

Description of myxosporeans (Cnidaria: Myxozoa) infecting the popular food fish *Notopterus notopterus* (Pisces: Notopteridae) in Malaysia and India

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ABSTRACT

This study was a co-operative investigation of myxosporean infections of *Notopterus notopterus*, the bronze featherback, which is a popular food fish in the South Asian region. We examined fish from Lake Kenyir, Malaysia and the River Ganga, Hastinapur, Uttar Pradesh, India, and observed infections with two myxosporeans: *Myxidium* cf. *notopterum* (Myxidiidae) and *Henneguya ganapatiae* (Myxobolidae), respectively. These species were identified by myxospore morphology, morphometry and host tissue affinity, and the original descriptions supplemented with small-subunit ribosomal DNA sequences and phylogenetic analysis. Free myxospores of *M. cf. notopterum* were found in the gallbladder, and measured $14.7 \pm 0.6 \mu\text{m}$ long and $6.3 \pm 0.6 \mu\text{m}$ wide; host, tissue and myxospore dimensions overlapped with the type, but differed in morphological details (spore shape, valve cell ridges) and locality (Malaysia versus India). Plasmodia and spores of *H. ganapatiae* were observed in gills, and myxospores had a spore body $9.7 \pm 0.4 \mu\text{m}$ long, $4.5 \pm 0.5 \mu\text{m}$ wide; sample locality, host, tissue, spore morphology and morphometry matched the original description. Small-subunit ribosomal DNA sequences were deposited in GenBank (*M. cf. notopterum* MT365527, *H. ganapatiae* MT365528) and both differed by >7% from congeneric species. Although the pathogenicity and clinical manifestation of myxozoan in humans are poorly understood, consumption of raw fish meat with myxozoan infection was reported to be associated with diarrhea. Identification of current parasite fauna from *N. notopterus* is an essential first step in assessing pathogen risks to stocks of this important food fish.

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1. Introduction

The bronze featherback, *Notopterus notopterus* Pallas, 1769 (Osteoglossiformes) is a member of a group commonly called “knife fishes”, which are distributed widely in Africa, South and Southeast Asia (Talwar and Jhingran, 1991). Knife fish have commercial value for recreational anglers, and species of *Notopterus*, *Chitala*, *Papyrocranus* and *Xenomystus* have been categorized as a com-

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mercially important fish by the Food and Agriculture Organization of the United Nations for food and the ornamental trade (Casavas et al., 1996). *Notopterus notopterus* is a particularly important food fish, with high commercial value. For example, in Cambodia this species has had the highest export value of any fish (Mille et al., 2016). The fish is also valued as a decorative species in the aquarium trade (Mohanty and Samanta, 2016).

Wild populations of bronze featherback are declining, and it is regarded as a threatened species (CAMP, 1998). Natural populations are impacted by excessive harvesting, and pollution from industrial, domestic and agricultural sources, which has led to increased concentrations of heavy metals in fish tissue (Ngor et al., 2003; Shah et al., 2009; Mohanty and Samanta, 2016). Initial attempts have been made to culture the fish (Rahmatullah et al., 2009; Mohanty and Samanta, 2018). Identification of parasites that infect *N. notopterus* is a fundamental part of assessing disease risks to both wild and cultured stocks of this important food fish.

The parasite fauna of bronze featherback is poorly studied. The ciliophoran protozoa, *Trichodina monopteri* was described by Mitra and Haldar (2004). Two cystidicolid nematodes, *Pseudopropleptus notopteri* and *Spinitectus notopteri* were described by Karve and Naik (1951) and redescribed by Moravec et al. (2016). One isopod, *Alitropus typus* Edwards, 1840, was recorded from the bronze featherback by Ahmad et al. (2016) in the Lake Chasma, Pakistan. The myxosporean fauna of this fish is relatively better studied, as Sarkar (1996) described *Myxobolus meglitschus* from the gills and *Myxidium notopterum* from the liver. Three *Henneguya* species are known from India: Quadri (1965, 1970) described *H. notopterae* and *H. ganapatiae*, while Lalita Kumari (1969) described *H. singhi*. Myxozoa is one of the economically most important groups of microscopic metazoan parasites, as they infect fishes from both food and pet trades. Stocks of food fish species impacted by myxozoan infections include finfish in Mediterranean aquaculture, by *Enteromyxum* spp. (Palenzuela et al., 2002), catfish by *Henneguya ictaluri* (Pote et al., 2000), and salmonids by *Myxobolus cerebralis* and *Ceratonova shasta* (AFS-FHS, 2014). In addition to reducing food fish stocks, myxozoans can affect humans directly. Several species of *Kudoa* are known to degrade fillet quality after catch (Henning et al., 2012; Langdon, 1991) and another *Kudoa*, *K. septempunctata*, can cause diarrhea and emesis after consumption of raw infected olive flounder (Harada et al., 2012). The pathogenicity of *K. septempunctata* was demonstrated in an in vitro experiment on human intestinal cells, which were rapidly invaded by sporoplasms (Ohnishi et al., 2013). Other *Kudoa* species evoke allergic reactions in consumers (Martínez de Velasco et al., 2008). Importantly, as both diversity and pathogenic effects of myxozoans are still being revealed, surveillance and detection of novel species are important for assessing risks to and from food fish.

