**Henneguya** (Cnidaria: Myxosporea: Myxobolidae) infections of cultured barramundi, *Lates calcarifer* (Perciformes: Latidae) in an estuarine wetlands system of Malaysia: description of *Henneguya setiuensis* n. sp., *Henneguya voronini* n. sp. and *Henneguya calcarifer* n. sp.

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Abstract

Examination of 35 barramundi (*Lates calcarifer*) from aquaculture cages in Setiu Wetland, Malaysia, revealed a single fish infected with three *Henneguya* spp. (Cnidaria: Myxosporea). Characterization of the infections using tissue tropism, myxospore morphology and morphometry and 18S rDNA sequencing supported description of three new species: *Henneguya setiuensis* n. sp., *Henneguya voronini* n. sp. and *H. calcarifer* n. sp. Myxospores of all three species had typical *Henneguya* morphology, with two polar capsules in the plane of the suture, an oval spore body, smooth valve cell surfaces, and two caudal appendages. Spores were morphometrically similar, and many dimensions overlapped, but *H. voronini* n. sp. had shorter caudal appendages compared with *H. calcarifer* n. sp. and *H. setiuensis* n. sp. Gross tissue tropism distinguished the muscle parasite *H. calcarifer* n. sp. from gill parasites *H. setiuensis* n. sp. and *H. voronini* n. sp.; and these latter two species were further separable by fine-scale location of developing plasmodia, which were intralamellar for *H. setiuensis* n. sp. and basal to the filaments for *H. voronini* n. sp. Small subunit ribosomal DNA sequences distinguished all three species: the two gill species *H. setiuensis* n. sp. and *H. voronini* n. sp. were only 88% similar (over 1708 bp), whereas the muscle species *H. calcarifer* n. sp. was most similar to *H. voronini* n. sp. (98% over 1696 bp). None of the three novel species was more than 90% similar to any known myxosporean sequence in GenBank. Low infection prevalence of these myxosporeans and lack of obvious tissue pathology from developing plasmodia suggested none of these parasites are currently a problem for barramundi culture in Setiu Wetland; however additional surveys of fish, particularly at different times of the year, would be informative for better risk assessment.

Keywords Cnidaria · *Henneguya* · Setiu Wetlands · Barramundi · South China Sea

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Introduction

Myxosporeans (Cnidaria: Myxosporea) are common, diverse parasites of marine and freshwater fishes (Lom and Dyková 2006). The genus Henneguya Thélohan, 1892, in family Myxobolidae, is the third most speciose myxosporean genus after Myxidium and Myxobolus, with > 200 Henneguya species described (Eiras 2002; Eiras and Adriano 2012; Székely et al. 2018). While most Henneguya species do not cause overt disease in their hosts, a few are economically important pathogens. Infections can cause mortalities in fish populations if parasites replicate to high intensities on the gills and cause respiratory insufficiency, especially in juvenile fish (Haaparanta et al. 1994; Whitaker et al. 2005). For example, H. ictaluri Pote, Hansan et Shivaji, 2000, causes proliferative gill disease in cultured channel catfish (Ictalurus punctatus Rafinesque) with up to 50% mortality (Bowser and Conroy 1985; Bosworth et al. 2003), and H. lateolabracis Yokoyama, Kawakami, Yasuda et Tanaka, 2003, is the causative agent of fatal cardiac henneguyosis in cultured Japanese seabass (Lateolabrax spp.) (Pote et al. 2000; Yokoyama et al. 2003).

Henneguya infection in the muscle can reduce the quality and marketability of the fish (Patashnik and Groninger 1964; Boyce et al. 1985), while other Henneguya species, for example, H. testicularis and H. oviperda, which are commonly found in the gonads of farmed fish, may affect reproductive health (Sitjá-Bobadilla 2009; Sokolov et al. 2019). Henneguya prevalence of infection can reach 75–100%, with an intensity of up to 8,000 cysts in one fish (Nie 1996; Fomena and Bouri 1996). Whether intense or not, exposure of the fish host to other stressors can exacerbate disease effects of the parasite, for example, under stressful or aggregated conditions, mortality of 95–100% has been reported in farm-reared channel catfish (Bowser and Conroy 1985; Minchew 1972).

Previous studies have demonstrated that Henneguya spp. are the most common myxosporean parasites in non-cyprinid fishes in Southeast Asia (Molnár et al. 2006a, 2006b; Székely et al. 2009a, 2009b). From Malaysia, the few known Henneguya species are from freshwater host species and/or habitats (Shariff 1982; Molnár et al. 2006a, 2006b; Székely et al. 2009a, 2009b). Six species are described from non-cyprinid fishes in Malaysia: H. sharifi Molnár, Székely, Mohamed and Shaharom-Harrison, 2006, in Pangasianodon hypophthalmus Sauvage (Pangasidae: Siluriformes); H. mystusia Sarkar, 1985, in Hemibagrus nemurus Valenciennes (Bagridae: Siluriformes); H. basifilamentalis Molnár, Székely, Mohamed and Shaharom-Harrison, 2006, in H. nemurus; H. shaharini Shariff 1992, in Oxyleotris marmorata (Bleeker) (Eleotridae: Perciformes); and H. hemibagri Tchang et Ma, 1993, in H. nemurus, all from the municipality of Kuala Terengganu, and H. daoudi Székely, Shaharom-Harrison, Mohamed and Molnár, 2009 in Trichopodus trichopterus (Pallas) (Osphronemidae: Perciformes), from Machang municipality. This research on the biodiversity of Henneguya spp. in freshwater habitats contrasts sharply with the paucity of information on these parasites from marine or estuarine fishes (Eiras 2002; Dyková et al. 2011).

