

Morphological and Molecular Description of a New Species of *Myxobolus* (Myxosporea: Myxobolidae) Infecting *Planiliza Macrolepis* (Smith, 1846) From India

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Abstract

The present paper describes a novel species of *Myxobolus* parasitizing the gill filaments of the largescale mullet, *Planiliza macrolepis* from Cochin backwaters, Kerala, India. The parasite develops in the gill filaments; plasmodia elongated, milky white, measured 1.37 – 2.18 (1.78 ± 0.35) mm x 0.07-0.12 (0.10 ± 0.02) mm in size. Mature myxospores ovoid in valvular view, biconvex in sutural view with smooth shell valves and measured 6.24 - 7.02 (6.63 ± 0.23) × 5.01 - 6.18 (5.68 ± 0.25) µm in size. Polar capsules equal, oval with pointed anterior ends, 3.07 – 3.58 (3.33 ± 0.12) × 1.68 – 2.42 (2.09 ± 0.18) µm in size. Polar filaments with 4 coils, measured 29.61 ± 4.75 µm in length when extruded. Sporoplasm binucleate with a rudimentary nucleus and a vacuole. A comparison with related *Myxobolus* species revealed significant morphological & morphometric differences. In BLASTN and genetic distance analysis, the present parasite showed high divergence with other myxosporean sequences, indicating its molecular uniqueness. In Maximum Likelihood and Bayesian Interference analysis, the present species stands out with *M. ramadus* as sister branch within the *Myxobolus* clade. In infected gill filaments, the plasmodia caused swelling/deformation, compression of lamellae and reduction in respiratory surface area. Three of 222 *P. macrolepis* screened were infected, indicating a prevalence of 1.3%. Considering the morphological, morphometric, molecular and phylogenetic differences with the previously described species of myxosporeans, along with the dissimilarities in host and geographical locations, the present parasite is treated as a new species and the name *Myxobolus cochinensis* n. sp. is proposed.

Introduction

Myxosporeans are a diverse, exclusively microscopic, group of metazoan parasites belonging to the phylum Cnidaria. They mainly parasitize fishes and sometimes reptiles, amphibians and even higher animals, and have a complex life cycle that alternates between vertebrate (fish) and invertebrate (annelid) hosts (Wolf and Markiw 1984). Many myxosporeans are known to cause reduced growth and productivity, and even serious mortalities and economic losses in farmed fish worldwide (Abidi et al. 2015).

The genus *Myxobolus* Bütschli, 1882, the largest myxosporean genus, contributes to almost one-third of the myxosporean species reported so far (Eiras et al. 2014; Chandran et al. 2019). Eiras et al (2014) have compiled 905 nominal species of *Myxobolus* parasitizing various fishes, and till date 80 more species have been added to this list. Kaur & Singh (2012) have reported 131 species of *Myxobolus* from Indian waters and since then 33 species have been added to this list. The genus *Myxobolus* is characterized by bivalvular myxospores which are ellipsoidal to ovoid or rounded in valvular view and biconvex in sutural view; with smooth shell valves and two pyriform, sometimes unequal polar capsules and binucleate sporoplasm, often with an iodophilous vacuole. They are histozoic in nature, infecting various tissues as intercellular or sometimes intracellular cysts (Lom & Dykova 2006).

