Parasitol Res (2014) 113:29–37
DOI 10.1007/s00436-013-3622-x

ORIGINAL PAPER

Myxobolus ophiocarae sp. n. (Myxozoa: Myxosporea: Bivalvulida) infecting the gill of wild goby, *Ophiocara porocephala* (Perciformes: Gobioidei) in Malaysia

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Received: 14 June 2013 / Accepted: 23 September 2013 / Published online: 6 October 2013

Abstract The authors studied the myxosporean infection of wild gobiid fishes (Perciformes: Gobioidei) in the Merang Estuary of Terengganu, Malaysia, and described *Myxobolus ophiocarae* sp. n. in *Ophiocara porocephala*. Several myxosporean plasmodia were found intralamellarly within the gill filaments. The spores differed from those of other *Myxobolus* species previously recorded on gobiid fishes. They were round in valvular view and lens-shaped in sutural view, and had two equal-sized, pyriform polar capsules with polar filaments having six to seven turns. The spores measured 10.34×8.79×4.53 μm. The 18S rDNA sequence of *M. ophiocarae* sp. n., based on a contiguous sequence of 1,789 base pairs, differed from any other *Myxobolus* spp. in GenBank. Phylogenetic analysis of the 18S rDNA gene revealed that this species showed the closest similarity to *Myxobolus nagaraensis*, *Myxobolus lentisuturalis*, and *Myxobolus cultus*.

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Introduction

The suborder Gobioidei comprising the families Eleotridae and Gobiidae is a speciose, morphologically diverse group of teleost fishes, most of which are small, benthic, and marine (Thacker 2003). There are about 35 genera, and 135 species belonging to the family Eleotridae worldwide. These fishes mostly inhabit tropical and subtropical areas and are relatively rare in the temperate regions. Only six species of eleotrid fishes could be found in Malaysian waters, including *Ophiocara porocephala* (Valenciennes 1837), locally known as "ubi belontok". This fish species has high economic importance in Malaysia. It is common and usually inhabits brackish estuaries, river mouths, and freshwater creeks, with younger specimens often found in rocky creeks near the coast (Ambak et al. 2010).

In recent years, investigations of myxozoans from both wild and cultured Malaysian fishes (Molnár et al. 2006a, b; Székely et al. 2009a, b, 2012) have shown high taxonomic diversity in freshwaters, but little attention has been paid to the myxozoan infections of brackish water fishes in Malaysia. In Asian countries, several myxosporean species have been recorded from gobies: *Myxobolus acanthogobii* Hoshina, 1952, *Myxobolus luciogobii* Ishizaki, 1957, *Myxobolus gylactiformae* Wu, 1985, *Myxobolus rhinogobii* Chen in Chen and Ma (1998), *Myxobolus tongyaensis* Chen in Chen and Ma (1998), *Myxobolus asianensis* Chen in Chen and Ma (1998), *Myxobolus nagaraensis* Yokoyama et al. 2007, *Henneguya pseudorhinogobii* Kageyama et al. 2009, *Henneguya miyazakii* Hoshina, 1952, *Henneguya rhinogobii* Li & Nie, 1973, and *Henneguya sichuanensis* (Chen and Ma 1998; Eiras 2002; Eiras et al. 2005; Kageyama et al. 2009;

Yokoyama et al. 2004, 2007). Myxosporean parasites known from gobies of other regions are *Kudoa camarguensis* (Pampoulie et al. 1999), *Ellipsomyxa gobii* (Køie 2003), *Myxobolus gobii* Naidenova in Gaevskaya, 1975, *Myxobolus albi* (Picon-Camacho et al. 2009), and a *Myxobolus* sp., each from France, Denmark, Russia, Scotland, and Brazil, respectively (Pampoulie et al. 1999; Køie 2003; Eiras et al. 2005; Picon-Camacho et al. 2009; Velasco et al. 2012).

In this study, a *Myxobolus* species is described from the gills of the estuarine fish *O. porocephala* as *Myxobolus ophiocarae* sp. n. Besides the morphological description, data are presented on the 18S rDNA of this new myxosporean.