Fishes of the genus Lates include species that are economically important such as Lates calcarifer (Barramundi) or Lates niloticus (Nile perch), which have been cultured globally as popular food fish (Tantikitti et al. 2005; Biswas et al. 2010; Aloo et al. 2017). Barramundi is cultured primarily in sea cages near river mouths or estuaries and harvested throughout the year in the Indo-pacific region (Boonyaratpalin 1997; Tantikitti et al. 2005; Biswas et al. 2010). Despite their importance, very little myxosporean research has been conducted on these fishes. Only one Henneguya species is known from barramundi, H. latesi Tripathi 1952, from India, together with several other myxosporeans: Myxidium calcariferi Chakravarty, 1943, Myxidium procerum var. calcariferi Chakravarty, 1943, and Myxobolus calcariferum Basu and Haldar, 2003 (Tripathi 1952; Eiras et al. 2005; Eiras et al. 2011).

Henneguya has never been documented formally in cultured barramundi in Setiu Wetland, Terengganu, Malaysia, despite recurring observations (2009–2012) of spores in gills, but never from the muscle (Shaharom-Harrison pers. comm.). These observations prompted us to formally identify the species and determine if the parasite/s were causing any pathology in the barramundi, which are cultured at high densities with other important fish species like groupers (Epinephelus sp.). We conducted a limited survey of myxosporean parasites in cultured barramundi and identified three new Henneguya species: H. setiuensis n. sp. and H. voronini n. sp. from different locations in the gills and H. calcarifer n. sp. from the skeletal muscle.

Materials and methods

Host and parasite samples

Cultured barramundi, Lates calcarifer (n = 35; length 15–30 cm), were sampled from estuarine net cages from Setiu Wetland, Malaysia (5°40’53.30”N, 102°42’43.93”E). Specimens were transported live to the Institute of Tropical Aquaculture (AKUATROP), University Malaysia Terengganu (UMT), in March 2013, and maintained in an aerated fish tank. Within 5 days of collection, fish were sedated using clove oil and killed by a cervical cut, according to institutional animal treatment protocols. Fish were necropsied and surveyed for myxosporean parasites with a Leica EZ4 stereomicroscope and a Leica DM750 compound microscope. We examined the body surface, oral cavity, gills, heart, gill bladder, kidney and skeletal muscle for overt myxosporean
pseudocysts (plasmodia). When suspected myxosporean plasmodia were found, they were excised using fine forceps and then ruptured in a drop of phosphate-buffered saline (PBS) and the contents examined in wet mount preparations. Parasites were examined and photographed using a Nikon Model Eclipse 80i advanced light microscope. Spores were checked for the presence of an iodonophilous vacuole after adding a drop of Lugol’s iodine solution. At least 2 dimensions (length, width or thickness) were measured from 30 or more spores from each plasmodium, 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 from individual plasmodia were preserved in 80% ethanol for subsequent molecular analysis. For histological examination, tissue samples from infected organs containing mature plasmodia were fixed in Bouin’s solution, embedded in paraffin wax, sectioned at 5–6 μm and stained with haematoxylin and eosin.

**Molecular and phylogenetic analyses**

At the IVMR CAR HAS, Hungary, DNA was extracted from the ethanol fixed spores using a DNeasy tissue kit (animal tissue protocol; Qiagen, Germany) according to the manufacturer’s instructions. Two rounds of PCR were performed: round 1 with universal eukaryotic small subunit ribosomal DNA (18S rDNA) primers ERIB1 and ERIB10 and fully nested round 2 with primers Myx1F and SphR (Table 1). First-round amplifications were performed in 25 μl reactions that comprised 2 μl extracted DNA (~ 0.2 μg), 0.2 mM of each dNTP (MBI Fermentas), 0.5 μM each of the forward and reverse primers, 2.5 μl 10× Taq buffer (MBI Fermentas), 2 U Taq polymerase (MBI Fermentas) and 15 μl of water. Thermal cycling comprised initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 50 s, 50 °C for 50 s and 72 °C for 1 min 40 s, with terminal extension of 72 °C for 10 min, and then rest at 4 °C. All PCRs were performed in a PTC-200 thermocycler (MJ Research).

Amplified DNA was purified with EZ-10 Spin Column PCR Purification Kit (Bio Basic Inc., USA) and then sequenced in both directions using an ABI BigDye Terminator v3.1 Cycle Sequencing Kit with an ABI 3100 Genetic Analyser (IVMR, HAS). Initial short (~ 800 bp) sequences inspired development of new *Henneguya*-specific sequencing primers HMF1, HMF2, HMR1 and HMR2 (Table 1).