Myxidium is one of the largest myxozoan taxa (Class Myxosporea) with over 232 nominal species (Eiras et al., 2011). It is a polyphyletic genus of typically coelozoic (rarely histozoic) parasites, with most species described from the gall bladder, with some from the kidneys or urinary bladder. Histozoic *Myxidium* species are known from the gills and skin (Eiras et al., 2011; Heiniger and Adlard, 2014). Genus *Henneguya* Thélohan, 1892 has 189 species (Eiras, 2002; Eiras and Adriano, 2012) and is the second largest group within the Myxozoa. *Henneguya* spp. are common parasites of marine and fresh-water fish and are typically histozoic in different organs and tissues, particularly the gills, skin, kidney, musculoskeletal system or gastrointestinal tract (Kent et al., 2001; Eiras, 2002; Bahri and Marques, 2008). In India, Kalavati and Nandi (2007) report 24 *Henneguya* species, the majority of which are from West Bengal, with three species (*H. ganapatiae*, *H. notopterae*, *H. singhi*) described from *N. notopterus*. However, like most myxozoans, many *Henneguya* species have been described on the basis of morphological and morphometric characters only, and presently lack corresponding DNA sequence data, which makes accurate re-identification challenging. The importance of species from this genus as pathogens of freshwater fish has been described by several authors (Dyková and Lom, 1978; Kalavati and Narasimhamurti, 1985; Lom and Dyková, 1995; Martins and Souza, 1997; Martins et al., 1999). With several species causing economic impacts on fish farm activities (Feist and Longshaw, 2006).

In this present study, we describe myxozoans from *N. notopterus* from India and Malaysia, using morphology and small-subunit ribosomal DNA (ssrDNA) sequencing. We found a *Myxidium* species, *M. cf. notopterum*, from the gall bladder of fish from Lake Kenyir, Malaysia, and we re-described *Henneguya ganapatiae*, from gills of *N. notopterus* from River Ganga, Hastinapur, Uttar Pradesh, India.

2. Materials & methods

2.1. Collection and morphological examination of bronze featherback in Malaysia

Notopterus notopterus were collected with gill nets in the Tasik Kenyir Water Reservoir (4°48'33.45"N, 102°47'10.45"E) in May 2011. Fish (N = 13; length 21–24 cm; weight 1.0–1.5 kg) were transported live to the Institute of Tropical Aquaculture (AKUATROP), University Malaysia Terengganu (UMT), and maintained in an aerated aquarium. Within 2 days after capture, fish were pithed, dissected and examined for the presence of myxosporeans using a stereomicroscope and a compound microscope. Emphasis was placed on examining organs typically associated with myxozoan development: gills, fins, muscle, kidneys, gall bladder, and intestine. Bile was smeared on a slide, and examined wet by microscopy. When suspected myxospores were found, they were studied with an advanced light microscope (Nikon Eclipse 80i). Thirty fresh spores from a single host were measured and characterized according to the guidelines of Lom and Arthur (1989); with the exception that we use the more structurally accurate term “polar tubule” instead of “polar filament”. Spores were preserved in 80% ethanol for subsequent molecular analysis.

2.2. Collection and morphological examination of bronze featherback in India

Fish were purchased at a fish market, but purportedly were caught in the River Ganga, in Hastinapur (29°01' N, 77°45' E), Meerut, Uttar Pradesh, India in February 2018. Fish (N = 20; body length 20–25 cm) and kept on ice until necropsy. Each fish

was examined for myxozoan parasites with special attention to infections of the gill. Due to degradation of the gill tissue, location and structure of plasmodia could not be characterized in detail. Spores were collected from damaged plasmodia by scraping gill filaments, then preserved in 4% formalin for morphological studies, and in 90% ethanol for molecular analysis. Twenty two fixed spores from a single fish were measured and described for *Myxidium* species using Nomarski differential interference contrast and photographed. All measurements are expressed in micrometers (Tables 2 and 3).

2.3. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from spores preserved in ethanol. The spores were centrifuged at 9600 ×g for 10 min and the supernatant removed. For the *Myxidium* spores, total DNA was extracted from the spore pellet using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), while for *Henneguya*, total DNA was extracted using a Genaid Tissue Genomic DNA Mini Kit (New Taipei City, Taiwan), following the manufacturers' instructions, with a 100 µl final elution step. *ssrDNA* was amplified using a nested PCR described in detail by Cech et al. (2015). Universal eukaryotic primers ERIB1 and ERIB10 (Barta et al., 1997) were used in the first round PCR. Myxozoan specific primers Myx1F (Hallett and Diamant, 2001) and SphR (Eszterbauer and Székely, 2004) were used in the second round PCR. The primer sequences are listed in Table 1. Amplicons were analysed by electrophoresis in a 1% agarose gel. The PCR products were excised from the gel, purified with the Gel/PCR DNA Fragments Extraction Kit (Geneaid, New Taipei City, Taiwan) and sequenced directly using the BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies) with an ABI PRISM 3100 Genetic Analyser (Life Technologies), using the amplification and inner primers.

