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ORIGINAL PAPER

Myxobolus turpisrotundus (Myxosporea: Bivalvulida) spores with caudal appendages: investigating the validity of the genus *Henneguya* with morphological and molecular evidence

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Abstract Spores of the myxozoan parasite *Myxobolus turpisrotundus* Zhang 2009 were observed for the first time bearing caudal appendages. Most spores had the typical *Myxobolus* spp. morphology, but approximately 10% of spores possessed a spore body that was slightly elongated with a short tail projecting from the spore valve. In other spores, the tail was much more clearly visible and elongate. The spore body of these unusual spores is consistent in morphology and dimension to the normal spores of *M. turpisrotundus*. Both spore types were found within individual cysts, and the small subunit ribosomal RNA (ssrRNA) gene sequence from parasite cysts of this type was nearly identical to the previously published sequence of *M. turpisrotundus* from allogynogenetic gibel carp *Carassius auratus gibelio* (Bloch). The phenomenon of *Myxobolus* spores with caudal appendages provides additional evidence that the use of this character to separate *Myxobolus* and *Henneguya* into distinct genera is not reflective of an evolutionarily accurate classification scheme. Phylogenetic analysis of ssrDNA sequence from *Myxobolus* and *Henneguya* species showed clustering of species in some locations of the tree, but ultimately these genera are intermixed. The use of a single character to delineate species in the two most species-rich myxozoan genera has been consistently challenged where DNA analyses are used. The present finding of a single species bearing both *Myxobolus*-type and *Henneguya*-type spores emphasizes the inadequacy of this classification scheme, and highlights the need for careful consideration of these variable characteristics when describing myxozoan species.

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Introduction

Myxosporean parasites have a significant role as pathogens of freshwater and marine fishes in wild and cultured stocks with over 2,180 described species (Lom and Dyková 2006; Cuadrado et al. 2007; Al Quraishy et al. 2008). Most species are identified on the basis of the myxospore (Lom and Arthur 1989; Eiras et al. 2009; Molnár et al. 2009). However, with the use of these classical zoological methods, it is very difficult to distinguish morphologically similar myxosporean species that may infect identical tissues and that develop in hosts species that are closely related taxonomically (Molnár et al. 2002, 2009). Fortunately,

molecular biological methods have become increasingly applied to parasitological studies and the taxonomic classification of myxosporeans has been expanded to incorporate phylogenetic analyses. The most revealing aspect of analyses based on small subunit ribosomal DNA (ssrDNA) sequence is the incongruence of phlylogenetic trees with classification based on spore morphology alone (Smothers et al. 1994; Andree et al. 1999; Kent et al. 2001; Bahri et al. 2003; Yokoyama et al. 2005; Fiala 2006; Bartošova et al. 2009).

An example of the incongruity between molecules and morphology is as follows. According to Lom and Dyková (1992), the genera *Myxobolus* and *Henneguya* are separate groups, distinguished by the presence of a caudal appendage found on *Henneguya* spores, but absent on *Myxobolus* spores. However, the ssrDNA sequence data does not support a phylogenetic separation of these two genera (Smothers et al. 1994; Andree et al. 1999; Kent et al. 2001; Fiala 2006; Bartošova et al. 2009). Specifically, the genus *Myxobolus* is not monophyletic (Andree et al. 1999) and likewise, *Henneguya* is polyphyletic (Kent et al. 2001). Andree et al. (1999) and Kent et al. (2001) suggested caudal appendages of *Henneguya* might be not a valid character to separate these species-rich groups of myxobolids into two separate genera. Yet with incomplete genetic data on the approximately 1,000+ species in these two genera, it is difficult to know what taxonomic revision is required to reflect the evolutionary history of these species.

During routine parasitological examination of allogynogenetic gibel carp *Carassius auratus gibelio* (Bloch), we observed the spores of *Myxobolus turpisrotundus* intermixed with atypical spores bearing caudal appendages making them appear as *Henneguya* species spores. The potential plasticity of this character further highlights the challenging differentiation of these two genera into a classification scheme that is not arbitrary. The objective of the present study was to investigate the phenomenon of *Myxobolus* spores with caudal appendages and the relationship of genera *Myxobolus* and *Henneguya* based on ssrDNA sequence data.

Materials and methods

Collection of myxospores

Ten specimens of allogynogenetic gibel carp weighting 300–500 g with visible cysts were collected from Yezhi Lake in Wuhan city, Hubei province, China (30° 27′ 5″ N, 114° 21′ 2″ E). All fish were transported to the laboratory alive and held in aquaria. Fish were euthanised by neural pithing. Cysts were removed from the skin of the host, placed in a cavity block and ruptured. A drop of spores was placed on a microscope slide, covered with a cover-slip and examined using a light microscope. The tailed-spores were collected as selectively as possible and pooled together to where their concentration was approximately 50% of the subsample. These concentrated spore samples were preserved in 100% ethanol for DNA analysis.