Materials and methods

In an effort to survey the myxosporean fauna of estuarine fishes in Malaysia, specimens of *O. porocephala* Valenciennes, 1837, commonly known as the northern mud gudgeon (size range, 5–8.2 cm; weight range, 1–5.5 g), were net-fished at a water depth of about 0.5 m in the Merang Estuary (5°32′ 2.26″N, 102°56′46.89″E) of the Kuala Terengganu district in the state of Terengganu, Malaysia, in August 2012. In addition, some other gobiids—six juveniles of *Periophthalmus chrysospilos* Bleeker, 1852 and three specimens of *Acentrogobius caninus* Valenciennes, 1837—were fished and examined for myxozoan infection. The live specimens were transported to the laboratory of the Institute of Tropical Aquaculture (AKUATROP), Universiti Malaysia Terengganu (UMT), maintained in an aerated aquarium and freshly dissected within 2 days. Examinations for the presence of myxosporeans were carried out using a Leica EZ4 dissecting microscope and a Leica DM750 compound microscope at different magnifications appropriate for the analysis of parasites. Other organs (kidneys, gallbladder, and intestine) were also examined under a microscope. Gill filaments infected with myxozoan plasmodia were examined in a Nikon model MM-800 microscope. Plasmodia were carefully removed with a fine needle and dissected on a slide. Fresh preparations of ruptured plasmodia containing spores were further studied using a Nikon Model Eclipse 80i advanced light microscope. Thirty spores were measured according to the guidelines provided by Lom and Arthur (1989). Plasmodia-containing spores were preserved separately in 80 % ethanol for molecular biology analysis. A voucher sample of *M. ophiocarae* sp. n. spores was deposited in the collection of the Zoological Department of the Hungarian Natural History Museum of Budapest, Hungary. Measurements and drawings of *M. ophiocarae* sp. n. spores were compared to data of *Myxobolus* spp. reported from gobiid hosts (Table 2) as well as to species that were congeners to *M. ophiocarae* sp. n. on the phylogenetic tree (Table 2).

Molecular methods

DNA was extracted from the spores preserved in ethanol using a DNeasy™ tissue kit (animal tissue protocol; QIAGEN, Germany) according to the manufacturer's instructions. The 18S rDNA was amplified with a set of universal eukaryotic primers ERIB1, 5′-ACC TGG TTG ATC CTG CCAG-3′ and ERIB10, 5′-CTT CCG CAG GTT CAC CTA CGG-3′ (Barta et al. 1997). PCR was carried out in a 25-μl reaction mixture comprising 2 μl of extracted genomic DNA, 5 μl of 1 mM deoxyribonucleotide triphosphates (dNTPs; MBI Fermentas), 0.25 μl of each primer, 2.5 μl of 10× Taq buffer (MBI Fermentas), 0.1 μl of Taq polymerase (2 U; MBI Fermentas) and 15 μl of water. The following profile was used to amplify the 18S rDNA region: an initial denaturation step at 95 °C for 3 min, followed by 35 cycles at 95 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min, completed with terminal extension at 72 °C for 7 min and then storage at 4 °C.

This was followed by a second round of PCR with the Myx1F, 5′-GTG AGA CTG CGG ACG GCT CAG-3′ and SphR, 5′-GTT ACC ATT GTA GCG CGC GT-3′ primer pair. PCR reactions were conducted with a volume of 50 μl consisting of 1 μl of amplified DNA, 10 μl of 1 mM dNTPs (MBI Fermentas), 0.5 μl of each primer, 5 μl of 10× Taq buffer (MBI Fermentas), 0.2 μl of Taq polymerase (2 U; MBI

Table 1 Primers used for PCR and sequencing

Primer	Sequence	Application	Source
ERIB1	5′-ACC TGG TTG ATC CTG CCA G-3′	First round PCR	Barta et al. (1997)
ERIB10	5′-CTT CCG CAG GTT CAC CTA CGG-3′	First round PCR	Barta et al. (1997)
Myx1F	5′-GTG AGA CTG CGG ACG GCT CAG-3′	Second round PCR	Hallett and Diamant (2001)
SphR	5′-GTT ACC ATT GTA GCG CGC GT-3′	Second round PCR and sequencing	Eszterbauer and Székely (2004)
MC5	5′-CCT GAG AAA CGG CTA CCA CAT CCA-3′	Sequencing	Molnár et al. (2002)
MB5r	5′-ACC GCT CCT GTT AAT CAT CAC C-3′	Sequencing	Eszterbauer (2004)
MB5f	5′-GAT GAT TAA CAG GAG CGG TTG G-3′	Sequencing	Eszterbauer (2004)
ACT1fr	5′-TTG GGT AAT TTG CGC GCC TGC TGC C-3′	Sequencing	Hallett and Diamant (2001)