Mulletts are euryhaline fishes belonging to the family Mugilidae, inhabiting the coastal, temperate and tropical waters and have a worldwide distribution. Mulletts are a preferred species in many parts of the world and many of them are considered potential candidates for aquaculture. Thirteen species of grey mullets have been reported from Indian waters, of which eight are commercially important (Luther 1973). The largescale mullet, *Planiliza macrolepis* Smith 1846 inhabits the shallow, brackish and coastal waters along the southwest coast of India and commands high market value. Parasitological studies carried out in mullets indicate that they are infected with large number of parasites, especially myxosporeans (Özer et al. 2016). Yurakhno & Ovcharenko (2014) have reported 36 species of myxosporeans representing eight genera infecting *Mugil cephalus*, of which 21 belonged to the genus *Myxobolus*. World over, 51 species of *Myxobolus* have been reported from mullets, of which, 22 were found infecting the gills (Yurakhno & Ovcharenko 2014; Marcotegui & Martorelli 2017; Cardim et al. 2018; Rocha et al. 2019). Eleven species of *Myxobolus*, *M. narasii* (Narasimhamurti 1970), *M. lizae*, *M. macrolepi* and *M. sphaeralis* (Narasimhamurti & Kalavati 1979), *M. mugcephalus* (Narasimhamurti et al. 1980), *M. anili* (Sarkar 1989), *M. bankimi* (Sarkar 1999), *M. mugilii* (Halder et al. 1996) and *M. goensis* (Eiras & D'souza 2004) have been reported infecting mullets from India. The present study describes a new species of *Myxobolus* infecting the gill filaments of *P. macrolepis* from Cochin backwaters, Kerala, India.

Materials And Methods

Fish sampling

Two hundred and twenty-two *P. macrolepis* were collected from Cochin backwaters (9.9312° N, 76.2673° E) using Chinese nets/gill nets from October 2017 to September 2019. The fish ranged from 7.5 - 21.4 cm in length and 10 – 120 g in weight. The collected fish were brought live to the laboratory and were either examined immediately or maintained in tanks and studied at convenience. The fish were killed by neural pithing and the external surface was examined under a Nikon SMZ1000 stereozoom microscope (Nikon, Japan). The fish were dissected following standard necropsy procedures and their internal organs were separated and examined in detail for the presence of parasites. The gills were transferred to a cavity block with 0.75% saline and after removing the excess mucus, the gill filaments were examined in detail. Cysts were transferred to a slide, ruptured using a fine needle and examined under a Nikon Eclipse 80i microscope

(Nikon, Japan). A few infected gill filaments were preserved in 95% ethanol for molecular analysis, while mature myxospores released from ruptured cysts were used for preparing Giemsa-stained permanent smears.

Morphology and morphometry

Fresh myxospores were studied in detail using a Nomarski Differential Interference Contrast (DIC) objective at $\times 100$ magnification and photographed using a Nikon DS-Fi1c camera (Nikon, Japan). Myxospores ($n = 30$) were measured with the help of Nikon-Elements BR software, as suggested by Lom and Arthur (1989). Nikon Y-IDT drawing tube (Nikon, Japan) was used to make line drawings. Measurements are given in micrometres (μm) as range followed by mean \pm standard deviation in parentheses.

Histology

Infected tissue samples were preserved overnight in 10% NBF, and processed for histology as per standard procedures. Using a microtome (Leica, Wetzlar, Germany), 5 μm thick sections were taken, stained with haematoxylin and eosin and photomicrographs were taken at various magnifications (Miller, 2012).

Molecular analysis

Genomic DNA was extracted from ethanol-fixed plasmodium on gill filaments using a Multi-Sample DNA Purification Kit (HiPurA™). Briefly, plasmodia were lysed in lysis buffer and Proteinase K, DNA was captured on to a membrane, washed with ethanol, eluted in nuclease free water and kept at -20°C until further use. A small fragment of the SSU rDNA was amplified by nested PCR. The first round of amplification was performed using the primers, ERIB1 (5'-ACCTGGTTGATCCTGCCAG-3') and ERIB10 (5'-CTTCCGCAGGTTACCTACGG-3') (Barta et al. 1997) followed by a second round using ERIB1 - ACT1R (5'-AATTTACCTCTCGCTGCCA-3') (Barta et al. 1997; Hallet & Diamant, 2001) and Myxgen4F (5'-GTGCCTGAATAAATCAGAG-3') - ERIB10 (Barta et al. 1997; Diamant et al. 2004) primers. PCR was performed in 30 μl total reaction volume containing 15 μl Dream Taq Green PCR Master Mix 2X (Thermo Scientific, USA), 12 μl nuclease-free water, 0.6 μl of each primer, 1.8 μl template DNA. PCR was carried out in a ProFlex PCR system (Applied Biosystems, USA) with an initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 60 s, 56°C for 60 s and 72°C for 2 min and a final elongation at 72°C for 10 minutes for first round of amplification and the second round was performed with an initial denaturation at 95°C for 3 min/5 min, followed by 35 cycles of 94°C for 60 s, 50°C for 2 min/ 56°C for 60s and 72°C for 3 min/1.30 min and a final elongation at 72°C for 10/5 minutes. PCR products were resolved on 1.5% agarose gel in Tris EDTA buffer and stained with ethidium bromide. The positive amplicons were removed, purified using a HiPurA™ Quick Gel Purification Kit (HIMEDIA, India) and Sanger sequencing carried out using ERIB1, ACT1R, ERIB10 and MYXGEN4F primers by a commercial company.

