7.0 – 9.4)	3.7 ± 0.3 (3.1 – 4.5)	Nagasawa et.al 1994
M. arcticus	*Oncorhynchus masou masou* Mena River,Japan	Medulla oblongata / spinal cord	11.1 ± 0.6 (9.9-11.9)	8.9 ± 0.5 (7.9 -9.9)	6.4 ± 0.5 (5.1 – 7.1)	6.1 ± 0.4 (5.3 – 6.9)	3.0 ± 0.1 (3.0 -3.5)	Urawa et.al 2009
M. arcticus	*O. masou masou* Chitose River Japan,	Medulla oblongata / spinal cord	14.1 ± 0.5 (13.3 – 15.6)	8.7 ± 0.4 (7.8- 9.4)	6.4 ± 0.3 (5.6 – 7.0)	8.4 ± 0.3 (7.5 – 9.0)	3.1 ± 0.2 (2.6 – 3.5)	Urawa et.al 2009
M. arcticus	*O. nerka* Chitose River, Japan	Medulla oblongata / spinal cord	13.2 ± 0.6 (11.9 – 14.4)	8.4 ± 0.5 (7.8 – 9.4)	6.6 ± 0.3 (5.5 – 7.0)	8.3 ± 0.5 (7.0 – 9.5)	3.1 ± 0.2 (2.3 – 3.7)	Urawa et.al 2009
M. arcticus	*O. keta* Chitose River, Japan	Medulla oblongata / spinal cord	14.0 ± 0.4 (13.3 – 14.8)	9.3 ± 0.3 (8.6 – 10.1)	6.3 ± 0.2 (5.9 – 7.0)	7.3 ± 0.3 (6.6 – 7.8)	3.1 ± 0.2 (2.7 – 3.9)	Urawa et.al 2009
M. arcticus	*Salvelinus alpines* Laksadal River System, Norway	Medulla oblongata / spinal cord	14.4 ± 0.6 (13.1 – 16.0)	10.6 ± 0.6 (9.8 – 11.1)	7.8 ± 0.2 (7.4 – 8.2)	7.8 ± 0.5 (7.2 – 8.6)	3.8 ± 0.3 (3.1 – 4.3)	Urawa et.al 2009
M. arcticus	*S. leucomaenis* Lake Shikotsu, Japan	Medulla oblongata / spinal cord	13.5 ± 0.8 (11.9 – 15.0)	9.8 ± 0.5 (8.4 – 10.9)	7.7 ± 0.4 (6.9 – 8.9)	7.2 ± 0.6 (5.8 – 8.5)	3.7 ± 0.3 (3.0 – 4.2)	Urawa et.al 2009
M. saraswatii	*Clarias batrachus*	Kidneys	10–20	7.8–11	-	3.5–9.5	2–3.5	Kaur & Singh, 2012
M. shaoch - ingensis	*C. batrachus, C. argus*	kidneys,intes tine,stomach	14.6 (12–15.6)	8.5 (7.2–9.0)	6.4 (6.0–6.7)	6.4 (6.0–6.7)	2.7 (2.6–3.0)	Eiras & Molnar 2005
M. koumin - gensis	*C. batrachus*	gills, kidney liver, spleen gonads	15.8 (15–16.2)	9.9 (8.4- 10.8)	6.2 – 6.5	7.1 (6–7.8)	3.5 (3–3.6)	Eiras & Molnar 2005
M. clariae	*C. batrachus*	cornea	10.53 (10.2–11.5)	7.03 (6.8– 7.7)	----------	3.67 (3.4– 4.3)	2.38 (1.7– 2.6)	Hemananda et.al 2009
M. clarii	*C. batrachus*	liver, testes	11.3–12.4	10.3	6.1	6.1	3.0	Eiras & Molnar 2005
M. kwangtu - ngensis	*C. batrachus*	gall-bladder	17.0 (15.8–18.6)	11.9 (10.8–13)	8.1 (7.8-8.6)	8.4 (7.8–9.6)	3.8 (3.6–3.8)	Eiras & Molnar 2005
M. leqin- gensis	*C. batrachus*	gills, intestine	13.6 (12.9-14.2)	9.4 (9–9.6)	5.5 (5.2-5.8)	6.2 (5.8–6.4)	3.3 (3.0–3.6)	Eiras & Molnar 2005
M. bivacuo - latus	*C. batrachus*	Intestinal wall	8–11 (9.0)	9	4.2	3–4.5 (4.2)	2.6–4 (3.0)	Kaur & Singh,2012
M. magurii	*Clarias magur*	Accessory Respiratory organ	14.13 (13–15)	7.75 (6.5–8)	----------	7.53 (7–8)	2.34 (2–3)	Kaur & Singh, 2012
M. tripathii	*Clarias sp.*	Wall of gut & visceral organs	10.1 (9.8–10.2)	13.0 (12.0-13.5)	----------	5.5 (5–6)	2.5	Kaur & Singh, 2012