BLAST searches were conducted to determine affinities to other myxosporeans in NCBI GenBank, and we selected the 33 18S rDNA sequences that had above 80% similarity to our three novel *Henneguya* species. These sequences were aligned using Clustal W (Thompson et al. 1994). Alignments were corrected manually in MEGA X (Kumar et al. 2018). The dataset was tested using MEGA X for the nucleotide substitution model of best fit as indicated by the Akaike Information Criterion (AIC). Phylogenetic relationships were inferred using the Maximum Likelihood (ML) method with the GTR + G + I substitution model and bootstrapping with 1000 replicates. *Chloromyxum cyprini* (AY604198) was chosen as the outgroup, as this represents one of the most primitive lineages of the myxosporean group that includes *Henneguya* spp. Genetic distances were determined using the p-distance model matrix in MEGA X.

**Table 1** Primers used for PCR and sequencing

| Primer   | Sequence     | Application     | Reference          |
|----------|--------------|-----------------|--------------------|
| ERIB1    | 5′-ACCTGGTTGATCCTGCGGAGC-3′ | 1st round PCR   | Barta (1997)       |
| ERIB10   | 5′-CTTCCGAGGTCCCTACGG-3′  | 1st round PCR   | Barta (1997)       |
| Myx1F    | 5′-GGT AGA CTT CGG AGC GCT CAG-3′ | 2nd round PCR   | Hallett and Diamant (2001) |
| SphR     | 5′-GTT ACC ATT GTA GCG CGG GT-3′  | 2nd round PCR and sequencing | Eszterbauer and Szekely (2004) |
| MC5      | 5′-CCT GAG AAA CGG CTA CCA CAT CCA-3′ | Sequencing | Molnár et al. (2002) |
| MC3      | 5′-GAT TAG CCT GAC AGA TCA CTC CTC A-3′ | Sequencing | Molnár et al. (2002) |
| HMF1     | 5′-GAT CTG GTG AGT AGT GGT GCA T-3′  | Sequencing | Present study       |
| HMF2     | 5′-GAG TGG TCC TAC GAT CGG GAT-3′   | Sequencing | Present study       |
| HMR1     | 5′-GGC CAT CCT AGC CAA GTA CAC A-3′  | Sequencing | Present study       |
| HMR2     | 5′-GCA AGC TCG AAC CAA AGC GAT-3′   | Sequencing | Present study       |
Scanning electron microscopic (SEM)

Plasmodia of Henneguya voronini n. sp. were compressed and ruptured onto 25 × 40 mm glass slides, fixed in 2.5% glutaraldehyde, then washed several times in 0.1 M sodium cacodylate buffer and postfixed in cold 1% (w/v) osmium tetroxide. Fixed tissue was then dehydrated in an alcohol series of 35% to 100% for 10- to 15-min each step, and then critical point dried in carbon dioxide (Baltic CPD 030). The glass slide containing the dried specimens was cut into smaller pieces, mounted onto aluminium stubs with double-sided tape and then sputter-coated with gold (Jeol JFC 1600). The specimens were examined in a Jeol SEM (Model JSM 6360LA).

Results

Thirty-five barramundi were examined, and myxosporean infections were found in only a single fish. Plasmodia were found in two different organs, the gills and the muscle. Plasmodia in the gills occurred in two well-differentiated locations: spherical plasmodia 50–70 μm in diameter developed intra-lamellarly, whereas ellipsoidal plasmodia 250–300 × 130–150 μm were located basi-filamentally between gill filaments. A third type of plasmodium, which was ovoid and 300–400 μm long, was found within the skeletal muscle. All three types of plasmodia contained mature myxospores, which were morphologically similar, with compact, ellipsoidal spore bodies of typical Henneguya morphology, with two polar capsules in the plane of the suture, smooth surfaced valve cells, with caudal appendages that extended about four times longer than the spore body. Morphometrics (Table 2) overlapped among myxospores from the three different tissue locations. Our diagnosis of three different species was based on morphological comparisons with existing records, specific tissue site of development and 18S rDNA sequence data (see below for taxonomic summaries).

Henneguya setiuensis n. sp.

Type host: barramundi, Lates calcarifer (Bloch 1790).

Site of infection: Within gill lamellae.

Locality: Setiu Wetlands, Terengganu, Malaysia.

Prevalence of infection: 2.8% (1/35).

Vouchers: Digital images of syntype spores and histological sections deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, collection no. HNHM-71892. The 18S rDNA sequence was deposited in GenBank under accession number MH743111.

Etymology: The species is named after the collection location, Setiu Wetlands, Malaysia.

Description of spores: Spores symmetric, with two equal caudal appendages, fusiform sporebody, two equal-sized pyriform polar capsules (Fig. 1). Spore wall thin (0.3–0.4 μm), smooth and composed of two equal valves. Apical end of spore body blunt, caudal end tapered and extends into the caudal appendages. Fresh myxospores 36–41 μm long, spore length 8.9 ± 0.4 (8.3–9.5) μm, width 5.9 ± 0.3 (5.8–6.0) μm and thickness 4.1 ± 0.1 (4.1–4.2) μm. Two polar capsules, pyriform, blunt at the posterior end and taper anteriorly, length 3.3 ± 0.2 (3.1–3.5) μm and width 2.1 ± 0.1 (2.0–2.2) μm. Polar tubules coiled in 6 turns perpendicular to the long axis of the polar capsules. Sporoplasm binucleate with a small iodinophilous vacuole. Caudal appendages straight, tapering, 30.5 ± 1.6 (28.0–32.0) μm. Plasmodia spherical, diameter 50–75 μm.