Phylogenetic analysis

Partial sequences of the three isolates (approximately 920bp, 776 bp and 863bp generated using ERIB1-ACT1R, and 1098bp, 1162bp, and 1161bp generated using MYXGEN4F-ERIB10 respectively) were assembled from two overlapping fragments, edited using BioEdit V7.2.5 (Hall 1999), and the resulting sequences with 1902bp, 1955bp and 1908bp length deposited in NCBI GenBank (Accession numbers: MT305662, MT305663 and MT305666). BLASTN analysis of the isolates was performed in NCBI and phylogenetic analysis carried out using partial 18S SSU rDNA sequences of 15 species of *Myxobolus* selected based on the similarity scores, and 13 other representative myxosporeans downloaded from GenBank. Sequences were aligned using Clustal W module in MEGA 7 (Kumar et al. 2016). Genetic distance analysis was carried out using P-distance model. Best-fit model for phylogenetic analysis appeared to be GTR+I+G based on Akaike's information criterion. Maximum Likelihood (ML) tree was constructed with the sequence of *Buddenbrockia plumatellae* (Accession no. KJ741235) as out group with rapid bootstrap method using a random starting tree and 1000 ML bootstrap replicates. Bayesian Inference (BI) was conducted in MrBayes, version 3.1.2 (Ronquist & Huelsenbeck 2003) using Markov Chain Monte Carlo searches of two simultaneous runs of four chains of 20,00,000 generations, with every 1000th tree being sampled. The first 25% trees were discarded as burn-in, and the posterior probability of each node was calculated for the remaining trees. The resulting ML and BI trees were edited and annotated in Figtree v1.4.0 (Rambaut 2008) and Adobe Photoshop (Adobe Systems Inc. San Jose, CA) respectively.

Results

Three of 222 *P. macrolepis* screened harboured cysts of a species of *Myxobolus* indicating a prevalence of 1.3%. Elongated, milky-white plasmodia were found developing within the gill filaments (Fig. 1 a); measured 1.37 – 2.18 (1.78 ± 0.4) mm in length and 0.07-0.12 (0.10 ± 0.02) mm in width.

Morphological description of myxospores

Mature myxospores were bivalvular with thick shell valves and a prominent suture; ovoid in valvular view and biconvex in sutural view; with a rounded anterior end and slightly narrow posterior end; measured 6.24 - 7.02 (6.63 ± 0.23) μm in length and 5.01 - 6.18 (5.68 ± 0.25) μm in width ($n=30$) (Fig. 1 b,c,f). Polar capsules equal, oval in shape with pointed anterior ends and occupied the anterior half of the spore; measured 3.07 - 3.58 (3.33 ± 0.12) μm in length and 1.68 - 2.42 (2.09 ± 0.18) μm in width ($n=30$) (Fig. 1 b-h). Polar filaments formed 4 coils, and measured 23.22 - 36.22 (29.61 ± 4.75) μm in length, when extruded (Fig. 1 d,g). Sporoplasm homogenous, binucleate, with a rudimentary nucleus and a vacuole, and occupied the whole extracapsular space behind the polar capsules (Fig. 1 c,h). Deformed spores with caudal appendages were observed infrequently (Fig. 1e).