Myxospore identification was performed from the fresh spores according to Lom and Noble (1984) and Lom and Arthur (1989). Descriptions and measurements of spores were performed, using a light microscope equipped with an ocular micrometer and image analysis software (Motic images 3.2) at ×1,000 magnification. Mean and standard deviations of each spore characteristics from 60 spores with caudal appendages (*Henneguya*-type) were calculated for each spore dimension. These data were compared to data from 60 normal (*Myxobolus*-type) spores as determined by Liu et al. (2010). Because spores are microscopic, not all dimensions could be obtained for each individual spore (i.e., spores observed in the valvular aspect are useful for length, width, and polar capsule measurements, but thickness cannot be determined for that particular spore). Mann-Whitney tests were used to test for significant differences ($p<0.05$) between means in spore length, width, thickness, and polar capsule length and width, comparing tailed spores versus non-tailed spores. Type specimens were deposited in the Laboratory of Fish Diseases, College of Fisheries, Huazhong Agricultural University.

DNA isolation and sequencing

The ssrRNA gene of the myxozoan from one allogynogenetic gibel carp was sequenced. Samples fixed in ethanol were spun for 5 min at 5,000 g to pellet the spores; the ethanol was removed and the pellet air-dried. Spore pellets were suspended in 500 μl lysis buffer (100 mM NaCl, 10 mM Tris, 10 mM EDTA, 0.2% SDS and 0.4 mg ml^{-1} Proteinase K) and incubated at 55°C overnight. Total DNA was extracted using a phenol-chloroform protocol as previously described (Eszterbauer et al. 2001). To amplify the ssrDNA gene, the primer pairs MX5 (5′-CTGCGGA CGGCTCAGTAAATCAGT-3′) and MX3 (5′-CCAGGA CATCTTAGGGCATCACAGA-3′) were used (Andree et al. 1999). The total volume of the polymerase chain reaction (PCR) was 50 μl, which contained 2.5 μl of extracted genomic DNA, 5 μl of 1×EasyTaq PCR Reaction Buffer (MBI Fermentas, Vilnius, Lithuania), 0.75 μl of dNTPs (MBI Fermentas), 1.0 μl of each primer, and 0.5 μl of 2.5 *U* EasyTaq DNA polymerase (MBI Fermentas) in MilliQ purified water. A PTC-100 DNA Thermocycler (MJ Research Inc., Watertown, MA, USA) was used for amplification with the conditions: 35 cycles of (95°C for 50 s, 54°C for 50 s, and 72°C for 60 s), and a terminal extension at 72°C for 10 min. The PCR products were isolated from agarose analytical gel and purified for sequencing using the High-Pure PCR Product Purifica-

tion Kit (Omega Bio-Tek, Inc., Norcross, GA, USA). Purified products were sequenced in both directions using the ABI PRISM® 3730 DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA). Sequences were assembled using BioEdit (Hall 1999) and verified as myxozoan species via BLAST search of Genbank.

Phylogenetic analysis

Almost all *Myxobolus* and *Henneguya* species representing the major divisions of myxobolid species for which ssrDNA (>1,000 nucleotides (nt)) was available were included in our phylogenetic analysis (names and GenBank accession numbers, Fig. 2). This included 66 *Myxobolus* spp., 23 *Henneguya* spp., four *Thelohanellus* spp., two *Sphaerospora* spp., and three ssrDNA sequences from unclassified actinospore stages. *Kudoa thyrsites* and *Kudoa alliaria* were used as outgroup taxa, representing the ancestral marine myxozoan lineage. A total of 106 sequences were used in our final alignment. Nucleotide sequences were aligned using ClustalX version 1.8 (Thompson et al. 1997) with default settings. Manual adjustments were done by eye to correct the alignment using BioEdit (Hall 1999). Ambiguous positions were removed from the dataset.

Phylogenetic analyses were carried out on the 1,301 character ssrDNA alignment as follows. The edited alignment was analyzed by jModeltest (Posada 2008) which identified the optimal evolutionary model using the Akaike information criteria, as the general time reversible model (GTR+I+G). Nucleotide frequencies were estimated from the data (A=0.2564, C=0.1869, G=0.2831, T=0.2736); six rates of nucleotide substitution were (AC)=1.6036, (AG)=4.2019, (AT)=2.0380, (CG)=0.7572, (CT)=6.1237, (GT)=1.0000; proportion of invariable sites=0.0290; gamma distribution=0.4230. These parameters were used for both maximum likelihood (ML) and Bayesian analysis. ML analysis was performed using PhyML (Guindon and Gascuel 2003). Bootstrap confidence values were calculated with 100 replicates. Bayesian analyses were conducted in Mr. Bayes (Ronquist and Huelsenbeck 2003) using the evolutionary model as above, with 10^6 generations, tree sampling every 100 generations, with a burn-in of 100 trees. Trees were initially examined in TreeView X (Page 1996) and edited and annotated in Adobe Illustrator (Adobe Systems Inc. San Jose, CA).