Remarks: We identified H. setiuensis n. sp. as a new species based on host, site of development (intra-lamellar in gills), morphology and morphometrics. The spores were most similar morphometrically and by tissue tropism (gill) to a previously described species H. latesi Tripathi 1952, from barramundi in India; however, the description of this earlier species has limited informative characters for comparison of spore measurements, and no 18S rDNA sequence data (Table 2). From the limited data available for comparison, H. setiuensis n. sp. was distinct in spore body shape (rounder vs oval), total length (36–41 μm vs 26–36 μm) and spore thickness (4.1 μm vs 5.4 μm). Determination of whether H. latesi represents a taxon distinct from any that we observed in Malaysian barramundi would require rediscovery of parasite material from the type locality in India, with molecular and fine-scale tissue tropism observations. Compared with Henneguya spp. known from Lates species hosts, H. setiuensis n. sp. was unique in most dimensions (Table 2) and had the smallest plasmodia; however molecular data and specific tissue site of infection are not available for these other species for more precise comparison. H. setiuensis n. sp. was morphometrically similar to the other two species found in the same fish in the current study, except spore width, polar capsule width and plasmodium size, but distinguishable based on fine-scale tissue tropism and DNA sequence.

Molecular analysis: Our consensus 18S rDNA sequence of 1708 bp was shown by BLAST search to be most similar to other Henneguya species in GenBank, but all < 89%. Pairwise comparison showed H. setiuensis n. sp. to be no more than 91% similar to the other Henneguya species found in the same fish: 89.9%, p-distance 0.101 with H. calcarifer n. sp.; 90.1%, p-distance 0.099 with H. voronini n. sp.

Histology: Plasmodia observed in different parts of the gill filaments, inside the multilayered epithelium between lamellae (Fig. 4a–b). Only local damage of the lamellae was observed, with no gross pathology of the gill filaments.
Comparison of hosts, localities, tissues and myxospore dimensions of *Henneguya* species described from *Lates* sp. SL: spore length, SW: spore width, AL: caudal appendage length, ST: spore thickness, PCL: polar capsule length, PCW: polar capsule width, NC: number of coils of unfired polar tubule. All measurements are in μm.

| Species          | Host Locality References | Shape          | PLasmoid dimension | Site of infection | References |
|------------------|--------------------------|----------------|---------------------|-------------------|------------|
| *H. mandouri*    | Ellipsoidal Gill filaments | Elliptical or oval Gill filaments | Egypt             | Rabie et al. 2009 |
| *H. massii*      | Gill filaments           | Spherical      | Gill filaments      | L. niloticus      | Chad et al. 2001 |
| *H. ghaffari*    | Gill filaments, intestinal muscles | Spindle shaped in the gill round to oval in the intestine | L. niloticus      | Ali et al. 1999  |
| *H. mbakouensis* | Gill filaments, oral. Gill filaments | Spherical Gill filaments | Gill filaments      | L. niloticus      | Bouin 2000  |
| *H. setiuensis*  | Gill filaments, oral. Gill filaments | Spherical Gill filaments | Gill filaments      | L. niloticus      | Present study |
| *H. voronini*    | Gill filaments, oral. Gill filaments | Spherical Gill filaments | Gill filaments      | L. niloticus      | Present study |
| *H. calcarifer*  | Gill filaments, oral. Gill filaments | Spherical Gill filaments | Gill filaments      | L. niloticus      | Present study |

* oval in frontal view with rounded anterior end
** oval in frontal view with rounded anterior and posterior ends

Type host: *barramundi, Lates calcarifer* (Bloch 1790).

Type locality: Base of the gill filament.

Type material: Digital images of syntype spores and histological sections were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, collection no. HNHM 71893. The 18S rDNA sequence was deposited in GenBank under accession number MH73110.

**Etymology:** The species is named in honour of V. A. Voronin, an eminent Russian fish parasitologist

**Description:** Symmetrical, with two equal caudal appendages and equal-sized polar capsules (Fig. 2). Spore wall 0.3–0.4 μm thick and composed of 2–equal valves. A small pyramid shaped, blunt at the posterior end and tapered to a smooth, rounded apex (Fig. 2). Spore wall thickness: 2.2 ± 0.1 μm. Total length 35.9 ± 10.4 μm. Length 5.9 ± 0.3 (5.8–6.0) μm. Thickness could not be measured as no spores were observed in sutural plane. Polar capsules pear shaped, blunt at the posterior end and tapered to a smooth, rounded apex. Total length 3.7 ± 2.1 (2.9–4.0) μm. Length of polar tubules 27.2 ± 1.4 (25.9–29.0) μm. About 4 times as long as the spore body length. Pinopodia length 5.9 ± 0.3 (5.8–6.0) μm. Polar tubules peeled in 6 turns perpendicular to the long axis of the polar capsule. Sporopyle breaches the long axis of the capsule. Caput appendages with a small, thin, non-continuous vacuole. Caudal appendages in two equal valves. Intra and extracellular developmental stages were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum.
moved into the gill filaments and was covered by a multilayered epithelium as it matured.

Microscopy: SEM revealed that the valve cell surfaces were smooth, which is a morphological feature typical of genus *Henneguya*.