2.4. Phylogenetic analysis

The sequence fragments were assembled using MEGA 6 (Tamura et al., 2013) and ambiguous bases were clarified by visual examination of the corresponding ABI chromatograms. Sequences of the discovered myxozoans were aligned with reference sequences from the NCBI GenBank database (based on a BLAST similarity >88% and coverage >75% for *H. ganapatiae*, and similarity >89% and coverage >77% for *M. cf. notopteron*) with CLUSTAL W (Thompson et al., 1994). Final alignments of 24 sequences for *M. cf. notopteron* and 34 sequences for *H. ganapatiae* were tested using MEGA 6 for the nucleotide substitution model of best fit as indicated by the Akaike Information Criterion (AIC). Phylogenetic relationships for *M. cf. notopteron* and *H. ganapatiae* were inferred using the maximum likelihood (ML) method with the G + I and GTR + G + I substitution models respectively, and bootstrapped with 1000 replicates. Initial trees for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Nearest Neighbor Interchange (NNI) approach for *M. cf. notopteron* and Maximum Composite Likelihood (MCL) approach for *H. ganapatiae*. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories; +G, parameter = 0.3388). The rate variation model allowed for some sites to be evolutionarily invariable ([+I]; 0.0000% sites). All alignments with <75% site coverage were eliminated. *Chloromyxum cristatum* and *Ceratonova shasta* were chosen as the outgroups for *H. ganapatiae* and *M. cf. notopteron* analyses, respectively.

3. Results

3.1. *Myxidium cf. notopteron*

Bronze featherback (N = 13; 21–24 cm total length) were examined from the Tasik Kenyir water reservoir in Malaysia. Free-floating mature *Myxidium* spores were found in the gall bladders of 2 fish (15.4%). Although the myxospore morphometry most closely resembled *M. notopteron* Sarkar, 1996, from the same type host in India, they differed somewhat in morphology (described below). No *ssrDNA* sequence was available from the type species to compare with the novel sequence data we provide here.

Table 1
Primers used in PCRs and sequencing.

Primer name	Sequence (5'-3')	Used to	Application	Reference
ERIB1	A'CCTGGTTGATCCTGCA	Both	1st round PCR	Barta et al., 1997
ERIB10	CTTCCGCAGGTTACCTACCG	Both	1st round PCR	Barta et al., 1997
Myx1F	GTG AGA CTG CGG ACG GCT CAG	Both	2nd round PCR	Hallett and Diamant, 2001
SphR	GTT ACC ATT GTA GCG CGC GT	Both	2nd round PCR & sequencing for <i>Henneguya</i>	Eszterbauer and Székely, 2004
ACT1FR	TTG GGT AAT TTG CGC GCC TGC	Both	Sequencing	Hallett and Diamant, 2001
CR1 R	GAT YAG ATA CCG TCS TAGT	<i>Henneguya</i>	Sequencing	Székely et al., 2015
CR1 F	CGA AGA CGA TCA GAT ACC GTC CTA	<i>Henneguya</i>	Sequencing	Székely et al., 2015
ACT3F	CAT GGA ACG AAC AAT	<i>Henneguya</i>	Sequencing	Hallett and Diamant, 2001
Myxgen4R	ACC TGT TAT TGC CAC GCT	<i>Myxidium</i>	Sequencing	Kent et al., 2000
Myxgen3F	GGA CTA ACR AAT GCG AAG GCA	<i>Myxidium</i>	Sequencing	Kent et al., 2000
MTseg2F	GCA AGA GGT GAA ATT CTT G	<i>Myxidium</i>	Sequencing	Kent et al., 2000
MB5r	ACC GCT CCT GTT AAT CAT CAC C	<i>Myxidium</i>	Sequencing	Eszterbauer, 2004
MC5	CCTGAGAAACGGCTACCACAT	<i>Henneguya</i>	Sequencing	Molnár et al., 2002

Description of spores: In valvular view spores fusiform with acuminate tips, in sutural view sigmoid, with twist on longitudinal axis giving reniform appearance. Longitudinal suture line thin (Fig. 1A, B, C). Spore valve with 8–10 longitudinal surface ridges. Length 14.7 ± 0.6 (13.8–16.0 μm), width 6.3 ± 0.6 (5.5–7.7 μm). Polar capsules pyriform, at either end of spore, having equal dimensions: length 5.7 ± 0.5 (4.6–6.4 μm), width 4.7 ± 0.4 (3.6–5.3 μm) (Table 2). Polar tubules with 3–4 coils perpendicular to the long axis of each polar capsule (Fig. 1A, B, C).

Type host: Bronze featherback, local name “belida”, *Notopterus notopterus* (Pallas) (Notopteridae).

Site of infection: Gall bladder

Prevalence of infection: 15.4% (2/13)

Reference materials: Digitized photos of syntype spores were deposited in the collection of Fish Pathology and Parasitology Group, Centre for Agricultural Research, Institute for Veterinary Medical Research, Budapest, Hungary.

Molecular data: Small subunit rDNA sequence data of 1667 bp from a single host fish, has been deposited in NCBI GenBank (accession no. MT365527). Sequences with highest similarity were *Myxosporea* gen. sp. PBS-2015 (93.0%; KP030767) and *Myxidium cuneiforme* (90.6%, DQ377709).