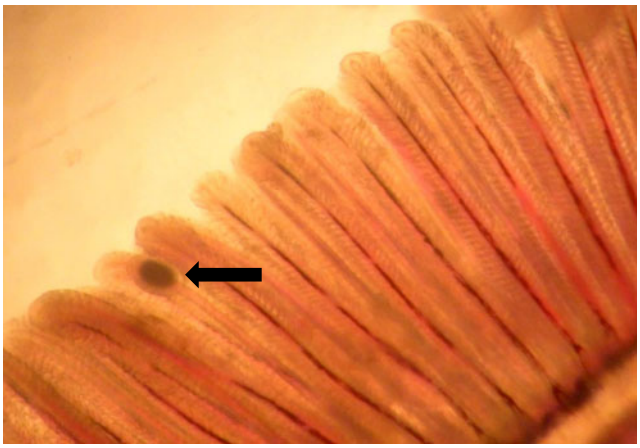


Fig. 1 *Myxobolus ophiocarae* sp. n. plasmodia infecting the gill lamellae develop in the epithelium (*arrow*) of the infected gill lamellae

Fermentas), and 33 μl of water. Amplification conditions in the second round were carried out with the following profile: 95 °C for 3 min, then 35 cycles at 95 °C for 50 s, 50 °C for 50 s, 72 °C for 1 min 40 s, terminated with an extension period at 72 °C for 7 min and then resting at 4 °C.

PCR cycles were run in a PTC-200 thermocycler (MJ Research). The PCR products were electrophoresed in 1 % agarose gels in Tris–acetate–EDTA buffer gel stained with 1 % ethidium bromide. Amplified DNA was purified with the EZ-10 Spin Column PCR Purification Kit (Bio Basic Inc). Purified PCR products were sequenced with the primers listed in Table 1, using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit with an ABI 3100 Genetic Analyser.

Sequences were aligned using the computer program Clustal W (Thompson et al. 1994) and corrected manually using the alignment editor of the software MEGA 5.10 (Tamura et al. 2011). DNA pair-wise distances were calculated with the MEGA 5.10 software using the Tamura–Nei substitution model. The phylogenetic position of goby myxobolids was estimated and compared to other topologies by maximum likelihood (ML) and Bayesian inference (BI) analyses. ML analyses were performed in MEGA 5.10. The dataset was tested using MEGA 5.10 for the nucleotide substitution model of best fit, and the model was shown by the Akaike Information Criterion as the best-fitting one was chosen: GTR + G + I. Bootstrap values based on 1,000 resampled datasets were generated. BI was computed by Topali 2.5 (Milne et al. 2008). The substitution models were tested by Bayesian Information Criterion and GTR + G was chosen. Posterior probabilities were estimated over 1,000,000 generations via two independent runs of four simultaneous MCMCMC chains with every 100th tree saved. The burn-in was 25.

Sequences of species related to *M. ophiocarae* sp. n. were downloaded from GenBank for the phylogenetic analysis. Taxa were selected on the basis of Basic Local Alignment Search Tool (BLAST) matches, with most being gill-infecting species; however, species from other organs and tissues are also represented. Additionally, several alignments of the *Myxobolus* clade described by Fiala (2006) were also used to clarify the position of the new sequence of *M. ophiocarae* sp. n. in the myxosporean phylogenetic tree. *Ceratomyxa shasta* Noble, 1950 (AF001579) was used as an outgroup in the final alignment.

Results

Only one out of the 12 examined fish specimens (8.3 %) was infected with this myxosporean species. Small, white, spherical plasmodia measuring less than 1 mm in diameter were found. The plasmodia on the gill filaments of *O. porocephala* were easily detected under both the dissecting and the

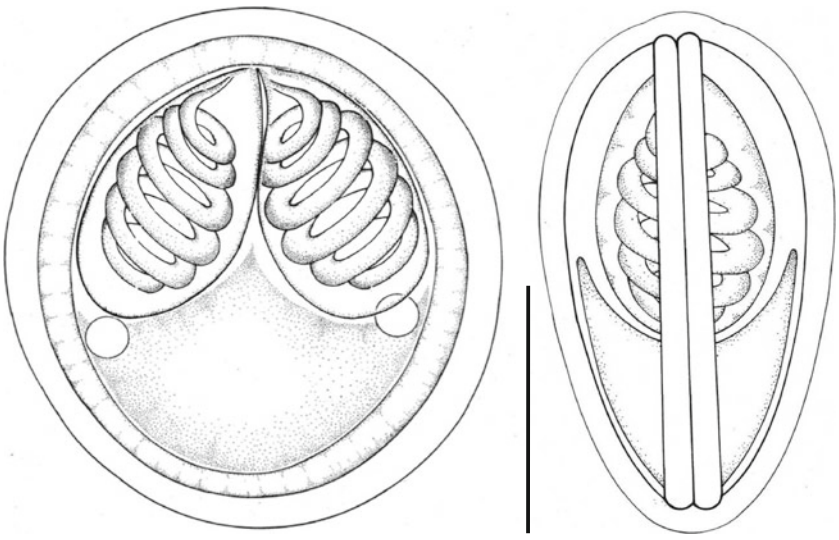


Fig. 2 Line drawings of the myxospore of *M. ophiocarae* sp. n. in frontal view (*left*) and sutural view (*right*); *bar* 5 μm