Results

Morphology

Mature spores of the parasite from allogynogenetic gibel carp were round in frontal view, lemon-shaped in lateral

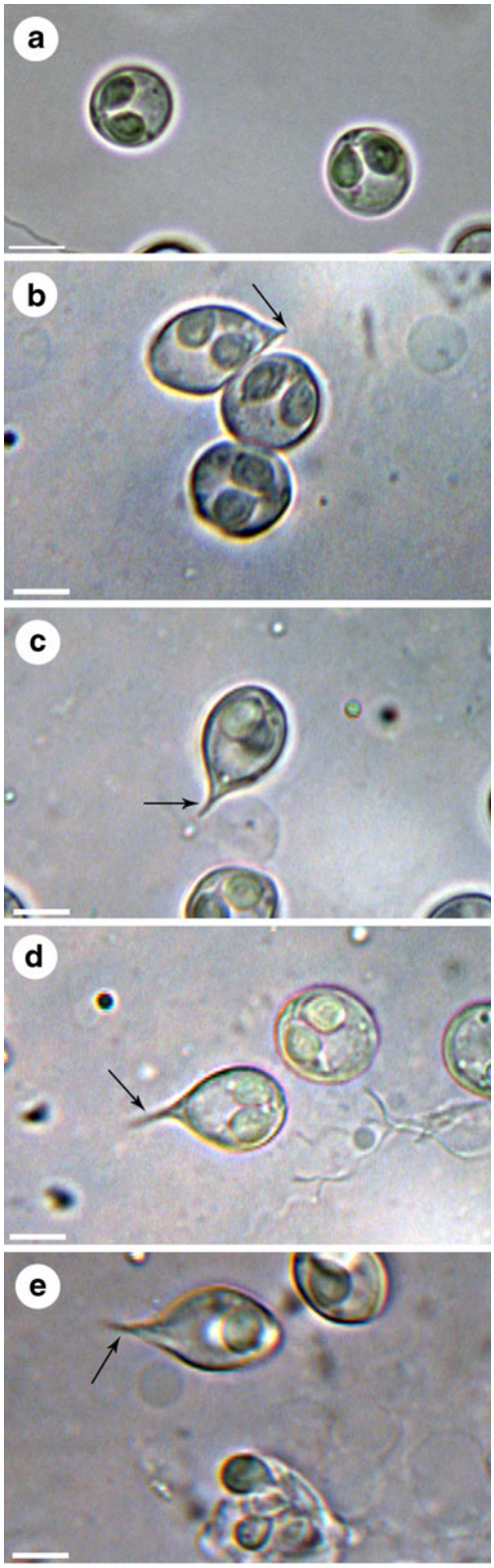


Fig. 1 Photomicrographs of fresh spores of *M. turpisrotundus*, normal spores (**a**) and spores with caudal appendages (**b**, **c**, **d**, **e**). Caudal appendages indicated by *arrows*. Bar=5 μm

view (Fig. 1a). Some spores had 5–6 "V" shaped folds along the sutural ridge distributed toward the posterior end of the shell valves. Two polar capsules were pyriform and of equal size, situated at the anterior extremity of the spore. Intercapsular appendix was present. Polar filaments coiled, with 5 to 6 turns. These observations are consistent with the morphological description of *M. turpisrotundus* which includes morphometric data for the 60 non-tailed spores (Liu et al. 2010).

Observation of fresh smears of *M. turpisrotundus* originating from individual cysts showed the presence of about 10% of the spores with a caudal appendage, reminiscent of a *Henneguya* species (Fig. 1b, c, d and e). In some spores the spore body is slightly elongated with a short tail (Fig. 1b). In others, the tail is more obvious (Fig. 1c, d, e). The spore body is elongate oval or rounded oval in front view, lemon-shaped in lateral view, measuring 7.8–9.5 (8.8±0.1) μm in length, 7.4–8.9 (8.0±0.1) μm in width, and 6.1–7.4 (6.3±0.2) μm in thickness. The two polar capsules, which corresponded more or less half the length of the spore body, were pyriform and of equal size with a rounded posterior extremity and tapering anteriorly; they were 3.7–4.8 (4.3±0.1) μm in length by 3.0–4.2 (3.4±0.1) μm in width and the polar filament formed 5–6 coils obliquely to the axis of the polar capsule. The total length of tailed-spores is 11.9–14.8 (13.4±0.2) μm, and the length of tail was 4.1–5.3 (4.6±0.2) μm. There is significant overlap, if not almost complete overlap, in dimensions of spores from tailed and non-tailed forms of *M. turpisrotundus* (Table 1) and statistical analyses failed to find statistically significant differences between any dimensions in the two spore forms (Table 1).

Sequence analysis

The ssrDNA sequence obtained for *M. turpisrotundus* was 1,542 nt in length, not inclusive of primers MX5 and MX3, and the contiguous sequence was deposited in GenBank under the accession number: GU570996. A BLAST search revealed that the ssrDNA sequence of *M. turpisrotundus* was nearly identical to the previously published sequence of *M. turpisrotundus* from allogynogenetic gibel carp (EF690299; 99.9% similarity). The two sequences of *M. turpisrotundus* differed only at a single nt position (compared 1,542 nt), −/at 724/838 in the sequences GU570996/EF690299, respectively.