**Henneguya calcarifer** n. sp.

Type host: barramundi, *Lates calcarifer* (Bloch 1790).

Site of infection: Skeletal muscle.

Type locality: Setiu Wetlands, Terengganu, Malaysia.

Prevalence of infection: 2.8% (1/35).

Type material: Digital images of syntype spores were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, collection no. HNHM-71894. The 18S rDNA sequence was deposited in GenBank under accession number MH743109.

Etymology: The species is named after the host.

Description of spores: Fig. 3. Myxospores symmetric, with two equal caudal appendages, and equal-sized polar capsules. Spore wall 0.3–0.4 μm, smooth and composed of two equal valves. Apical end of spore body blunt, the caudal end tapers and extends into the caudal appendages, total length 34–45 μm. Spore length 9.4 ± 0.6 (8.3–10.0) μm, width 5.2 ± 0.3 (4.8–5.5) μm and thickness 3.8 ± 0.1 (3.7–4.0) μm. Two polar capsules pear shaped, blunt at the posterior end and taper anteriorly, length 3.4 ± 0.2 (3.1–3.7) μm and width 1.4 ± 0.2 (1.1–1.7) μm. Polar tubules coiled in 6 turns perpendicular to the long axis of the capsule. Sporoplasm binucleate with a small iodinophilous vacuole. Caudal appendages straight, tapering, length 30.9 ± 3.0 (28.0–35.0) μm, ~ 4 times longer than the spore body. Plasmodia spherical 300 × 400 μm.

Remarks: *H. calcarifer* n. sp. resembles morphologically and morphometrically both *H. setiuensis* n. sp. and *H. voronini* n. sp. (Table 2) but has different tissue site of development (muscles not gill). This is the first muscle-infecting *Henneguya* described from *Lates calcarifer* and is relatively distinct from the only other
species from a *Lates* congener: *H. ghaffari* from muscle and gills of *L. niloticus* in Egypt (Table 2).

Molecular analysis: 1696 bp 18S rDNA were sequenced. A BLAST search indicated that the most similar species were other *Henneguya* species, but all < 89%. Pairwise analysis showed *H. calcarifer* n. sp. was molecularly very similar (97.7% over 1696 bp) with *H. voronini* n. sp., described from the same fish (above).

Histology: Low intensity of parasite plasmodia in the host skeletal muscle meant no plasmodia were visible in histological sections.

**Phylogenetic analysis**

Phylogenetic analyses of 43 myxosporean sequences revealed a topology that showed correlation of myxosporeans
clustering with host order, with *Henneguya* spp. that infect Perciformes clustering into three distinct clades. The three species described herein cluster together within a subclade that includes another species that infect Perciformes (Fig. 5).

**Discussion**

*Henneguya* is histozoic myxosporean parasite of freshwater and marine fishes and one of the most species-rich genus of myxosporeans with more than 200 species described (Lom and Dyková 2006; Eiras 2002; Eiras and Adriano 2012). We used characters of specific host, tissue, myxospore morphology and 18S rDNA sequences to describe three *Henneguya* species in barramundi, *Lates calcarifer*, from the east coast of Peninsula Malaysia. *Henneguya setiuensis* n. sp. and *H. voronini* n. sp. developed between gill lamellae and at the base of the gill filaments, respectively, while *H. calcarifer* n. sp. developed in skeletal muscle from the flank of the fish. Discovery of the *Henneguya* infections in gills was not unexpected, as this organ is a common site of myxobolid infections; however, *H. calcarifer* n. sp. is the first myxosporean with a preference for muscle in a species of *Lates*. Muscle tropism of *Henneguya* species is less common than gills, being a character of 8 of some 180 species, including only 1 from a host in the Asian region: *H. ophicephali* Chakravarty, 1939, in *Ophiocephalus punctatus* from India (Eiras 2002; Eiras and Adriano 2012).

*Henneguya* has the distinctive morphological character of two caudal appendages, which distinguishes it from the other genera of the family Myxobolidae. However, molecular (SSU rDNA) evidence shows that genus *Henneguya* is polyphyletic, and this does not support the hypothesis that the presence of caudal appendages is a valid character for distinguishing species of *Henneguya* from *Myxobolus* (see comprehensive analysis in Liu et al. 2019). While we acknowledge the weak support for *Henneguya* as a distinct genus, formal taxonomic revision of genera within Myxobolidae is still lacking and is outside the scope of the present study. Accordingly we described the three novel species herein as *Henneguya*, based on the historical morphological character-based definition, augmented with DNA data.
Lower than prevalences of other Henneguya species. This was a positive for all three. We observed infection in only 1/35 fish (2.8%), but this individual was positive for all three Henneguya species. This was lower than prevalences of other Henneguya infections in Lates spp.: H. mandouri 32/40, 80%; H. ghaffari 65/188, 34.6%; H. latesi 6/20, 30%; and H. massii 3/67 (4.4%) (Tripathi 1952; Ali 1999; Kostoingue et al. 2001; Rabie et al. 2009). In addition to low prevalence, the single fish that we observed with the infections did not have any observable pathology associated with the parasites. This is consistent with the Henneguya infections from congeneric hosts, with none of these reported to cause henneguyosis (Tripathi 1952; Ali 1999; Kostoingue et al. 2001; Rabie et al. 2009).