Taxonomic summary

Phylum: Cnidaria

Unranked Subphylum: Myxozoa Grassé, 1970

Class: Myxosporea Bütschli, 1881

Order: Bivalvulidae Shulman, 1959

Family: Myxobolidae Thelohan, 1892

Genus: *Myxobolus* Bütschli, 1882

Species: *Myxobolus cochinchensis* n. sp.

Host: *Planiliza macrolepis* (Mugilidae) Smith, 1846

Type locality: Cochin backwaters, Southwest coast of India (9.9312° N, 76.2673° E)

Site/Organ: Gill filaments

Prevalence: 3 of 222 *P. macrolepis* were infected (1.3%)

Etymology: Named after the locality from where the parasite was recovered.

Type material

Voucher specimen (air-dried, Giemsa-stained myxospores) deposited in the parasite collections of the Marine Biodiversity Museum, Central Marine Fisheries Research Institute, India (Accession number: CD. 1. 1. 1. 2) and partial sequences of 18S SSU rDNA gene deposited in NCBI GenBank (Accession numbers: MT305662, MT305663 and MT305666).

Remarks

Morphological features place the present myxosporean under the genus *Myxobolus*. Among the species of *Myxobolus* possessing spores with rounded anterior and slightly narrow posterior ends, two equal polar capsules and without sutural ornamentation, the present species exhibit close similarities with *M. discrepans* infecting *Ictiobus bubalis* (Rice et al. 1943), *M. stomum* infecting *Plectorhincus gaterinus* (Ali et al. 2003), *M. cycloides* and *M. mulleri* infecting *Leuciscus cephalus* (Molnár et al. 2006), *M. pethericii* infecting *Ctenopoma petherici* (Fomena et al. 2007), *M. agolus* and *M. clarii* infecting *Oreochromis niloticus* (Mohammed et al. 2012) and *M. nigeriae* infecting *Schizothorax niger* (Dar et al. 2016). A comparison of the above eight species with the present myxosporean (Table 1) indicate that, except *M. nigeriae*, all the above species differ from the present species in having larger myxospores and polar capsules. Though *M. nigeriae* closely resembles the present species in spore dimensions, it differs in the width of the polar capsule, number of polar filament coils and infects a fresh water fish host. The present species differs from all the above species except *M. pethericii* in the number of polar filament coils. Further, *M. discrepans*, *M. cycloides*, and *M. mulleri* can be differentiated based on the presence of intercapsular process, while *M. stomum* and *M. cycloides* differs in the site of infection. Among the compared species, the present myxosporean infect *P. macrolepis* which belong to family Mugilidae, and except *M. nigeriae*, all the compared species have been reported from different geographic locations. Though *M. ramadus*, *M. cerveirensis*, *M. episquamalis* and *M. exiguus* comes close to the current species in BLASTN analysis, they exhibit strong morphological and morphometric differences with the present species. Considering the differences in morphology and morphometry the present myxosporean is treated as a new species, and named *Myxobolus cochinchensis* n. sp.

Histology

In histological preparations, the elongated, intrafilamental-epithelial (FE) type plasmodia were lodged within the body of the gill filament. Infected gill filament appeared slightly dilated/swollen and deformed (Fig. 2a). The cyst wall was moderately thick and enclosed mature and immature spores (Fig. 2b). Pathological changes included lamellar hypertrophy and total destruction of infected gill lamellae. Compression of lamellae on the adjacent gill filaments was also noticed. (Fig. 2c).

Molecular analysis

Partial 18S SSU rDNA sequences (~1902bp, 1955bp and 1908bp) of the three isolates of the present myxosporean were identical (Accession numbers: MT305662, MT305663 and MT305666) and exhibited 0.1% to 0.2% divergence (Table 2). In sequence similarity analysis using BLASTn tool, the sequences showed a highest identity of 89.77% with *M. ramadus* (MK203074) infecting the gill lamellae of *Chelon ramada*, followed by 87.76% with *M. cerveriensis* (MK203079) parasitizing the intestine of *Chelon ramada*, 87.42% with *M. episcquamalis* (JF810537) infecting the scales of *Mugil cephalus* and 86.99% with *M. exiguus* (MH236070) infecting the peritoneum of *Chelon ramada*. In genetic distance analysis using P-distance model, isolates of the present myxosporean showed a divergence of 11.9% to 12.1% with *M. ramadus*, the closest myxosporean sequence in BLASTN analysis. Other compared sequences exhibited still lower molecular identities and higher divergence values, suggesting the molecular uniqueness of the present species.