Fig. 6. Table showing comparison of *Myxobolus arcticus* along with other morphologically similar species

based on maximum parsimony and maximum likelihood analyses of present species (accn. no. KF662475) along with sequences of *M. arcticus* infecting *Oncorhynchus masou* (accn. no. JN003830) and *O. nerka* (accn. no. JN003829); *M. cerebralis* random sample (accn. no. U96493) and *M. cerebralis* clone (accn. no. AY479924); freshwater myxosporean out group *Thelohanellus wuhanensis* (accn. no. JQ690370) and *T. kitauei* (accn. no. JQ690367); and marine out group *Zschokkella nova* (accn. no. DQ377690) and *Z. parasiluri* (accn. no. DQ377689). The maximum parsimony tree shows bootstrap confidence level of present *M. arcticus* as 100 in relation to *M. arcticus* from both hosts- masu salmon of Japan (accn. no. JN003830) and sockeye salmon of Canada (accn. no. JN003829) confirming the closeness of these three geographically distant populations (Fig. 4). The maximum likelihood tree yields same results with bootstrap confidence level of 97 to other *M. arcticus* species and place them as closely related clade (Fig. 5).

Thus it can be inferred that *M. arcticus* Pugachev and Khokhlov, 1979 isolated from kidney of *C. batrachus* is same species as the *M. arcticus* found in salmons of Far East Asia and North America and all these parasites are conspecific. Presence of the exotic myxozoan, *M. arcticus* Pugachev and Khokhlov1979, a brain specific parasite of salmonids, in kidney of native fish *C. batrachus* is the first record of *M. arcticus* from India. Recently in the year 2012, Kaur and Singh prepared a synopsis of 131 nominal species of *Myxobolus* Bu¨tschli, 1882 reported from India including six exotic species but *M. arcticus* is not mentioned in this synopsis and thus it supports our results. The present *M. arcticus* isolated from kidney of *C. batrachus* is morphologically very much similar to all the conspecific *M. arcticus* populations isolated from salmonids (Urawa *et al*., 2009; Nagasawa *et al*., 1994) except *M. arcticus* from masu salmon, *O. masou masou* sampled from river Mena, Japan; which is smaller than present species. However in the same species *O. masou masou* and other salmonids collected from another river 'Chitose' of Japan, the spores are quite bigger; resemble to each other and; to conspecific *M. arcticus* from salmonids of Norway, Russia and Eastern Siberia (Fig.6-Table). The minute morphometric differences in spore shape and size of present parasite *M. arcticus* in comparison to various *M. arcticus* species isolated by earlier workers (Urawa *et al*., 2011; Kaur & Singh, 2012) in distant geographical locations can be explained by interpretation of Moser (1977), who studied more than 700 species of 30 myxosporean genera and found that spore morphology is more constant among isolates of the same myxosporean species of different unrelated geographical origins whereas species from different tissues and different hosts displayed greater diversity of spore morphology. He concluded that spore size and shape is determined by selective forces imposed by host behavior and the particular environment within the respective host tissues. Hervio and his colleagues in the year 1997 analyzed 18S rDNA sequences of 4 *Kudoa* species and revealed that parasites are grouped according to geographical locations rather than spore morphology. Ferguson and his coworkers (2008) suggested that Myxosporeans can be distinguished from each other by the site of infection, SSU rDNA sequence and spore length. Andree and his coworkers (1999) reported that ten *Myxobolus* sp. groups based on 18S rRNA sequences data are more similar to species grouped on the basis of tissue tropism rather than spore morphology or on the basis of geographical origin but the degree of tissue tropism or degree of tissue specificity of host with which it interacts to a parasite is variable in Myxosporeans.