Correlation between particular myxosporean species and tissue tropism in the vertebrate host is well-established (e.g. Molnár 1994), particularly in species of Myxobolidae, with fine-scale tissue preferences being observed in the gills (Molnár 2002; Eszterbauer 2004). Alternatively, many myxobolids and myxosporean species in general tend to cluster according to the family/order of the fish host, with this character being useful to distinguish genetically closely related species (e.g. Ferguson et al. 2008; Carriero et al. 2013; Vieira et al. 2017; Naldoni et al. 2018; Holzer et al. 2018; Liu et al. 2019). In the present study, our phylogenetic analysis showed that the three novel Henneguya species formed a well-supported sub-clade of perciform-infecting myxobolids, which included Henneguya sp. 3 PBS-2015 (KP030761), from gills of Lates niloticus from Lake Turkana, Kenya, and Henneguya daoudi (EU643625), from gills of another perciform fish, Trichogaster trichopterus from Malaysia (Fig. 5). The nearest sister clade included Myxobolus neurophilus (KF588564) and Henneguya chaudhuyi (KT279402), both of which infect perciform fish: Perca flavescens (yellow perch) and Channa punctata (spotted snakehead), respectively. The analysis thus revealed a stronger correlation between all three novel Henneguya spp. and their host, rather than the specific tissue in which they developed a pattern consistent across multiple groups of Myxobolidae as demonstrated previously (Liu et al. 2019).

Our taxonomic diagnosis incorporated consideration of whether either gill-infecting Henneguya species had already been described. Specifically, we considered their similarity with the only Henneguya known from gills (and mouth) of L. calcarifer: H. latesi Tripathi 1952, in barramundi from India. The description of that parasite is limited to host, tissue and morphometry (no DNA, no micrographs; Tripathi 1952). The taxon shares host and one of the two reported tissues (gills) with H. setiuensis n. sp. and H. voronini n. sp. but differs in most myxospore dimensions. We concluded that there was insufficient evidence to unambiguously identify either of our Malaysian gill-infecting species as H. latesi. Furthermore, given that we observed two genetically distinct Henneguya species within the gills, it is possible that the original description of H. latesi for “gills and mouth” could actually represent two distinct species. Determination of whether H. latesi represents a single taxon, distinct from any that we observed in Malaysian barramundi, would require redescription H. latesi from the type locality in India, with molecular and fine-scale tissue tropism observations.

**Fig. 4** Histological sections of gill filaments from Lates calcarifer infected by plasmodia of H. setiuensis n. sp. and H. voronini n. sp. (a) Plasmodia of H. setiuensis n. sp. (arrows) producing compression and damage to the lamellae (*). (b) Development of plasmodia of H. voronini n. sp. (arrows) in the sub-epithelial layer at the base of the filament. Note that the plasmodia also impinge into the gill arch (g).

Our PCR approach for genetically characterizing the parasites was based on previous work to amplify DNA from another Malaysian Henneguya species, H. daoudi, in which its 18S rDNA was sequenced using “freshwater” myxosporean primers SphF-SphR, MC3-MC5 and MB5r-MB5f (Székely et al. 2009a). Although we were able to amplify ~ 800 bp fragments using SpHR, MC3 and MC5, these did not overlap sufficiently to construct contigs. Accordingly, we used our sequence information from H. calcarifer n. sp. to design new barramundi-Henneguya-specific primer pairs HMF-HMF2 and HMR1-HMR2, to give sufficient coverage to assemble contigs of 1696–1708 bp, including terminal primers MYX1f and SpHR.

Henneguya species can cause disease and mortality in farmed fish species, for example, H. exilis in catfish (Current and Janovy 1977) and H. piaractus in pacu (Martins et al. 1997). Our study of parasites of L. calcarifer was to assess the baseline occurrence of myxosporeans and any associated pathology in this important aquaculture species in Malaysia. We observed infection in only 1/35 fish (2.8%), but this individual was positive for all three Henneguya species. This was lower than prevalences of other Henneguya infections in Lates spp.: H. mandouri 32/40, 80%; H. ghaffari 65/188, 34.6%; H. latesi 6/20, 30%; and H. massii 3/67 (4.4%) (Tripathi 1952; Ali 1999; Kostoingue et al. 2001; Rabie et al. 2009). In addition to low prevalence, the single fish that we observed with the infections did not have any observable pathology associated with the parasites. This is consistent with the Henneguya infections from congeneric hosts, with none of these reported to cause henneguyosis (Tripathi 1952; Ali 1999; Kostoingue et al. 2001; Rabie et al. 2009).