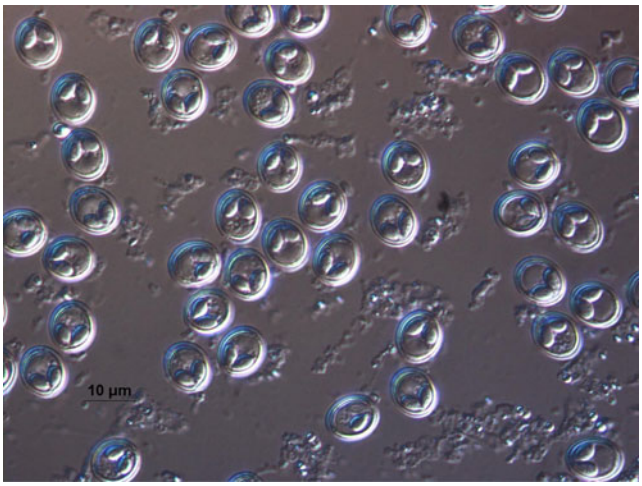


Fig. 3 Fresh myxospores of *M. ophiocarae* sp. n. in frontal view

compound microscopes. Spores obtained from mature plasmodia showed the typical characteristics of a *Myxobolus* sp. This newly isolated species differs both morphologically and genetically from the known *Myxobolus* spp. (Fig. 5) and is described as a new species under the name of *M. ophiocarae* sp. n. Pathological changes or deformities were not observed, and no myxosporean infection was found in other organs of this fish.

Diagnosis of *M. ophiocarae* sp. n. (Myxosporea; Bivalvulida)

Spherical to ellipsoidal plasmodia located intralamellarly contained numerous spores and some advanced sporogonic stages (Fig. 1).

Description

Spores (Figs. 2 and 3) round in valvular view and biconvex or lens shaped in sutural view; length=10.34±0.47 (9.29–11.35; $N=30$), width=8.79±0.37 (7.98–9.53; $N=30$), and thickness=4.53±0.44 (4.05–5.2; $N=10$). Two polar capsules pyriform, equal in size, 4.72±0.42 (3.93–5.45; $N=30$) long and 2.85±0.27 (2.23–3.29; $N=30$) wide. Polar filament, five or six turns, is situated perpendicular to the longitudinal axis, ~60 µm when extruded (Fig. 4). Evident mucous envelope surrounded every spore and appeared like a halo (Fig. 3).

Fig. 5 Phylogenetic tree generated by maximum likelihood analysis of the 18S rDNA sequences of *M. ophiocarae* sp. n. and some related species. *Numbers at nodes* indicate the bootstrap values (ML) and posterior probabilities (BI). *Ceratomyxa shasta* was used as the outgroup

Type host Northern mud gudgeon, *O. porocephala* Valenciennes, 1837 (Perciformes: Gobioidei: Eleotridae), juvenile.

Type locality Merang Estuary, Terengganu, Malaysia. Position: latitude=5°32′2.26″N; longitude=102°56′46.89″E.

Site of tissue development Gill lamellae.

Prevalence of infection One of 12 of 5- to 8.2-cm-sized fish.

Type material Digitised photos of syntype spores were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-70395. The 18S rDNA sequence was deposited in GenBank under accession numbers KF211435 and KF211436.

Etymology The species name refers to the genus of the vertebrate type host *Ophiocara*.

Trophozoites Mature plasmodia, spherical or ellipsoidal, containing spores were found intralamellarly in the gills.

Molecular analyses Two samples of *M. ophiocarae* n. sp. were sequenced (1787-KF211435 and 1789-KF211436 bps).