Fig. 2 Phylogenetic tree resulting from maximum likelihood (ML) analysis of myxobolid species ssrDNA sequences. Genbank accession numbers are listed adjacent to species names. Support values in percent units at branching points are listed as: Bootstrap values from ML analysis/Bayesian posterior probabilities. *Asterisks* are shown where values exceeded 95%. *Dashes* are shown for values under 65%. *Branches with dashed lines* indicate weakly supported lineages. *Arrows* indicate *Myxobolus* species for which 'tailed' spores have been documented. *H Henneguya* species, *M Myxobolus* species

Phylogenetic analysis

Phylogenetic analysis was based on a final edited alignment which was 1301 characters in length and contained 106 taxa. With the exception of *Myxobolus acanthogobii*, the remaining myxobolid sequences analyzed form a single well-supported lineage (Fig. 2, clade "A"). This includes *Myxobolus*, *Henneguya*, *Thelohanellus*, and *Sphaerospora* species. The two *Sphaerospora* species are sister to each other, nested within the large clade "D" comprised of mostly *Myxobolus* species. All *Thelohanellus* species are found in clade "D" as well, but the genus is polyphyletic, appearing on two branches. *Henneguya doneci* and *Henneguya cutanea* also appear in clade "D" in two distinct locations. The other large clade "B" is composed of both *Henneguya* and *Myxobolus* species, but the majority of *Henneguya* species are found here. There is instability at the base of clade "B" regarding the branching of the *Henneguya salminicola* group, the *Myxobolus insidiosus* group, and the *Myxobolus portucalensis* group. Neither maximum likelihood nor Bayesian analyses provided strong support for the placement of these groups at either

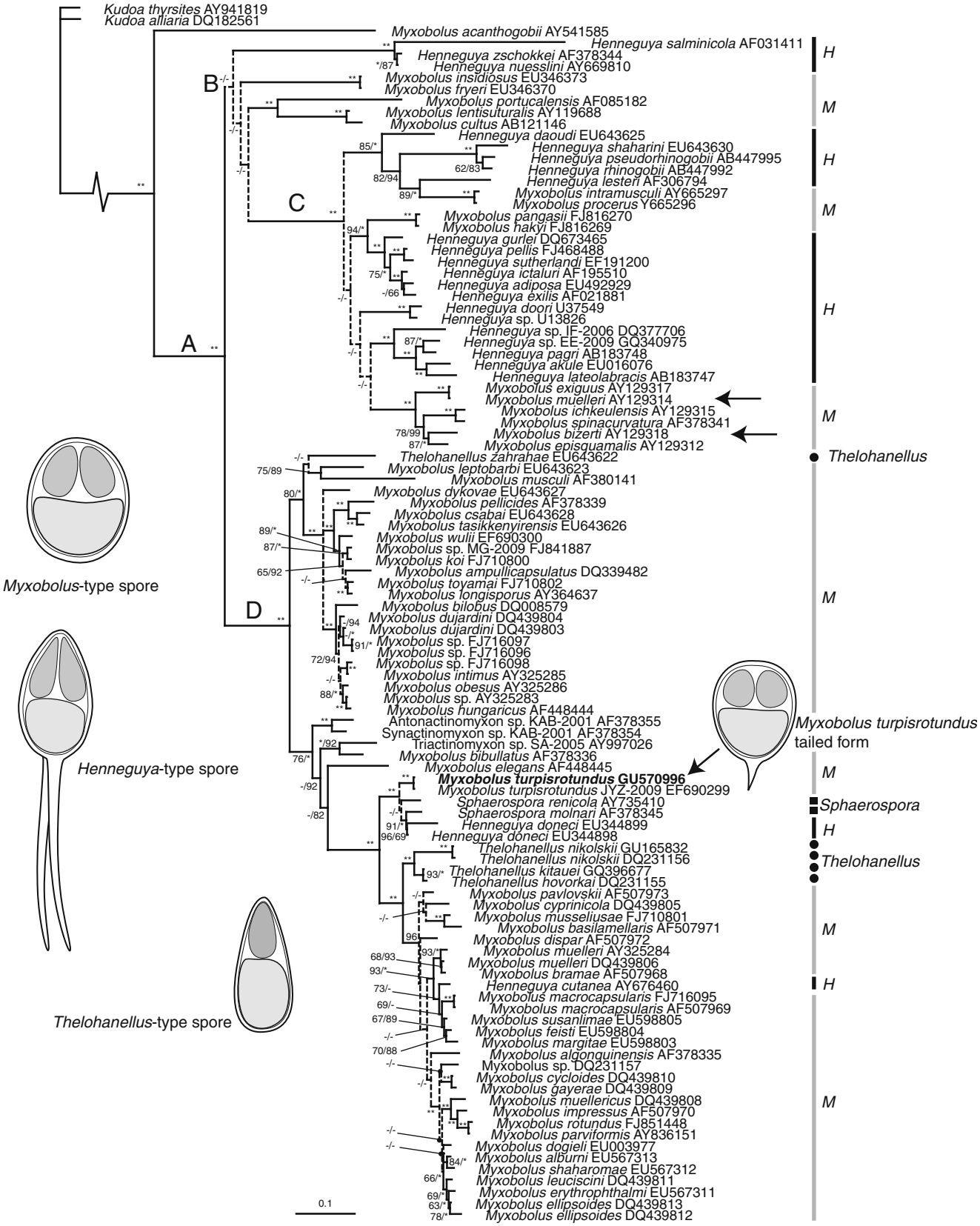
Table 1 Spore measurements for *Myxobolus turpisrotundus* and statistical analysis comparing tailed and non-tailed spores