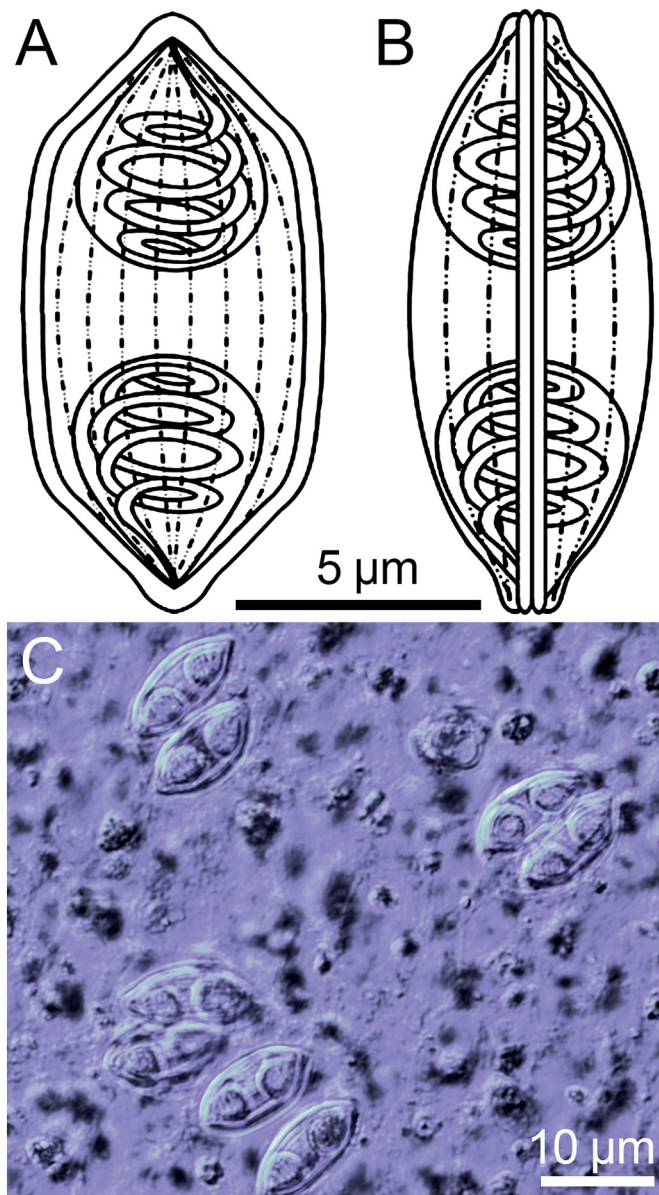


Fig. 1. Myxospores of *Myxidium* cf. *notopterus*. A–B: Line drawings of mature myxospores in frontal and valvular view showing polar capsules with coiled polar tubules and the longitudinal grooves. C: Fresh, unstained myxospores in frontal view showing the two pyriform polar capsules.

Table 2

Comparison of hosts, infection site, myxospore dimensions and localities of *Myxidium cf. notopterus* and other closely related congeners. All measurements are in micrometer (μm).

Species	Host	Infection site	Spore body		Polar capsule		No. of polar tubule coils	Locality	Reference
			Length	Width	Length	Width			
<i>Myxidium cf. notopterus</i>	<i>Notopterus notopterus</i>	Gall bladder	13.8–16.0 (14.7 ± 0.6)	5.5–7.7 (6.3 ± 0.6)	4.6–6.4 (5.7 ± 0.5)	3.6–5.3 (4.7 ± 0.4)	3–5	Malaysia	Present study
<i>M. incompaverni</i>	<i>Diplectanocotyla gracilis</i>	Parenchymal tissue	11.3–11.8 (11.6)	4.2–5.6 (4.9)	2.4–3.3 (2.9)	1.8–2.0 (1.9)	2–3	Malaysia	Freeman and Shinn, 2011
<i>M. notopterus</i>	<i>Notopterus notopterus</i>	Liver	13.5–16.0 (15.37)	7.0–9.0 (8.3)	4.5–6.0 (5.65)	4.5–5.5 (5.05)	3–4	India	Eiras et al., 2011
<i>M. cuneiforme</i>	<i>Cyprinus carpio</i>	Gall Bladder	12.0–13.1 (12.5 ± 0.3)	4.8–6.1 (5.4 ± 0.3)	4.0–4.6 (4.3 ± 0.2)	2.6–3.4 (3.1 ± 0.2)	5–6	China	Li et al., 2016
<i>M. amazonense</i>	<i>Corydoras melini</i>	Gall bladder	16.1–17.9 (17.0 ± 0.9)	3.0–4.4 (3.7 ± 0.7)	4.9–5.9 (5.4 ± 0.5)	2.8–4.0 (3.4 ± 0.6)	4–5	Brazil	Mathews et al., 2015
<i>M. scripta</i>	<i>Trachemys scripta elegans</i>	Gall bladder	16.6–20.4 (18.8)	4.6–5.9 (5.1)	5.1–7.8 (6.6)	2.6–4.1 (3.8)	6–8	USA	Roberts et al., 2008
<i>M. truttae</i>	<i>Salmo trutta fario</i>	Gall bladder	11–12	7.0–7.3	3.7 diameter		NA	France	Eiras et al., 2011