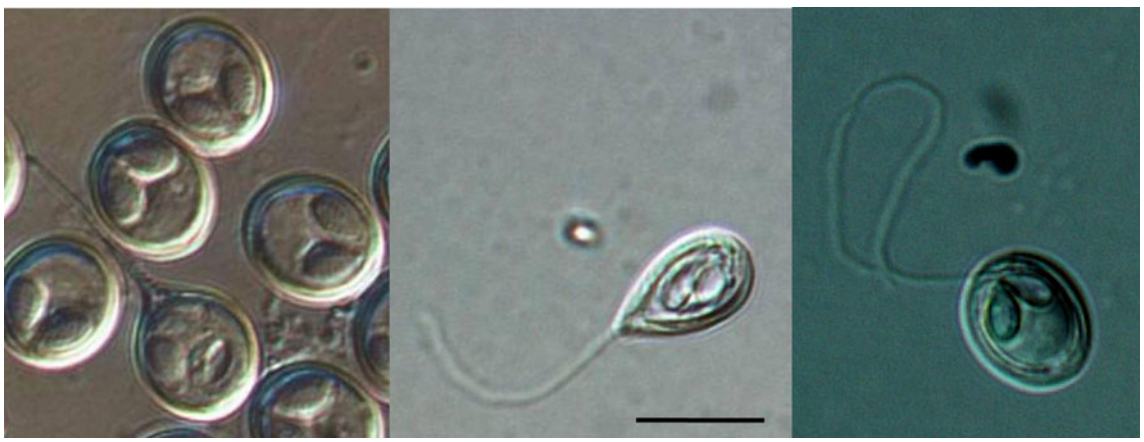
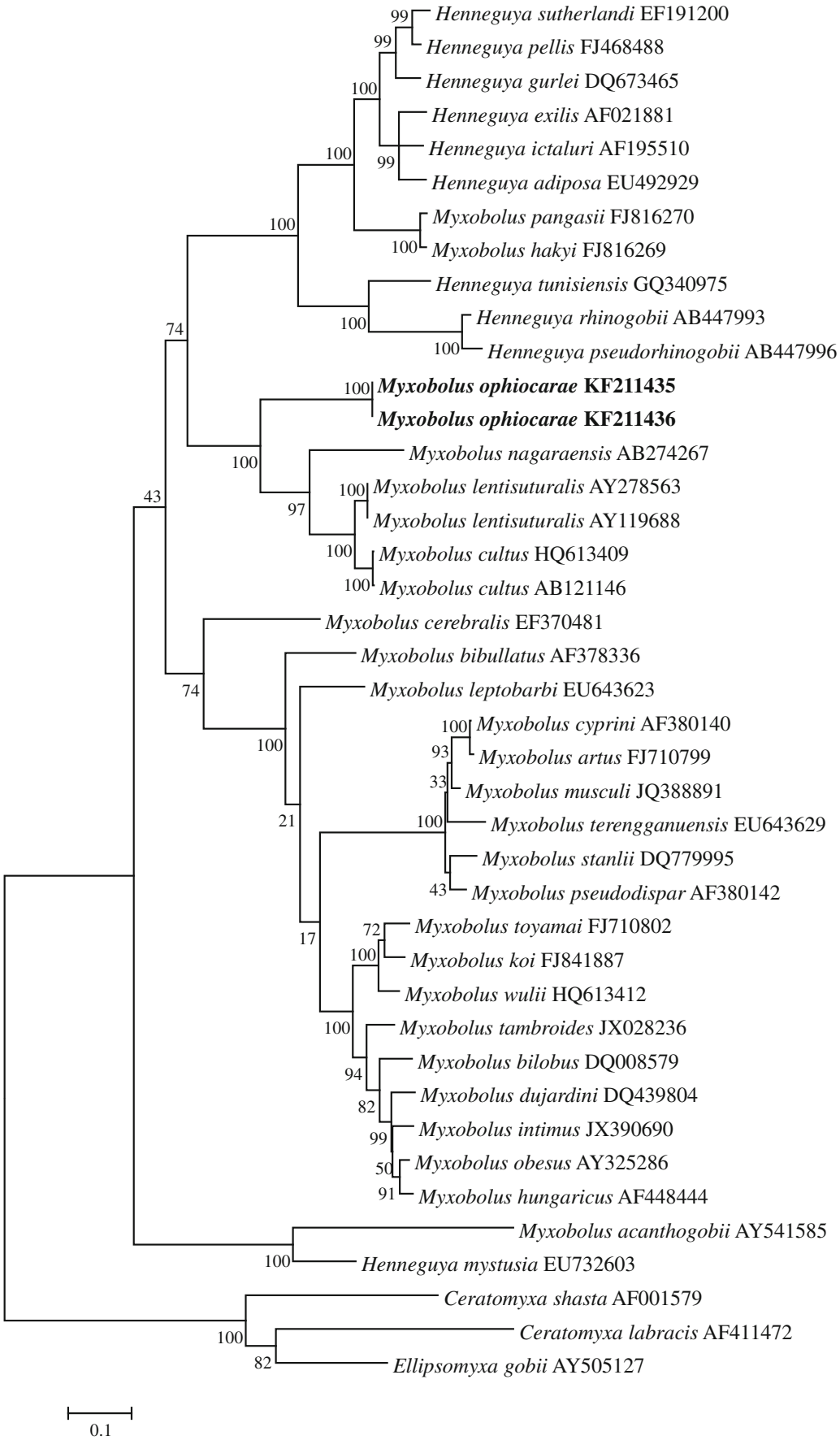