Source	*Myxobolus turpisrotundus*		Mann–Whitney test (*p* value)
	Present study	Liu et al. (2010)	
Morphotype	Tailed spores	Non-tailed spores	
Spore (body) length	7.8–9.5 (8.8±0.1)[a]	8–9.3 (8.7±0.4)	n.s.d. (p=0.16)
Spore (body) width	7.4–8.9 (8.0±0.1)	7.5–8.9 (8.2±0.3)	n.s.d. (p=0.43)
Spore (body) thickness	6.1–7.4 (6.3±0.2)	6.0–7.0 (6.2±0.3)	n.s.d. (p=0.41)
Polar capsule length	3.7–4.8 (4.3±0.1)	3.8–5.0 (4.4±0.4)	n.s.d. (p=0.44)
Polar capsule width	3.0–4.2 (3.4±0.1)	2.9–4.0 (3.3±0.4)	n.s.d. (p=0.41)
Total length	11.9–14.8 (13.4±0.2)	n.a.	n.a.
Filament coils	5–6	5–6	n.a.

All measurements reported in micrometers (μm)

n.s.d. no statistically significant difference, *n.a.* not applicable

[a] Minimum–maximum (mean±SD)


Kudoa thyrsites AY941819
Kudoa alliaria DQ182561
Myxobolus acanthogobii AY541585
Henneguya salminicola AF031411
Henneguya zschokkei AF378344
Henneguya nuesslini AY669810
Myxobolus insidiosus EU346373
Myxobolus fryeri EU346370
Myxobolus portucalensis AF085182
Myxobolus lentisuturalis AY119688
Myxobolus cultus AB121146
Henneguya daoudi EU643625
Henneguya shaharini EU643630
Henneguya pseudorhinogobii AB447995
Henneguya rhinogobii AB447992
Henneguya lesteri AF306794
Myxobolus intramusculi AY665297
Myxobolus procerus Y665296
Myxobolus pangasii FJ816270
Myxobolus hakyi FJ816269
Henneguya gurlei DQ673465
Henneguya pellis FJ468488
Henneguya sutherlandi EF191200
Henneguya ictaluri AF195510
Henneguya adiposa EU492929
Henneguya exilis AF021881
Henneguya doori U37549
Henneguya sp. U13826
Henneguya sp. IF-2006 DQ377706
Henneguya sp. EE-2009 GQ340975
Henneguya pagri AB183748
Henneguya akule EU016076
Henneguya lateolabracis AB183747
Myxobolus exiguus AY129317
Myxobolus muelleri AY129314
Myxobolus ichkeulensis AY129315
Myxobolus spinacurvatura AF378341
Myxobolus bizerti AY129318
Myxobolus episquamalis AY129312
Thelohanellus zahrahae EU643622
Myxobolus leptobarbi EU643623
Myxobolus musculi AF380141
Myxobolus dykovae EU643627
Myxobolus pellicides AF378339
Myxobolus csabai EU643628
Myxobolus tasikkenyirensis EU643626
Myxobolus wulii EF690300
Myxobolus sp. MG-2009 FJ841887
Myxobolus koi FJ710800
Myxobolus ampullicapsulatus DQ339482
Myxobolus toyamai FJ710802
Myxobolus longisporus AY364637
Myxobolus bilobus DQ008579
Myxobolus dujardini DQ439804
Myxobolus dujardini DQ439803
Myxobolus sp. FJ716097
Myxobolus sp. FJ716096
Myxobolus sp. FJ716098
Myxobolus intimus AY325285
Myxobolus obesus AY325286
Myxobolus sp. AY325283
Myxobolus hungaricus AF448444
Antonactinomyxon sp. KAB-2001 AF378355
Synactinomyxon sp. KAB-2001 AF378354
Triactinomyxon sp. SA-2005 AY997026
Myxobolus bibullatus AF378336
Myxobolus elegans AF448445
Myxobolus turpisrotundus GU570996
Myxobolus turpisrotundus JYZ-2009 EF690299
Sphaerospora renicola AY735410
Sphaerospora molnari AF378345
Henneguya doneci EU344899
Henneguya doneci EU344898
Thelohanellus nikolskii GU165832
Thelohanellus nikolskii DQ231156
Thelohanellus kitauei GQ396677
Thelohanellus hovorkai DQ231155
Myxobolus pavlovskii AF507973
Myxobolus cyprinicola DQ439805
Myxobolus musseliusae FJ710801
Myxobolus basilamellaris AF507971
Myxobolus dispar AF507972
Myxobolus muelleri AY325284
Myxobolus muelleri DQ439806
Myxobolus bramae AF507968
Henneguya cutanea AY676460
Myxobolus macrocapsularis FJ716095
Myxobolus macrocapsularis AF507969
Myxobolus susanlimae EU598805
Myxobolus feisti EU598804
Myxobolus margitae EU598803
Myxobolus algonquinensis AF378335
Myxobolus sp. DQ231157
Myxobolus cycloides DQ439810
Myxobolus gayerae DQ439809
Myxobolus muellericus DQ439808
Myxobolus impressus AF507970
Myxobolus rotundus FJ851448
Myxobolus parviformis AY836151
Myxobolus dogieli EU003977
Myxobolus alburni EU567313
Myxobolus shaharomae EU567312
Myxobolus leuciscini DQ439811
Myxobolus erythrophthalmi EU567311
Myxobolus ellipsoides DQ439813
Myxobolus ellipsoides DQ439812
A
B
C
D
0.1
H
M
H
M
H
M
Thelohanellus
M
Sphaerospora
H
Thelohanellus
M
H
M
Myxobolus-type spore
Henneguya-type spore
Thelohanellus-type spore
Myxobolus turpisrotundus
tailed form