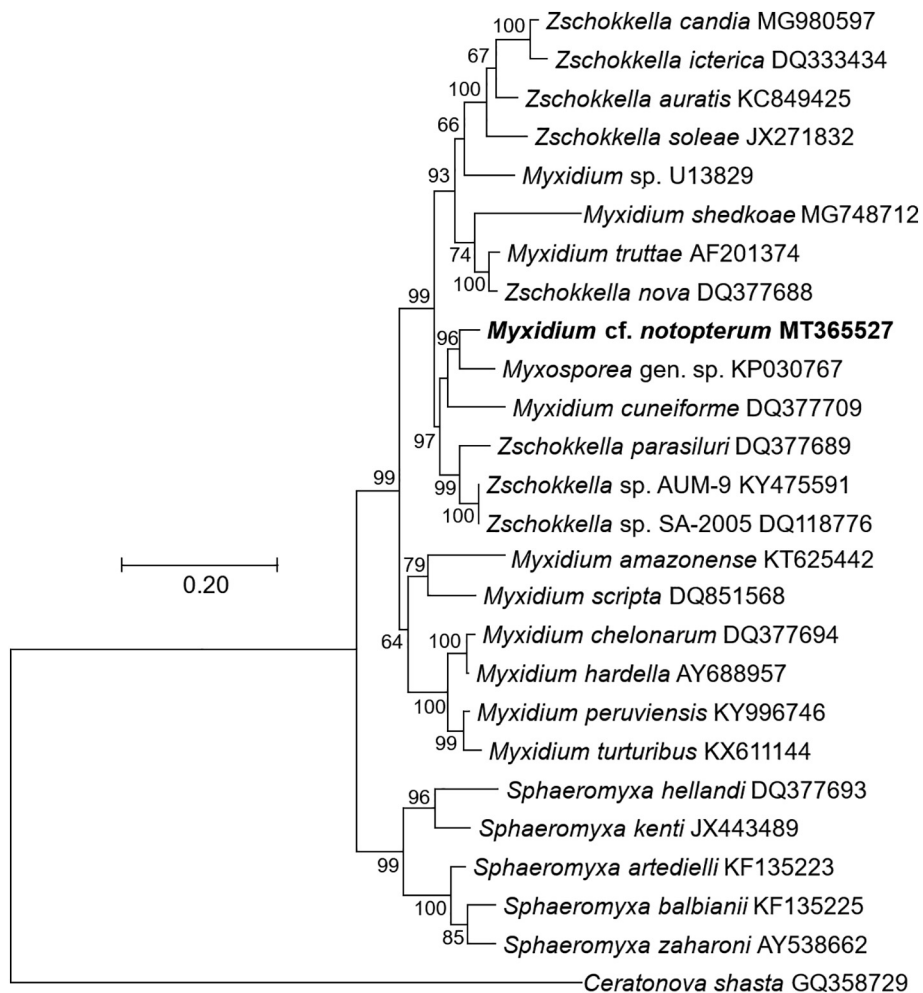


Fig. 2. Phylogenetic tree generated by maximum likelihood analysis of *ssrDNA* sequences of *Myxidium cf. notopterus*, and other closely-related myxosporean species; GenBank accession numbers shown after the species name. Novel data are in bold. Numbers at nodes indicate the bootstrap confidence values >50 (ML). *Ceratonova shasta* was used as an outgroup.

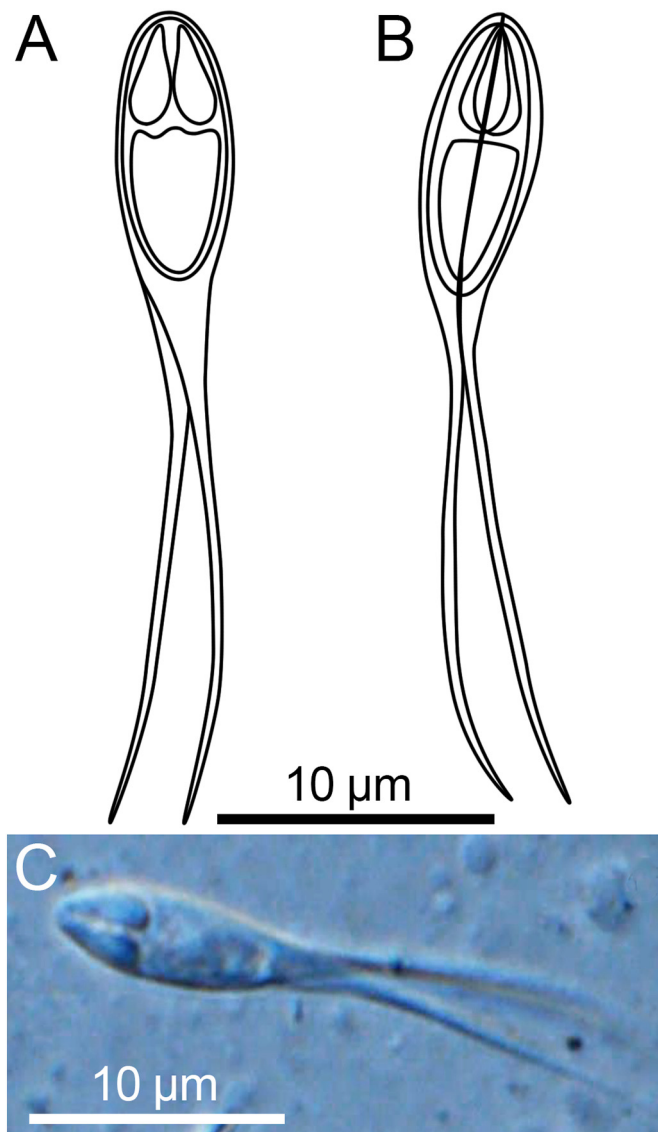


Fig. 3. Myxospores of *Henneguya ganapatiae*. A–B: Line drawings of mature myxospores in frontal and valvular view showing polar capsules and caudal processes. C: Fresh, unstained myxospore in frontal view showing the two pyriform polar capsules and caudal processes.