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Based on our limited sampling, we demonstrated the presence of at least three *Henneguya* species that parasitize *barramundi* in Malaysia. In our target population, these parasites occurred only at low prevalence and intensity, and without overt pathology. The health effects of any of these *Henneguya* species either singly or combined are unknown in populations of fish under conditions of stress or exposed to other health impacts from intense aquaculture, where *Henneguya*-related disease could emerge. Future work should concentrate on surveys from additional cultured fishes (both *barramundi* and grouper) from this region, under different stress levels and rearing conditions, to determine what other myxosporean species might be present and might be causing disease impacts.
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References

Ali MA (1999) Henneguya ghaffari sp. n. (Myxozoa: Myxosporea), infecting the Nile perch Lates niloticus (Teleostei: Centropomidae). Dis Aquat Org 28:225–230
Alou PA, Njiru J, Balirwa JS, Nyamweya CS (2017) Impacts of Nile Perch, Lates niloticus, introduction on the ecology, economy and conservation of Lake Victoria, East Africa. Lakes Reserv Manag 22(4):320–333
Barta JR (1997) Investigating phylogenetic relationships within the Apicomplexa using sequence data: the search for homology. Methods 13(2):81–88
Biswas G, Thirunavukkarasu AR, Sundaray JK, Kailasam M (2010) Optimization of feeding frequency of Asian seabass (Lates calcarifer) fry reared in net cages under brackish water environment. Aquaculture 305(1–4):26–31
Boonyaratpalin M (1997) Nutrient requirements of marine food fish cultured in Southeast Asia. Aquaculture 151(1–4):283–313
 Bosworth BG, Wise DJ, Terhune JS, Wolters WR (2003) Family and genetic group effects for resistance to proliferative gill disease in channel catfish: an ultrastructural study. Protistol 13:157–167
Dyková I, de Buron I, Roumillat WA, Fiala I (2011) Henneguya cynoscioni sp. n. (Myxozoa: Bivalvulida), an agent of severe cardiac lesions in the spotted searrot, Cynoscion nebulosus (Teleostei: Sciaenidae). Folia Parasitol 58(3):169–177
Eiras JC (2002) Synopsis of the species of the genus Henneguya Thélohan, 1892 Myxozoa: Myxosporea: Myxobolidae. Syst Parasitol 52(1):43–54
Eiras JC, Adriano EA (2012) A checklist of new species of Henneguya Thélohan, 1892 (Myxozoa: Myxosporea, Myxobolidae) described between 2002 and 2012. Syst Parasitol 83:95–104
Eiras JC, Molnár K, Lu YS (2005) Synopsis of the species of Myxobolus Büttschli, 1882 (Myxozoa: Myxosporea: Myxobolidae). Syst Parasitol 61:1–46
Eiras JC, Saraiva A, Cruz CF, Santos MJ, Fiala I (2011) Synopsis of the species of Myxidium Büttschli, 1882 (Myxozoa: Myxosporea: Bivalvulida). Syst Parasitol 80:81–116
Eszterbauer E (2004) Genetic relationship among gill-infecting Myxobolus species (Myxosporea) of cyprinids: molecular evidence of importance of tissue-specificity. Dis Aquat Org 58:35–40
Eszterbauer E, Székely C (2004) Molecular phylogeny of the kidney-parasitic Sphaerospora renicola from common carp (Cyprinus carpio) and Sphaerospora sp. from goldfish (Carassius auratus auratus). Acta Vet Hung 52(4):469–478
Ferguson JA, Atkinson SD, Whipp CM, Kent ML (2008) Molecular and morphological analysis of Myxobolus spp. of salmonid fishes with the description of a new Myxobolus species. J Parasitol 94:1322–1334
Fomena A, Bouix G (1996) New species of Henneguya Thélohan, 1892 (Myxozoa: Myxosporea) parasites of freshwater fishes in Cameroon. J Afr Zool 110:413–423
Fomena A, Bouix G (2000) Henneguya mbakaouensis sp. nov., Myxobolus nouensis sp. nov. and M. hydrocyoni Kostoiugue & Toguebaye, 1994, Myxosporea (Myxozoa) parasites of Centropomidae, Cichlidae and Characidae (Teleosts) of the Sanaga Basin in Cameroon (Central Africa). Parasite 7:209–214
Haaparanta A, Valtonen ET, Hoffman RW (1994) Pathogenicity and seasonal occurrence of Henneguya creplini (Protozoa, Myxosporea) on the gills of perch Perca fluviatilis in central Finland. Dis Aquat Org 20:15–22
Hallett SL, Diamant A (2001) Ultrastructure and small-subunit ribosomal DNA sequence of Henneguya lesteri n. sp. (Myxozoa), a parasite of sand whiting Sillago analis (Sillaginidae) from the coast of Queensland, Australia. Dis Aquat Org 46(3):197–212
Holzer AS, Bartošová-Sojková P, Born-Torrijos A, Lövy A, Hartigan A, Fiala I (2018) The joint evolution of the Myxozoa and their alternate hosts: A cnidian recipe for success and vast biodiversity. Mol Ecol 27:1651–1666
Kostoiugue B, Diebakate C, Faye N, Toguebaye BS (2001) Presence of myxosporidia (Myxozoa: Myxosporea) of the genus Henneguya Thélohan, 1892 in freshwater fishes from Chad (Central Africa). Acta Protozool 40:117–123
Kumar S, Stecher G, Li M, Kayz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549