the base of clade "C" (as shown, Fig. 2) or as sister to clade "D". Regardless of the instability in some areas of the tree, both *Henneguya* and *Myxobolus* species are intermingled, in some lineages with very strong support (Fig. 2). The placement of *M. turpisrotundus* in the tree is within the large "mostly *Myxobolus*" clade "D". It is a member of a clade containing three other species (*H. doneci*, *S. renicola*, and *S. molnari*), arguably one of the more unusual groupings within this larger lineage. There is weak support for the basal position of *M. turpisrotundus* in this grouping, it sometimes clustered with *S. renicola*, albeit with weak support as well.

Discussion

Among the Myxosporea Bütschli, 1,881, species of genus *Myxobolus* Bütschli, 1,882, and genus *Henneguya* Thélohan 1,892 together represent almost half of the described diversity of Myxozoa found in fish, with about 800 and 200 described species, respectively (Eiras 2002; Eiras et al. 2005). These two genera are considered separate groups with regard to their morphology, albeit by a single characteristic (Lom and Dyková 1992). In recent years, as taxonomic investigations are increasingly supplemented with molecular biological methods for myxosporeans, many papers have reported on the phylogenetic relationships within the suborder Platysporina (spores with one or two polar capsules at one pole in sutural plane, including *Myxobolus*, *Henneguya* and *Thelohanellus*) (Smothers et al. 1994; Andree et al. 1999; Kent et al. 2001; Fiala 2006; Bartošova et al. 2009). From these studies and our own, it is clear that the ssrDNA and large subunit ribosomal DNA (lsrDNA) sequence data do not support a phylogenetic separation of *Henneguya* and *Myxobolus*. Thus, as Kent et al. (2001) stressed, the caudal appendages of *Henneguya* are not a valid character for distinction of these two genera. The fact that this character is intermixed within the phylogeny of myxobolids suggests that the genetic capacity to develop spore tails exists broadly within this family, but only certain lineages express it.

The irregular occurrence of caudal appendages throughout the myxozoan tree of life is emphasized further by the appearance of this trait in spores of some species of *Myxobolus* as reported here for *M. turpisrotundus* (Fig. 1b, c, d and e). Bahri (2008) reported on this in *Myxobolus bizerti* and *Myxobolus mülleri* spores with caudal appendages. El-Mansy (2005) also observed the same phenomena in *Myxobolus heterosporus* and demonstrated that tailed-spores might be heteromorphs of this species. Shulman (1966) dedicated significant discussion to atypical spores of *Myxobolus kawabatae* and *Myxobolus gigi* that also possess these variable appendages. Considering the tailed versus non-tailed spores of *M. turpisrotundus*, there is obvious overlap in their dimensions and no statistically significant differences in morphology between them (Table 1), except of course for the caudal appendages. Moreover, the BLAST search revealed that the ssrDNA sequence of a specimen containing tailed-spores of *M. turpisrotundus* was nearly identical (99.9%) to the previously published sequence of *M. turpisrotundus*. This is well within the intraspecific sequence variation of what has been reported for species of myxozoa (Ferguson et al., 2008; Molnár et al. 2006; Whipps et al. 2004; Whipps and Diggles 2006; Whipps and Kent 2006).

Our phylogenetic data, as with previous studies (Fiala 2006) clearly demonstrate the polyphyletic nature of *Henneguya*, although this is not a completely haphazard pattern because many *Henneguya* species cluster together (Fig. 2). Given these findings and earlier observations on *Myxobolus* species with *Henneguya*-like tails (Shulman 1966; El-Mansy 2005; Bahri 2008), the caudal projections are clearly a trait that many of these species have the genetic capacity to express. Alternatively, this is a convergent trait having arisen multiple times in the evolution of the myxobolids. In either case, it begs the question why this trait might be displayed in some species and not others. *Thelohanllus* species appear in two lineages in our phylogeny (Fig. 2). These species bear spores with an overall *Myxobolus*-like morphology but possess a single polar capsule instead of two. This likely represents the loss and subsequent fixation of the one polar capsule trait at least twice. The occurrence of *Sphaerospora* species within our phylogeny is also interesting as other *Sphaerospora* species represent a basal lineage to the myxozoa (Jirků et al., 2007). Nonetheless, *Myxobolus* and *Sphaerospora* spores possess the simplest morphology and it would be interesting to investigate these two *Sphaerospora* species with regards to their ultrastructure and development to see if these are consistent with the myxobolids or the "true" *Sphaerospora* species at the base of the myxozoan tree of life.