Phylogenetic analysis: Maximum likelihood (ML) analysis (Fig. 2), with 26 species in the ingroup and *Ceratonova shasta* (GQ358729) as an outgroup, showed seven clades of Myxidiidae (*Myxidium* and *Zschokkella*), and Sphaeromyxidae (*Sphaeromyxa*), all of which are coelozoic species infecting the gall bladder or the bile duct of their fish hosts. Specifically, *M. cf. notopterum* clustered with *Myxidium* species that parasitize the gall bladder of fresh water fish hosts.

Remarks: Myxospores of *M. cf. notopterum* were morphometrically similar to *Myxidium notopterum* (Sarkar, 1996) described from the same fish in West Bengal, India. Spores of our species have a slightly sigmoidal shape, whereas *M. notopterum* spores are cylindrical with rounded ends. Polar capsules of *M. cf. notopterum* are more elongated than those of *M. notopterum*. Polar tubules have 3–4 turns in *M. notopterum* compared with 4–5 for *M. cf. notopterum* (Table 2). No ridges are reported on the spore surface of *M. notopterum*, whereas *M. cf. notopterum* has 8–10 striations.

3.2. Redescription of *Henneguya ganapatiae* Quadri, 1970

Bronze featherback (N = 20; 20–25 cm total length) were collected from River Ganga, Hastinapur, Uttar Pradesh, India. Mature myxospores of *H. ganapatiae* were observed in gills of 15/20 (75%) fish.

Table 3

Comparison of hosts, infection site, myxospore dimensions and localities of *Henneguya ganapatiae* and other closely related *Henneguya* congeners. All measurements are in micrometer (μm).

Species	Host	Infection site	Spore body		Polar capsule		Locality	Reference
			Length	Width	Length	Width		
<i>Henneguya ganapatiae</i>	<i>Notopterus notopterus</i>	Gills	9.7 ± 0.4 (9.3–10.0)	4.5 ± 0.5 (4.0–4.8)	3.3 ± 0.2 (3.2–2.6)	1.6 ± 0.1 (1.4–1.8)	Uttar Pradesh, India	Present study
<i>H. ganapatiae</i>	<i>Notopterus notopterus</i>	Gill contents	9.9–10.0	4.0–4.4	3.21–2.6	1.4–1.8	Andra Pradesh, India	Quadri, 1970
<i>H. bicaudi</i>	<i>Cirrihinus mrigala</i>	Gill filaments	21.0	6.2	3.1	2.0	Harike wetlands, Punjab, India	Kaur and Attri, 2015
<i>H. ophiocephali</i>	<i>Ophiocephalus punctatus</i>	Gills and muscles	41.5–52.5	6.2–7.2	6.2	9.3	West Bengal, India	Chakravarty, 1939
<i>H. chudharyi</i>	<i>Channa punctatus</i>	Gill lamellae	10.5–13.2 (11.6 \pm 1.1)	3.6–4.2 (3.8 \pm 0.2)	5.5–7.2 (6.5 \pm 0.6)	1.0–1.3 (1.1 \pm 0.1)	Uttar Pradesh, India	Bajpai and Haldar, 1982
<i>H. voronini</i>	<i>Lates calcarifer</i>	Base of the gill filament	9.9 \pm 0.3 (9.5–10.3)	5.9 \pm 0.3 (5.8–6.0)	3.7 \pm 0.2 (3.5–4.0)	2.1 \pm 0.1 (2.0–2.2)	Malaysia	Borkhanuddin et al., 2020
<i>H. setiuensis</i>	<i>Lates calcarifer</i>	Within gill lamellae	8.9 \pm 0.4 (8.3–9.5)	5.9 \pm 0.3 (5.8–6.0)	3.3 \pm 0.2 (3.1–3.5)	2.1 \pm 0.1 (2.0–2.2)	Malaysia	Borkhanuddin et al., 2020
<i>H. calcarifer</i>	<i>Lates calcarifer</i>	Skeletal muscle	9.9 \pm 0.3 (9.5–10.3)	5.9 \pm 0.3 (5.8–6.0)	3.7 \pm 0.2 (3.5–4.0)	2.1 \pm 0.1 (2.0–2.2)	Malaysia	Borkhanuddin et al., 2020
<i>H. bicornuata</i>	<i>Ophiocephalus punctatus</i>	Branchial epithelium	26.4–29.1	2.8–3.8	3.3–3.6	1.1–1.4	West Bengal, India	Ray-Chaudhuri and Chakravarty, 1970
<i>H. zahoori</i>	<i>Ophiocephalus punctatus</i>	Gill filaments	20.0–30.0	2.1–3.0	4.9–6.7	0.7–1.1	Uttar Pradesh, India	Bhatt and Siddiqui, 1964

Description of spores: Spore body ellipsoidal in both frontal and sutural views, with two slightly curved caudal processes. Spore valves have thin walls, surface smooth, without ridges (Fig. 3A, B, C). Spore body length 9.7 ± 0.4 (9.3–10.0 μm), width 4.5 ± 0.5 (4.0–4.8 μm). Two polar capsules, pyriform, approximately equal size, length 3.3 ± 0.2 (2.6–3.2 μm) and width 1.6 ± 0.1 (1.4–1.8 μm). Polar tubules not observed. Length of caudal processes 23.7 ± 1.4 (22.0–25.0 μm) (Table 3).