Fig. 4 Myxospores of *M. ophiocarae* sp. n. (**a**, **b**) Spore with tail-like structure; (**c**) extruded polar filament; *bar* 10 µm


Henneguya sutherlandi EF191200
Henneguya pellis FJ468488
Henneguya gurlei DQ673465
Henneguya exilis AF021881
Henneguya ictaluri AF195510
Henneguya adiposa EU492929
Myxobolus pangasii FJ816270
Myxobolus hakyi FJ816269
Henneguya tunisiensis GQ340975
Henneguya rhinogobii AB447993
Henneguya pseudorhinogobii AB447996
Myxobolus ophiocarae KF211435
Myxobolus ophiocarae KF211436
Myxobolus nagaraensis AB274267
Myxobolus lentisuturalis AY278563
Myxobolus lentisuturalis AY119688
Myxobolus cultus HQ613409
Myxobolus cultus AB121146
Myxobolus cerebralis EF370481
Myxobolus bibullatus AF378336
Myxobolus leptobarbi EU643623
Myxobolus cyprini AF380140
Myxobolus artus FJ710799
Myxobolus musculi JQ388891
Myxobolus terengganuensis EU643629
Myxobolus stanlii DQ779995
Myxobolus pseudodispar AF380142
Myxobolus toyamai FJ710802
Myxobolus koi FJ841887
Myxobolus wulii HQ613412
Myxobolus tambroides JX028236
Myxobolus bilobus DQ008579
Myxobolus dujardini DQ439804
Myxobolus intimus JX390690
Myxobolus obesus AY325286
Myxobolus hungaricus AF448444
Myxobolus acanthogobii AY541585
Henneguya mystusia EU732603
Ceratomyxa shasta AF001579
Ceratomyxa labracis AF411472
Ellipsomyxa gobii AY505127
0.1

A BLAST search presented no identical match and no close relationship was found with any other *Myxobolus* species represented in GenBank. The most similar 18S rDNA sequences in GenBank to *M. ophiocarae* n. sp. are those of *M. nagaraensis* (76.9 %), a myxozoan found in the body cavity and caudal peduncle of the freshwater goby *Rhinogobius* sp. from the Nagara River, Gifu Prefecture, Japan; *Myxobolus lentisuturalis* (79.0 %), a muscle-infecting myxozoan found in Prussian carp, *Carassius gibelio*, of China; and *Myxobolus cultus* (78.9 %), a myxozoan which was originally found through exposure of actinosporean spores from *Branchiura sowerbyi* to goldfish in Tokyo, Japan.

Phylogenetic analysis Maximum likelihood and Bayesian inference both placed *M. ophiocarae* sp. n. (KF211435 and KF211436) in a basal position to *M. nagarensis*, *M. lentisuturalis*, and *M. cultus*. Both maximum likelihood and Bayesian inference analyses gave highly similar tree topology of the position of *M. ophiocarae* n. sp. (Fig. 5). The new isolate clusters in the middle position of a clade containing the majority of *Myxobolus* spp. sequenced to date.

Taxonomic affinities *M. ophiocarae* sp. n. partially resembles the *Myxobolus* species listed in Table 2. *M. ophiocarae* sp. n. is similar to *M. albi* (Picon-Camacho et al. 2009) due to the presence of mucous envelope that appears as a halo surrounding the spores. However, it differs from *M. albi* in the shorter length of spores, smaller polar capsules, and fewer polar filament turns. *M. rhinogobii*, *M. asianensis*, and *M. acanthogobii* have larger and thicker spore body and bigger polar capsules. *M. luciogobi* has a smaller spore body and polar capsule. The polar capsules of *M. tongyaensis* and *M. nagaraensis* are longer than those of *M. ophiocarae* sp. n. No measurements were available from *Myxobolus* sp. collected from the Brazilian gobiid *Gobioides broussonnetii*.

Discussion

Myxobolus spp. are generally known as histozoic parasites of freshwater fishes. About 30 species live in marine habitats, mostly in estuarine fishes. When Lom and Dyková (2006) surveyed myxosporeans, they estimated the number of *Myxobolus* spp. to be around 792, including seven in amphibians. Among them, approximately 255 species have been reported to infect the gills (Eiras et al. 2005; Picon-Camacho et al. 2009).