Very few studies have examined the functional morphology of myxozoan spores but Shulman (1966) provides perhaps the most comprehensive discussion on the topic. Given the similarities between the spore body of *Myxobolus* and *Henneguya* spores, it is likely that they possess very similar qualities with regards to resistance to compression and resiliency when exposed other physical and chemical environmental factors. Also, because many species of these two genera are histozoic, it is difficult to envision how the presence or absence of caudal appendages might be adaptive within the fish host when the spores are the quiescent stage confined within the parasite cyst. What is most compelling is the role of caudal appendages in the settling rate of the myxospore once liberated from the fish (likely through predation and either direct release of spores

or following passage through the digestive tract of the predator). Shulman (1966) investigated the settling rates of numerous species and categorized their rates as either slow, intermediate, or rapid. He found *Myxobolus* species fell into all three of these categories; 41 *Myxobolus* species of 104 species tested (39%) were slow, 21 of 30 (70%) were intermediate, 23 of 42 (55%) were fast settling. However, all *Henneguya* species (12 tested) possessed spores with a slow settling rate (Shulman 1966). This characteristic was also described in detail for spores of *Henneguya doori* by McConnell and Cone (1992). The caudal projections help orient the *Henneguya* spore into the flow of any current and together with a slow settling rate, 1 m/33 h for *H. doori* (McConnell and Cone 1992), and these characteristics facilitate the dispersion of spores over great distances. It is important to note the slow settling rates were also observed for many *Myxobolus* species (Shulman 1966), so this is not a characteristic exclusive to *Henneguya* species. However, it does provide a reasonable explanation for the apparent gains and losses of the caudal appendages throughout the myxobolid tree of life as buoyancy and dispersal is something upon which selection can act under differing environmental conditions.

In the present study, the observation of *Myxobolus* spores with *Henneguya*-like caudal appendages creates a challenge for classification. Are these *Henneguya* species that present a tailless morphotype, or a *Myxobolus* species with a tailed morphotype? The contents of any given *M. turpisrotundus* cyst were dominated by the *Myxobolus* spore type (approximately 90%) and earlier descriptions have reported cysts where tailed forms were not observed (Liu et al. 2010), so it is reasonable to base classification on the dominant form where possible. The sheer number of described species within these genera makes a rigorous taxonomic revision extremely challenging. There is of course great utility in maintaining these genera for the purposes of communication within the scientific community. However, as previous studies have shown, caudal appendage as a character to separate *Myxobolus* and *Henneguya* into distinct genera is not valid from the perspective of an evolutionarily consistent classification scheme. This is problem not unique to these two genera within the Myxozoa. Until morphological characteristics that correlate more consistently with molecular phylogenies can be identified, it is imperative that species simply be described in the greatest detail possible and DNA data are included in these descriptions. Future investigations and reclassifications will hinge on the thorough efforts of today's parasitologists.

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References

Al Quraishy S, Koura E, Abdel-Baki A, Bashtar AR, El Deed N, Al Rasheid K, Abdel Ghaffar F (2008) Light and electron microscopic studies on *Kudoa pagrusi* sp. n. (Myxosporea: Multivalvulida) infecting the heart of sea bream *Pagrus pagrus* (L.) from the Red Sea. Parasitol Res 102:205–209

Andree KB, Székely C, Molnár K, Gresoviac SJ, Hedrick RP (1999) Relationships among members of the genus *Myxobolus* (Myxozoa: Bilvalvulidae) based on small subunit ribosomal DNA sequences. J Parasitol 85:68–74

Bahri S (2008) Abnormal forms of *Myxobolus bizerti* and *Myxobolus mülleri* (Myxosporea: Bivalvulida) spores with caudal appendages. Bull Eur Assoc Fish Pathol 28(6):252–255

Bahri S, Andree KB, Hedrick RP (2003) Morphological and phylogenetic studies of marine *Myxobolus* spp. from mullet in Ichkeul Lake, Tunisia. J Eukaryot Microbiol 50:417–424

Bartošova P, Fiala I, Hypša V (2009) Concatenated SSU and LSU rDNA data confirm the main evolutionary trends within myxosporeans (Myxozoa: Myxosporea) and provide an effective tool for their molecular phylogenetics. Mol Phylogenet Evol 53:81–93

Cuadrado M, Albinyana G, Padrós F, Redondo MJ, Sitjà-Bobadilla A, Álvarez-Pellitero P, Palenzuela O, Diamant A, Crespo S (2007) An unidentified epi-epithelial myxosporean in the intestine of gilthead sea bream *Sparus aurata* L. Parasitol Res 101:403–411

Eiras JC (2002) Synopsis of the species of the genus *Henneguya* Thélohan, 1892 (Myxozoa: Myxosporea: Myxobolidae). Syst Parasitol 52:43–54