Type host: Bronze featherback, local name “patra”, *Notopterus notopterus* (Pallas, 1769) (Notopteridae).

Site of infection: Gill filaments

Reference materials: Digitized photos of syntype spores retained in the collection of Fish Pathology and Parasitology Group, Centre for Agricultural Research, Institute for Veterinary Medical Research, Budapest, Hungary.

Prevalence: 75% (15/20)

Molecular data: Sequence data of the *ssrDNA* of *H. ganapatiae* (1660 bp) from a single host fish, was deposited in NCBI GenBank (accession number MT365528). Pairwise comparisons revealed that the most similar myxozoans were *H. chudharyi* (89.4%; from spotted snakehead fish *Channa punctata*; KT279402), and *H. setiuensis* (90.7%; MH743111), *H. calcariferi* (90.8%; MH743109) and *H. voronini* (90.4%; MH743110) described from barramundi *Lates calcarifer* from Malaysia.

Phylogenetic analysis: *Henneguya ganapatiae* clustered with other *Henneguya* species that parasitize fresh and brackish water fish hosts (Fig. 4).

Remarks: The parasite species observed in bronze featherback had morphology and measurements that correspond to *Henneguya ganapatiae* Quadri (1970).

4. Discussion

The parasite fauna of fishes belonging to Notopteridae is poorly studied, with data available only for the bronze featherback, *N. notopterus*. Our cooperative work on finding myxosporean infections in this host from Malaysia and India resulted in detecting two species. We used morphology, morphometry and *ssrDNA* sequencing, to identify these taxa as *M. cf. notopterum* and *H. ganapatiae*, respectively.

Myxidium notopterum Sarkar, 1996 was described from the liver of *N. notopterus* from India. We identified morphometrically very similar myxospores from the gall bladder of the same host, from Malaysia. We regard the tissue difference as minor, as spores in the gall bladder probably originated in the liver, and no developmental stages were observed in the gall bladder itself. Morphological features differed from the type species (overall sigmoid versus cylindrical shape, more elongate polar capsules, more tubule turns, and valve cell ridges), however no molecular data are available from the type species for comparison. Thus we regard the currently available data as insufficient for identifying *M. notopterum*, or describing a new species. We recommend that the parasite be collected and re-described from the original biotope in India, and a molecular comparison made. As for the other congeners, morphometry, host and geographic origin differentiate *M. cf. notopterum* from *M. cuneiforme*, *M. amazonense*, *M. scripta*, and *M. truttae*. Nevertheless, some measurements of spore features overlap among species, especially polar capsule dimensions and the count of polar tubule turns (Eiras et al., 2011; Li et al., 2016; Mathews et al., 2015; Roberts et al., 2008). This is