Phylogenetic analysis

In phylogenetic analysis genus *Myxobolus* appeared polyphyletic. Isolates of *M. cochinensis* n. sp. occupied an independent position within the *Myxobolus* clade with high nodal support in both BI and ML analyses (1/100). In ML tree, *M. ramadus* appeared as sister taxa to the present parasite with a bootstrap value of 0.93/99 (Fig. 2).

Discussion

The present study describes a new species of myxosporean, *M. cochinensis* n. sp. infecting the largescale mullet, *P. macrolepis* from Cochin backwaters. Although myxospore morphology and morphometry constitute the foundation for myxosporean systematics, recent advances in molecular systematics using 18S SSU rDNA based phylogenetic inferences have greatly improved the perception of myxosporeans (Ye et al. 2014). Moreover, molecular studies allow the separation of morphologically indistinguishable species (Cech et al. 2012), thereby resolving the taxonomic ambiguities existing in many species. Members of the genus *Myxobolus* are generally known to exhibit high levels of tissue and host specificities and hence these features are also considered important in the taxonomy of the group (Guo et al. 2016).

Molecular analysis of 18S SSU rDNA of the three isolates of *M. cochinensis* n. sp. revealed a divergence value of 0.1 to 0.2, demonstrating their conspecific nature. Sequences of *M. cochinensis* n. sp. exhibited a molecular divergence of 11.9% to 12.1% with *M. ramadus*, the closest reference sequence in BLASTN analysis, suggesting its molecular uniqueness (Table 2). Fiala et al. (2015) have remarked that *Myxobolus* clade appears as the largest lineage within the myxozoan phylogenetic tree and exhibits a polyphyletic origin. In the present study also, genus *Myxobolus* exhibited a polyphyletic origin in both ML and BI tree topologies with *M. ramadus*, appearing as a sister taxa. According to Fiala (2006), interspecific SSU variation in the Myxosporea is greater than 2%, while Ferguson et al. (2008) observed that in some genera like *Myxobolus*, it can be as low as 0.2%. In the present study, phylogenetic trees constructed with ML and BI methods were identical, with the isolates of *M. cochinensis* n. sp. forming a distinct cluster with high bootstrap value, indicating their identity as a unique species.

Mulletts appear to be one of the preferred hosts for myxosporeans, especially those belonging to the genus *Myxobolus* (Yurakhno & Ovcharenko 2014). The prevalence observed for *M. cochinensis* n. sp. in the present study was relatively low (1.3%). The prevalence reported for *Myxobolus* infections in fishes varied from 2.31% to 70% and 4.12% to 80% in freshwater and marine habitats respectively (Padma Dorothy & Kalavati 1992; Awakura et al. 1995; Zhang et al. 2010; Salman et al. 2017). In mullets, the prevalence of *Myxobolus* infection varied from 4.5% to 60% and 1.8% to 80% in freshwater and marine/brackishwater habitats respectively (Bahri et al. 1996; Ovcharenko et al. 2017; Cardim et al. 2018; Rocha et al. 2019). Variations in prevalence could be ascribed to factors including availability of definitive and intermediate hosts, climatic and environmental conditions and nutrient levels in their respective habitats (Yokoyama & Fukuda 2001).

Histopathology revealed that the infected gill filament appeared slightly swollen and deformed with compression/destruction of gill lamellae. These alterations in the gill surface architecture can reduce the gaseous exchange capacity of the gills. Similar observations were made by Cardim et al. (2018) in *M. bragantinus* infecting the gills of *M. rubrioculus*, and by Ahmed et al. (2019) in *M. himalayaensis* from the gills of *S. richardsonii*. Kaur & Katoch (2016) revealed that cysts of *Myxobolus* in the secondary gill lamellae induce hypertrophy and inflammation of secondary lamellae, leading to asphyxia in the host. Dar et al. (2017) reported inactivation of infected gill lamellae due to intra-lamellar vascular (LV3) type infection causing necrosis, hypertrophy and hyperplasia of the calciform cells. Marcotegui & Martorelli (2017) observed that cysts of *M. saladensis* infecting the gill epithelium of *M. liza* may interfere with the respiratory function of the host.