The *M. arcticus* has morphological and genetic diversity even among salmonids. Urawa and his coworkers (2009) revealed considerable genetic diversity in *M. arcticus* isolated from various host species and SSu rDNA analysis indicated 3.2 – 4.7 % sequence variation between Pacific salmon (genus *Oncorhynchus*) and Chars (genus *Salvelinus*). Intraspecific SSu rDNA sequence variations have been observed in geographically distant (allopatric) representatives of both *Kudoa amamiensis* and *K. thyrsites* (Whipps *et al*., 2003). Kent and Poppe (1998) reported that distant geographic isolates (North America and South Africa) of *K. thrysites* showed 99.4% homology in the 18S rDNA sequences. However Intraspecific SSU rDNA sequence deviations have been reported in sympatric myxozoans also from different host species, like *Myxidium lieberkuehni* and *M. pseudodispar* (Schlegel *et al*., 1996; Molnar *et al*., 2002). Andree and his coworkers (1999) reported 0.8% SSU rDNA sequence variation between geographic isolates of *M. cerebralis*. Thus, the molecular data indicated that *M. arcticus* isolates from the *C. batrachus* are slightly distinct biological individuals from the allopatric *M arcticus* of salmons of Japan and Canada and high sequence similarity of present *M. arcticus* to other *M. arcticus* from masu salmon of Japan and Sockeye salmon *O. nerka* of Canada does not necessarily equate to conspecificity, nor do the minor sequence differences mean they are separate species. Urawa and his coworkers (2011) also observed that differences in host specificity are not compelling evidence for distinct species or strains of the parasites. Presently there are no uniform criteria, whether morphological or molecular, for deciding boundaries between myxozoan species. The two distant allopatric populations may indicate dispersal or diversity but not speciation. Likewise genetic differences in a few specimens from a conserved gene such as the SSU rDNA are suggestive of an ecological separation, but not necessarily speciation. Different sequences may represent multiple alleles of the same gene.

Conclusion

Present record raises many questions like do all the allopatric populations of *M. arcticus* have same origin? Is it originated in marine or freshwater habitat as specific hosts are anadromous salmons? Do they really belong to one species? How this exotic species crossed the boundaries and entered in a totally different niche? How it parasitized kidney instead of brain? To answer these questions and to determine the patterns or routes of spread of myxozoan parasites, epidemiological study is required which should be based on highly variable nuclear and mitochondrial DNA regions like microsatellites sequences coupled with data regarding ultra-structure, life cycle, tissue tropism or host parasite interactions, geographical distribution of host species and alternate host. This will provide insight into evolution, life history and dispersal of these parasites. The trans-boundary movement of salmonids can also be a reason for presence of *M. arcticus* in India. Further studies should be undertaken to identify the parasite genes which control important processes as the recognition, attachment, multiplication and destruction of host tissues by the parasite. This will lead to a better understanding of the molecular mechanisms which determine the critical host parasite interaction.

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Conflict of interest

The authors declare that they have no conflict of interest.

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