Liu Y, Lövy A, Gu Z, Fiala I (2019) Phylogeny of Myxobolus (Myxozoa) and the evolution of myxospore appendages in Myxobolus clade. Int J Parasitol 49:523–530
Lom J, Arthur JR (1989) A guide for the preparation of species descriptions in Myxosporea. J Fish Dis 12:151–156
Lom J, Dyková I (2006) Myxozoan genera: a definition and notes on taxonomy, life-cycle terminology and pathogenic species. Folia Parasitol 53:1–36
Martins ML, Souza VN, Moraes FR, Moraes JRE, Costa AJ, Rocha UF (1997) Phylogeny and behavioral effects associated with Henneguya sp. (Myxozoa: Myxobolidae) infections of captive pacu Piaractus mesopotamicus in Brazil. J World Aquacult Soc 28:297–300
Minchew CD (1972) Identification and frequency of occurrence of four forms of Henneguya found in channel catfish. Proceedings of the 26th Annual Conference of the Southeastern Fish and Game Commissions
Molnár K (1994) Comments on the host, organ and tissue specificity of fish myxosporeans and on the types of their intrapiscine development. Parasitol Hung 27:5–20
Molnár K (2002) Site preference of fish myxosporeans in the gill. Dis Aquat Organ 48:197–207
Molnár K, Eszterbauer E, Székely C, Dán Á, Harrach B (2002) Morphological and molecular biological studies on intramuscular Myxobolus spp. of cyprinid fish. J Fish Dis 25(11):643–652
Molnár K, Székely C, Mohamed K, Shaharom-Harrison F (2006a) Myxozoan pathogens in cultured Malaysian fishes. I. Myxozoan infection of the sutchi catfish. Dis Aquat Organ 68:209–218
Molnár K, Székely C, Mohamed K, Shaharom-Harrison F (2006b) Myxozoan pathogens in cultured Malaysian fishes. II. Myxozoan infections of redtail catfish Hemibagrus nemurus in freshwater cage cultures. Dis Aquat Organ 68:219–226
Naldoni J, Maia AAM, Correa LL, Silva MRM, Adriano EA (2018) Two new myxosporean species parasite of Phractocephalus hemioliopterus from the Brazilian Amazon: morphology, ultrastructure and SSU-rDNA sequencing. Dis Aquat Organ 128:37–49
Nie P (1996) Co-occurrence and microhabitat of Ancyrocephalus mogurndae (Monogenea) and Henneguya weishanensis (Myxosporea) on gills of the mandarin fish, Siniperca chuatsi. Folia Parasitol 43:272–276
Pote LM, Hanson LA, Shivaji R (2000) Small subunit ribosomal RNA sequences link the cause of proliferative gill disease in channel catfish to Henneguya n. sp.. (Myxozoa: Myxosporea). J Aquat Anim Health 12(3):230–240
Rabie SA, Mohammed NL, Hussein AN, Hussein NM (2009) The infection of freshwater fishes with three species of Henneguya in Qena. Upper Egypt Egypt Acad J Biol Sci 1:11–19
Shariff M (1982) Henneguya shaharini sp. nov. (Protozoa: Myxozoa), a parasite of marble goby, Oxyeleotris marmoratus (Bleeker). J Fish Dis 5:37–45
Sitjá-Bobadilla A (2009) Can myxosporean parasites compromise fish and amphibian reproduction? P Roy Soc B-Biol Sci 276:2861–2870
Sokolov SG, Lebedeva DI, Murzina SA, Parshukov AN, Bystrova KA, Ieshko EP (2019) Morphology and phylogeny of Henneguya oviperda infecting oocytes of Esox lucius, with description of parasite-induced histopathology. Dis Aquat Org 133(2):91–98
Székely C, Shaharom-Harrison F, Cech G, Mohamed K, Molnár K (2009a) Myxozoan pathogens of Malaysian fishes cultured in ponds and net-cages. Dis Aquat Organ 83(1):49–57
Székely C, Shaharom-Harrison F, Cech G, Ostoros G, Molnár K (2009b) Myxozoan pathogens of fishes of the Tasik Kenyir Water Reservoir, Terengganu, Malaysia. Dis Aquat Organ 83(1):37–48
Székely C, Borzák R, Molnár K (2018) Description of Henneguya jaczoi sp. n. (Myxosporea, Myxobolidae) from Perca fluviatilis (L.) (Pisces, Percidae) with some remarks on the systematics of Henneguya spp. of European fishes. Acta Vet Hung 66(3):426–443
Tantikitti C, Sangpong W, Chiavareesaja S (2005) Effects of defatted soybean protein levels on growth performance and nitrogen and phosphorus excretion in Asian seabass (Lates calcarifer). Aquaculture 248(1–4):41–50
Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680
Tripathi YR (1952) Studies on parasites of Indian fishes I. Protozoa: Myxosporidia together with a check list of parasitic protozoa described from Indian fishes. Rec. Indian Museum 50:63–88
Vieira DHMD, Alama-Bermejo G, Bartholomew JL, Abdallah VD, Azevedo RK (2017) Morphological and molecular description of Myxobolus batalhensis n. sp. (Myxozoza, Myxosporea), a liver and ovary parasite of Salminus hilarii in Brazil. Parasitol Res 116:3303–3313
Whitaker JW, Pote LM, Hanson LA (2005) Assay to detect the actinospore and myxospore stages of proliferative gill disease in oligochaetes and pond water. N Am J Aquac 67:133–137
Yokoyama H, Kawakami H, Yasuda H, Tanaka S (2003) Henneguya lateolabracis (sp. n. Myxozoa, Myxosporea), the causative agent of cardiac henneguyosis in Chinese sea bass Lateolabrax sp. Fish Sci 69(6):1116–1120

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