Considering the striking morphological, morphometric, molecular and phylogenetic differences with existing species of myxosporeans, together with differences in host and geographical locations, the present species of *Myxobolus* recovered from *P. macrolepis* is viewed as a new species and the name *Myxobolus cochinchensis* n. sp. is proposed.

Declarations

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Author's contribution: The study was designed and guided by N.K. Sanil and P. Vijayagopal. Mary Soniya Correya carried out the study in detail. The manuscript was written by all the authors.

Compliance with ethical standards: All applicable institutional, national and international guidelines for the care and use of animals were followed in the present study.

Conflict of interest: The authors declare that that there is no conflict of interest between them.

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Tables

Table 1: Showing myxospore dimensions (µm) of *Myxobolus cochiniensis* n. sp. with morphologically similar species

Species	Myxospore measurements					Site of infection	Host	Geographic region	Reference
	SL	SW	PCL	PCW	NC				
<i>M. discrepans</i>	15.5	13.9	5.4	3.9	8-10	Gills	<i>Ictiobus bubalis</i>	USA	Rice et al., 1943
<i>M. stomum</i>	7.0-10.0 (8.5)	5.5-7.5 (6.5)	4.0-5.0 (4.4)	2.0-3.0 (2.4)	5-6	Oral cavity muscle	<i>Plectorhincus gaterinus</i>	Egypt	Ali et al., 2003
<i>M. cycloides</i>	13-15.2 (14.1)	9.8-11.1 (10.43)	4.1-6.5 (5.2)	3.0-4.0 (3.36)	6-7	Swim bladder	<i>Leuciscus cephalus</i>	Hungary	Molnar et al., 2006
<i>M. mulleri</i>	9.5-10.0 (9.8)	7.5-8.0 (7.5)	5.0-5.5 (5.2)	4.0-5.0 (4.6)	5-6	Gills	<i>Leuciscus cephalus</i>	Hungary	Molnar et al., 2006
<i>M. pethericii</i>	12.0-14.0 (12.6)	6.5-7.8 (7.0)	5.0-6.0 (5.3)	1.5-2.0 (1.8)	4-5	Gills	<i>Ctenopoma petherici</i>	Cameroon	Fomena et al., 2007
<i>M. agolus</i>	10.5-11.2	8.5-9.2	4.8-5.5	3.5-3.8	10-11	Gills	<i>Oreochromis niloticus</i>	Egypt	Mohammed et al., 2012
<i>M. clarii</i>	9.1-12.4	6.8-9.7	3.7-4.8	2.1-3.0	5	Gills	<i>Oreochromis niloticus</i>	Egypt	Mohammed et al., 2012
<i>M. nigeriae</i>	6.3-6.9 (6.6)	4.8-5.2 (5.0)	3.1-3.5 (3.3)	1.5-1.7 (1.6)	5	Gills	<i>Schizothorax niger</i>	India	Dar et al., 2016
<i>M. cochinensis</i> n. sp.	6.24 - 7.02 (6.63)	5.01 - 6.18 (5.68)	3.07 - 3.58 (3.33)	1.68 - 2.42 (2.09)	4	Gills	<i>Planiliza macrolepis</i>	India	Present study

(SL-spore length, SW-spore width, PCL-polar capsule length, PCW-polar capsule width, NC-no. of coils)

Table 2: Estimates of pairwise distance between the sequences of *Myxobolus cochinensis* n. sp. and other closest myxosporeans available in NCBI GenBank. The genetic distance values and the number of different nucleotides are given below and above the diagonal respectively.