Fishes of the suborder Gobioidei have been reported to be infected by members of several myxozoan genera such as *Kudoa* Meglitsch, 1960, *Ellipsomyxa* Køie 2003, *Henneguya* Thelohan, 1892, and *Myxobolus* Bütschli, 1882 (Pampoulie et al. 1999; Eiras 2002; Køie 2003; Yokoyama et al. 2004, 2007; Eiras et al. 2005; Kageyama et al. 2009; Picon-Camacho et al. 2009; Velasco et al. 2012). *Myxobolus* spp. identified from biotopes of China, Japan, Scotland, and Brazil infect eight gobiid host species: *Acanthogobius flavimanus*, *Gobius ophicephalus*, *Luciogobius guttatus*, *Synechogobius ommaturus*, *Rhinogobius giurinus*, *Rhinogobius* sp. (OR-type), *Pomatoschistus microps*, and *G. broussonnetii* (Table 2).

Differentiation of *Myxobolus* spp. is mostly based on their morphology and more recently on their DNA sequences. However, their host, organ, and tissue specificity has been described during identification. The importance of the infection site for the phylogenetic clustering of myxozoans has been recently demonstrated by a number of studies that found stronger linkages between Myxosporea infecting the same target organs as opposed to common host groups or species with similar spore morphology (Eszterbauer 2004; Holzer et al. 2004; Fiala 2006). The site selection of *Myxobolus* spp. in gobiid fishes varied among gills, brain and nervous system, urinary bladder, muscle, skin, kidneys, visceral cavity, nares, and palate. The present species may be compared with several *Myxobolus* species that develop plasmodia in the gills of gobiid fishes, namely *M. albi*, *M. asianensis*, and *M. rhinogobii* (Chen and Ma 1998; Picon-Camacho et al. 2009), although the last two species were also found on the skin and muscle of the host. Among them, the almost roundish shape of the spores of *M. ophiocarae* sp. n. most closely resembles *M. albi*, but they have dissimilar dimensions. However, *M. ophiocarae* sp. n. parasitizes the interlamellar sites of the gill filaments, whereas *M. albi* develops in the cartilage of the gill.

Phylogenetic analyses have shown that *M. ophiocarae* sp. n. is closely related to *M. nagaraensis*, which was clustered with *M. lentisuturalis* from *Carassius auratus auratus* (AY278563), *M. lentisuturalis* (AY119688) from *C. gibelio*, and *M. cultus* (Yokoyama et al. 1995) from *C. auratus* within a broad clade of *Myxobolus* species that predominantly infect the gills of their vertebrate hosts (Fig. 5). The adjacent root above *M. ophiocarae* sp. n. clustered *H. pseudorhinogobii* (AB447996) and *H. rhinogobii* (AB447993), both of which were collected from the freshwater goby *Rhinogobius* sp. (OR-type) off Japan. Comparisons of *M. ophiocarae* sp. n. with species previously described from gobiid fishes mainly relied upon spore measurement due to the paucity of available genetic data, with the exception of *M. nagaraensis* (AB274267; Yokoyama et al. 2007), *M. acanthogobii* (AY541585; Yokoyama et al. 2004), and *M. albi* (EU420055; Picon-Camacho et al. 2009), which was used for comparison. *M. albi* was removed from the phylogenetic tree because the length of its sequence was too short and was out-positioned in the phylogenetic tree. *M. nagaraensis*, *M. acanthogobii*, and *M. albi* are not closely related to *M. ophiocarae* n. sp. genetically, which allows us to regard the new isolate as an independent species.

Table 2 Comparison of the spore morphometric data of *Myxobolus ophiocarae* n. sp. with those of selected *Myxobolus* species reported from gobiid hosts