Eiras JC, Molnár K, Lu YS (2005) Synopsis of the species of *Myxobolus* Butschli, 1882 (Myxozoa: Myxosporea: Myxobolidae). Syst Parasitol 61:1–46

Eiras JC, Takemoto RM, Pavanelli GC (2009) *Henneguya corruscans* n. sp. (Myxozoa, Myxosporea, Myxobolidae), a parasite of *Pseudoplatystoma corruscans* (Osteichthyes, Pimelodidae) from the Paraná River, Brazil: a morphological and morphometric study. Vet Parasitol 159:154–158

El-Mansy A (2005) Revision of *Myxobolus heterosporus* Baker, 1963 (syn. *Myxosoma heterospora)* (Myxozoa: Myxosporea) in African records. Dis Aquat Org 63:205–214

Eszterbauer E, Benko M, Dan A (2001) Identification of fish parasitic *Myxobolus* (Myxosporea) species using a combined PCR-RFLP method. Dis Aquat Org 44:35–39

Ferguson JA, Atkinson SD, Whipps CM, Kent ML (2008) Molecular and morphological analysis of *Myxobolus* spp. of salmonid fishes with the description of *Myxobolus fryeri* n. sp. J Parasitol 94:1322–1334

Fiala I (2006) The phylogeny of Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis. Int J Parasitol 36:1521–1534

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symp Ser 41:95–98

Jirků M, Fiala I, Modrý D (2007) Tracing the genus *Sphaerospora*: rediscovery, redescription and phylogeny of the *Sphaerospora ranae* (Morelle, 1929) n. comb. (Myxosporea, Sphaerosporidae), with emendation of the genus *Sphaerospora*. Parasitology 134:1727–1739

Kent ML, Andree KB, Bartholomew JL, El-Matbouli M, Desser SS, Devlin RH, Feist SW, Hedrick RP, Hoffmann RW, Khattra J, Hallett SL, Lester RJG, Longshaw M, Palenzeula O, Siddall ME, Xiao C (2001) Recent advances in our knowledge of the Myxozoa. J Eukaryot Microbiol 48:395–413

Liu Y, Gu ZM, Luo YL (2010) Some additional data to the occurrence, morphology and validity of *Myxobolus turpisrotundus* Zhang, 2009 (Myxozoa: Myxosporea). Parasitol Res. doi:10.1007/s00436-010-1835-9

Lom J, Arthur JR (1989) A guideline for preparation of species descriptions in Myxosporea. J Fish Dis 12:151–156

Lom J, Dyková I (1992) Fine structure of Triactinomyxon early stages and sporogony: Myxosporean and Actinosporean features compared. J Protozool 39:16–27

Lom J, Dyková I (2006) Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. Folia Parasitol 53:1–36

Lom J, Noble ER (1984) Revised classification of the class Myxosporea Bütschli, 1881. Folia Parasitol 31:193–205

McConnell CJ, Cone DK (1992) Settling rates and density of spores of *Henneguya doori* (Myxosporea) in water. J Parasitol 78:427–429

Molnár K, Eszterbauer E, Székely C, Dan A, Harrach B (2002) Morphological and molecular biological studies on intramuscular *Myxobolus* spp. of cyprinid fish. J Fish Dis 25:643–652

Molnár K, Marton S, Eszterbauer E, Székely C (2006) Comparative morphological and molecular studies on *Myxobolus* spp. infecting chub from the River Danube, Hungary, and description of *M. muellericus* sp. n. Dis Aquat Org 73:49–61

Molnár K, Székely C, Hallett SL, Atkinson SD (2009) Some remarks on the occurrence, host-specificity and validity of *Myxobolus rotundus* Nemeczek, 1911 (Myxozoa: Myxosporea). Syst Parasitol 72:71–79

Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1253–1256

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Shulman SS (1966) Myxosporidia of the Fauna of the USSR. Nauka, Moscow (in Russian)

Smothers JF, Von Dohlen CD, Smith LH, Spall RD (1994) Molecular evidence that the myxozoan protists are metazoans. Science 265:1719–1721

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acids Res 25:4876–4882

Whipps CM, Diggles BK (2006) *Kudoa alliaria* in flesh of Argentinian Hoki *Macruronus magellanicus* (Gadiformes; Merlucciidae). Dis Aquat Org 69:259–263

Whipps CM, Kent ML (2006) Phylogeography of the cosmopolitan marine parasite *Kudoa thyrsites* (Myxozoa: Myxosporea). J Eukaryot Microbiol 53:364–474

Whipps CM, El-Matbouli M, Hedrick RP, Blazer V, Kent ML (2004) *Myxobolus cerebralis* internal transcribed spacer 1 (ITS-1) sequences support recent spread of the parasite to North America and within Europe. Dis Aquat Org 60:105–108

Yokoyama H, Itoh N, Tanaka S (2005) *Henneguya pagri* n. sp. (Myxozoa: Myxosporea) causing cardiac henneguyosis in red sea bream, *Pagrus major* (Temminck & Schlegel). J Fish Dis 28:479–487