Sl. No.	<i>Myxobolus</i> Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	<i>M. cochiniensis</i> (MT305663)		1	2	218	218	178	231	254	236	258	261	245	257	277
2.	<i>M. cochiniensis</i> (MT305662)	0.1		3	219	219	179	232	255	237	259	262	246	258	278
3.	<i>M. cochiniensis</i> (MT305666)	0.1	0.2		220	220	180	233	256	238	260	263	247	259	279
4.	<i>M. muscularis</i> (MK203075)	15.0	15.1	15.1		179	212	137	193	136	206	207	155	202	183
5.	<i>M. episquamalis</i> (JF810537)	15.0	15.0	15.1	12.0		205	200	173	197	184	188	208	183	241
6.	<i>M. ramadus</i> (MK203074)	11.9	12.0	12.1	14.5	14.0		229	234	237	242	244	241	239	281
7.	<i>M. cerveirensis</i> (MK203079)	16.0	16.1	16.1	9.0	13.6	15.8		226	153	225	224	176	226	197
8.	<i>Myxobolus</i> sp. WSK- 2013 (KC733438)	17.7	17.8	17.9	13.1	11.6	16.2	15.5		206	107	102	229	97	250
9.	<i>M. exiguus</i> (MH236070)	16.4	16.5	16.5	8.9	13.3	16.4	10.1	14.0		213	213	110	211	47
10.	<i>M. galaicoportucalensis</i> (MC203084)	18.1	18.1	18.2	14.0	12.4	16.8	15.5	6.9	14.6		39	239	99	257
11.	<i>M. mugiliensis</i> (MK203082)	18.3	18.4	18.5	14.1	12.7	17.0	15.4	6.6	14.6	2.4		241	90	256
12.	<i>M. pharyngobranchialis</i> (MK203073)	17.1	17.2	17.2	10.2	14.2	16.8	11.7	15.8	7.1	16.6	16.7		233	150
13.	<i>M. vesicularis</i> (MK203085)	18.0	18.1	18.1	13.7	12.3	16.6	15.5	6.3	14.4	6.4	5.8	16.1		257
14.	<i>Myxobolus</i> sp. k AC- 2020 (MT437045)	19.7	19.8	19.9	12.3	16.7	20.0	13.3	17.4	3.0	18.0	18.0	9.9	18.1	