Myxobolus species	SBL	SBW	SBT	LPC	WPC	PC	NPF	Site of infection	Original host(s)	Locality
Myxobolus ophiocarae sp. n.	10.3 (9.3–11.4)	8.8 (7.9–9.5)	4.53 (4.1–5.2)	4.7 (3.9–5.4)	2.9 (2.2–3.3)	=	5–6	Gills	*Ophiocara porocephala*	Malaysia
Myxobolus acanthogobii Hoshina, 1952	9.8 (7.9–11.8)	8 (6.6–9.5)	6.1 (5.3–7.1)	3.5 (2.9–4.2)	2.2 (1.8–2.9)	=	NA	Brain, nervous system	*Acanthogobius flavimanus*	Japan
Myxobolus luciogobii Ishizaki, 1957	9.0 (7.7–10.7)	7.7 (6.9–9.2)	6.4 (5.0–7.9)	3.7 (3.0–4.6)	2.6 (2.3–2.9)	=	6	Urinary bladder	*Luciogobius guttatus guttatus*	Japan
Myxobolus gobii Naidenova in Gaevskaya et al., 1975	9.8–10.7	9.8–10	7	3–4.9	2.8–3.5	=	NA	Nares	*Gobius ophicephalus*	Russia
Myxobolus gylactiformae Wu, 1985	10.6 (9.1–11.1)	8.3 (7.2–8.9)	7.8(7–8.4)	3.9 (3.6–4.1)	3.5 (3.3–3.6)	=	NA	Urinary bladder	*Synechogobius ommaturus*	China
Myxobolus rhinogobii Chen in Chen and Ma (1998)	12.7 (12.0–13.8)	8.9 (7.4–8.4)	5.9 (5.5–6.0)	5.9 (4.8–6.0)	3 (2.6–3.4)	=	6–7	Gills, muscle	*Rhinogobius giurinus*	Off China
Myxobolus asianensis Chen in Chen and Ma (1998)	11 (10.8–12.0)	9.6 (9.4–10)	6.2 (6.0–6.6)	5 (4.6–5.4)	3.4 (3.2–3.6)	=	5–6	Gills, skin	*Rhinogobius giurinus*	Off China
Myxobolus tongyaensis Chen in Chen and Ma (1998)	11 (9.2–11.6)	7.2 (6.2–7.7)	6 (5.7–6.3)	5.8 (4.6–6.9)	2.6 (2.3–3.1)	≠	6–7	Kidneys	*Rhinogobius giurinus*	Off China
Myxobolus nagaraensis[a] (Yokoyama et al. 2007)	11.9 (10.5–13.5)	9 (8.0–10.0)	6.5 (6.0–7.0)	5.5 (4.5–6.0)	3 (2.5–4.0)	=	5	Visceral cavity	*Rhinogobius* sp.	Japan
Myxobolus albi Picon-Camacho et al. 2009	8.3 (8.3–10.0)	9.1 (7.7–10.0)	6.6 (5.8–7.4)	3.9 (3.0–4.8)	2.8 (2.0–3.3)	=	4–5	Gill arch cartilage	*Pomatoschistus microps*	Scotland
Myxobolus sp. (Velasco et al. 2012)	NA	NA	NA	NA	NA	NA	NA	Palate	*Gobioides broussonnetii*	Brazil
Myxobolus lentisuturalis[a] (Dyková et al. 2002)	11.8 (11.2–12.4)	7.6 (7.2–8.4)	5.2	4.2 (4.0–4.4)	2.5 (2.0–2.8)	=	4	Muscle tissue	*Carassius gibelio*	China
Myxobolus lentisuturalis[a] Dykova, Fiala et Nie in Caffara et al. (2009)	10.5 (9.0–11.0)	6.6 (6.0–7.0)	3.9 (3.0–5.0)	3.7 (3.0–4.5)	2.2 (2.0–3.0)	=	4–5	Dorsolateral humps	*Carassius auratus auratus*	Italy
Myxobolus cultus[a] (Yokoyama et al. 1995)	10.2 (9.2–11.3)	6.0 (5.2–7.2)	4.3 (3.6–4.6)	4.0 (3.1–4.9)	1.9 (1.5–2.1)	=	3–5	Cartilage	*Carassius auratus*	Japan

All measurements are given in micrometers

SBL spore body length, *SBW* spore body width, *SBT* spore body thickness, *LPC* length of polar capsule, *WPC* width of polar capsule, *PC* relative size of the polar capsules (equal, different, or equal and different), *NPF* number of coils of polar filaments, *NA* not reported in the reviewed literature

[a] *Myxobolus* spp. having close phylogenetic relatedness to *M. ophiocarae* sp. n

Morphometric comparison of the species with the closest genetic similarity to *M. ophiocarae* sp. n. showed considerable difference in the spore length and spore thickness of *M. nagaraensis* (Yokoyama et al. 2007) and *M. lentisuturalis* (Dyková et al. 2002). An apparent similarity of spore length was found, however, to *M. cultus* (Yokoyama et al. 1995) and *M. lentisuturalis* (Caffara et al. 2009), but there were differences in spore width and polar capsule length. Moreover, the locality of infection was also different when compared to other species with close genetic similarity (Table 2). The actual prevalence and distribution of this parasite across Malaysia and in other areas where the host is found are yet unknown. Further studies are needed to determine the current distribution, population status, and ecological interactions that this wide-ranging genus may have on native hosts of Malaysian estuaries.

Acknowledgments The research was supported by the Hungarian Scientific Research Fund (OTKA) grant K 100132 and a Malaysian Governmental Scholarship for MHB. The authors are grateful to the staff members of AKUATROP and the Marine Science Biodiversity Laboratory (Universiti Malaysia Terengganu) for their assistance during the investigation.

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