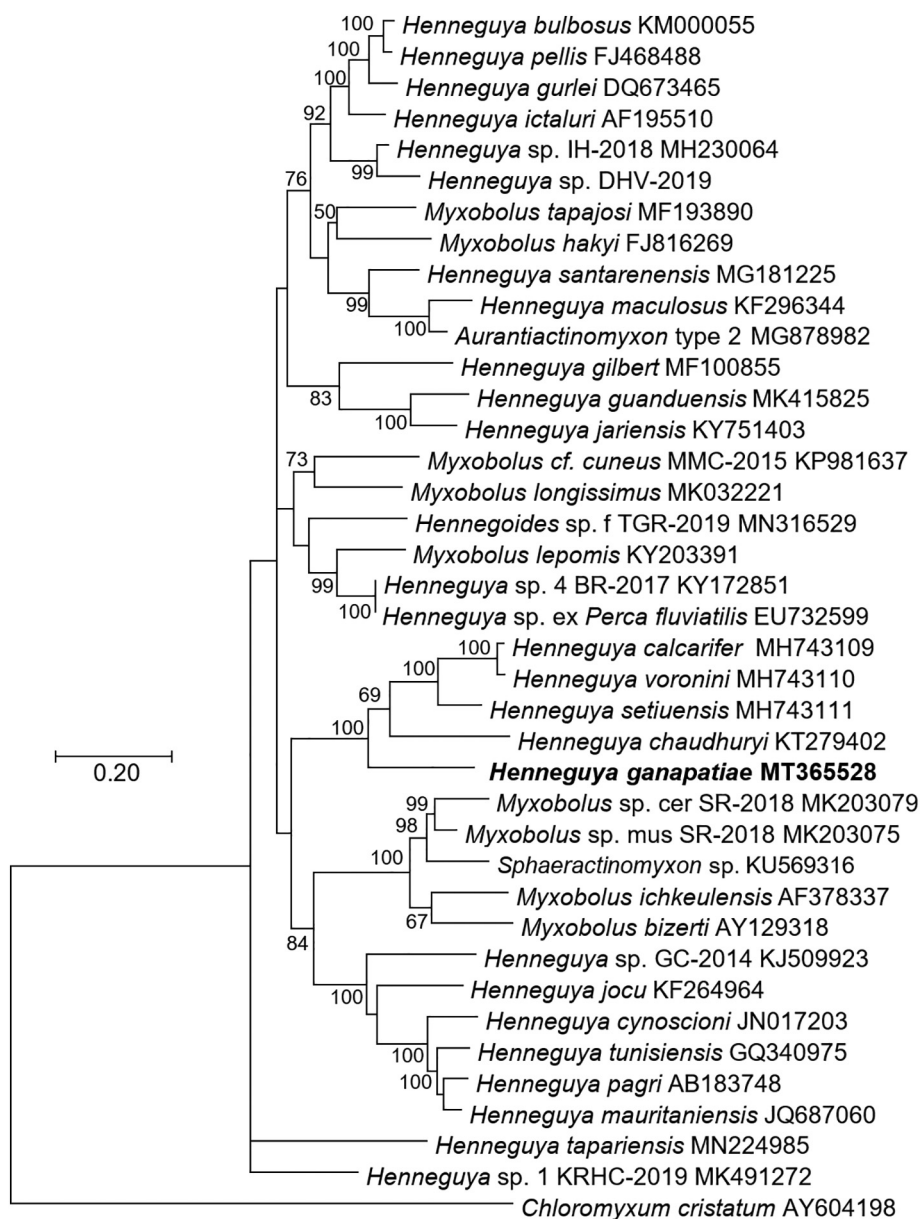


Fig. 4. Phylogenetic tree generated by maximum likelihood analysis of *ssrDNA* sequences of *Henneguya ganapatiae* and other closely-related myxosporean species; GenBank accession numbers shown after the species name. Novel data are in bold. Numbers at nodes indicate the bootstrap confidence values >50 (ML). *Chloromyxum cristatum* was used as an outgroup.

the first report of *Myxidium* species from a Malaysian freshwater fish, and only one other congener is known from this region, the marine *Myxidium incomptaverni*, which is a hyperparasite of *Diplectanocotyla gracilis* (Monogenea) (Freeman and Shinn, 2011).

Generally, myxospore morphology is a lesser important correlate in phylogenetic relationships, with myxozoans from vertebrate hosts yielding the strongest evolutionary signals, followed by aquatic hosts and tissue tropism (Carriero et al., 2013; Rocha et al., 2018; Kent et al., 2001; Eszterbauer, 2004; Holzer et al., 2004; Fiala, 2006). Due to their paraphyletic and polyphyletic nature, similar morphological features, and closely-related genetic identity, suggestions have been made to merge the genera *Myxidium* and *Zschokella* (Fiala, 2006; Li et al., 2016). Phylogenetic analysis from this study produced results that are congruent with previous observations of polyphyly of these two genera, with *M. cf. notopterus* in a sister clade to several *Zschokella* species. The phylogeny supported tissue tropism as an evolutionary signal for *M. cf. notopterus*, as this species clustered with other gall bladder/bile duct infecting myxosporeans. We found only weak correlation of vertebrate host with myxozoan species in this group, as multiple host taxa/groups (e.g. Siluriformes; Cypriniformes) were represented by myxozoans that clustered with *M. cf. notopterus* (host = Osteoglossiformes). Although we have added sequence data from the first *Myxidium* from an

osteoglossiform, further clarity of phylogenetic relationships should be gleaned from future sequencing of related myxozoans, particularly from South Asian fishes.

Henneguya spp. are important disease agents in both wild and farmed food fish, with a considerable increase in the number of novel *Henneguya* species identified recently (Eiras and Adriano, 2012). *Henneguya* is within the family Myxobolidae, and is distinguished on the basis of myxospores having two caudal processes. Five myxobolid myxozoans are known from the host, *Notopterus* spp.: three *Henneguya* spp. (*H. ganapatiae*, *H. notoapterae*, *H. singhi*) and two *Myxobolus* (*M. meglitschus* and *M. notoapterum*). As we only encountered one of the *Henneguya* spp. recorded from gills of the bronze featherback, we were unable to test the validity of the other two species with either a redescription of morphology, or addition of molecular data. We confirmed that the species we found, *H. ganapatiae*, shows morphometric differences to *H. notoapterae* and *H. singhi* in polar capsules and spore body. However, as with the *Myxidium* species we found, no sequence data were available from *Henneguya* spp. from *N. notoapterus* in India, for comparison. From available data, our phylogenetic analysis showed that *H. ganapatiae* was most similar (90.7%) to three *Henneguya* species from gill filaments, gill lamellae and muscles, respectively, of *Lates calcarifer* from Malaysia, and *Henneguya chaudharyi* (89.4%) from gill filaments of *Channa punctatus* from India.

Due to difficulties of obtaining live infected *N. notoapterus*, we were unable to prepare histological sections and thus could not study developmental stages or the specific site preference of *H. ganapatiae* on the gills; both of which are important non-molecular characters for species descriptions. We identified the parasite found as *H. ganapatiae* Quadri, 1970 on the basis of host, geographic locality, tissue tropism and similarity of myxospore morphology and morphometry. We supplement the type description with molecular data and analysis of the phylogenetic position of this species. Additional myxozoan surveys from South Asian fishes are needed to improve both taxonomy and broader context of known and novel myxozoan parasites from this region. Identification and surveillance for species that parasitize *N. notoapterus* is important for assessing risks to both wild and cultured stocks of this important food fish species.

Declaration of competing interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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