Figures

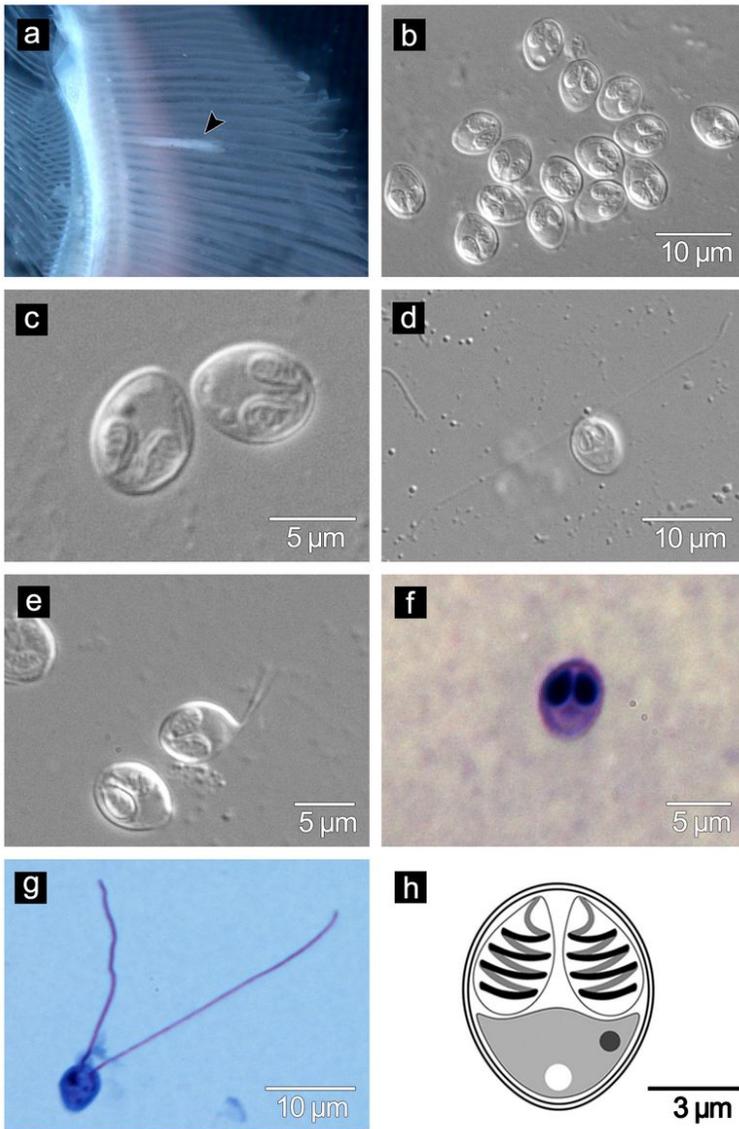


Figure 1

M. cochinensis n. sp. a) plasmodium on gill filaments; b) frontal view of myxospores; c) enlarged view of myxospores; d) myxospore showing extruded polar filaments; e) abnormal myxospore with caudal appendages; f) Giemsa-stained myxospore; g) Giemsa-stained myxospore with extruded polar filament; h) line drawing of myxospore.

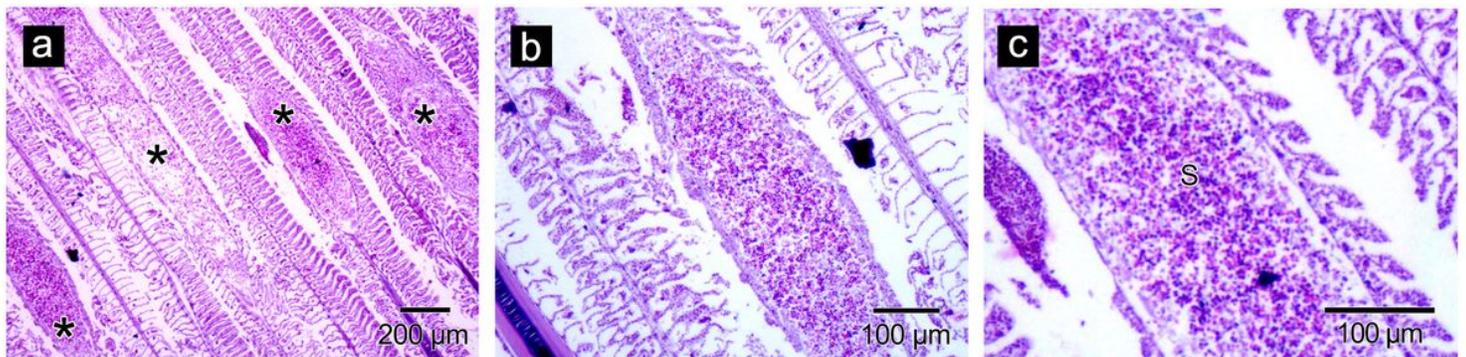


Figure 2

Histological changes caused by *M. cochinensis* n. sp. a) plasmodia on gill filaments (*); b) enlarged view showing cyst wall; c) lamellar hypertrophy and destruction of gill lamellae, S-spores.

Image not available with this version

Figure 3

Consensus phylogenetic tree showing the evolutionary relationship of *M. cochinensis* n. sp. (in bold italics) with other related species of myxosporeans. Each branch tip carries species name followed by its accession number, node values are posterior probability and bootstrap for BI and ML respectively. Dashes at nodes indicate bootstrap value of (ML) < 50. In histological preparations, the elongated, intrafilamental-epithelial (FE) type plasmodia were lodged within the body of the gill filament. Infected gill filament appeared slightly dilated/swollen and deformed (Fig. 2a). The cyst wall was moderately thick and enclosed mature and immature spores (Fig. 2b). Pathological changes included lamellar hypertrophy to total destruction of infected gill lamellae. Compression of lamellae on the adjacent gill filaments was also noticed. (Fig. 2c).