Parasitol Res (2010) 107:67–73
DOI 10.1007/s00436-010-1835-9

ORIGINAL PAPER

Some additional data to the occurrence, morphology and validity of *Myxobolus turpisrotundus* Zhang, 2009 (Myxozoa: Myxosporea)

Y. Liu · Z. M. Gu · Y. L. Luo

Received: 12 November 2009 / Accepted: 4 March 2010 / Published online: 30 March 2010

Abstract *Myxobolus turpisrotundus* Zhang, 2009, infects allogynogenetic gibel carp *Carassius auratus gibelio* (Bloch) and is always regarded as synonymous with *Myxobolus rotundus* Nemeczek, 1911, since its first report in goldfish *Carassius auratus auratus* (L.) in China in 1955. In this study, it was comprehensively examined by morphological and molecular biological methods. The round spores of *M. turpisrotundus* are similar to those of *M. rotundus* from common bream *Abramis brama* (L.) in morphology; however, we detected slight differences in morphometry. The ratios of the length and width of the spore to the length and width of the polar capsule of *M. turpisrotundus* are usually below 2.0 and 1.9, respectively, however these ratios are always above 2.0 and 1.9 in *M. rotundus*. The plasmodium size of *M. turpisrotundus* is 600-6,200 μm in diameter and that of *M. rotundus* is 60-180 μm in diameter. Scanning observation showed the spore surface of *M. turpisrotundus* was generally pitted. Yet the surface of *M. rotundus* is smooth. Sequence comparison revealed the small subunit ribosomal RNA gene sequence of *M. turpisrotundus* did not match any published sequences of *M. rotundus* (EU710583, 85% over 742 bp; FJ851447, 85% over 742 bp, FJ851448, 85% over 742 bp; FJ851449, 85% over 742 bp). Moreover, phylogenetic analysis showed *M. turpisrotundus* clustered with the species from allogynogenetic gibel carp with high bootstrap values (100% neighbor-joining, NJ; 100% maximum parsimony, MP) and *M. rotundus* from common bream composed a new cluster with high bootstrap values (100% NJ, 100% MP). From the morphological and molecular biological data, we gain enough evidences to support the validity of *M. turpisrotundus*.

Y. Liu · Z. M. Gu (✉) · Y. L. Luo
College of Fisheries, Huazhong Agricultural University,
Wuhan, Hubei Province 430070, People's Republic of China
e-mail: guzemao@mail.hzau.edu.cn

Introduction

Myxosporea Bütschli, 1881, are mainly fish parasites with over 2,180 described species (Munoz et al. 2000; Ali et al. 2002; Lom and Dyková 2006; Cuadrado et al. 2007; Al Quraishy et al. 2008). Among the myxosporea, species of the genus *Myxobolus* Bütschli, 1882, are most commonly found in fish, with 744 known species throughout the world (Eiras et al. 2005). These species have been identified principally on the basis of spore morphology, which was the primary identifying character for myxosporean species until the acceptance of molecular biological methods in myxozoan research. With the use of these classical zoological methods, it is very difficult to determine the validity of morphologically similar myxosporea with identical tissue affinity and that develop in taxonomically closely related host species (Molnár et al. 2002, 2009). Although host, organ, and tissue specificity are great help in differentiating morphologically identical or similar spores (Lom and Arthur 1989; Molnár 1994), molecular biological methods are often necessary to resolve species (Eszterbauer and Székely 2004; Székely et al. 2009b).

Myxobolus turpisrotundus is a common and specific parasite of allogynogenetic gibel carp *Carassius auratus gibelio* (Bloch) and was first reported in reared goldfish *Carassius auratus auratus* (L.) in China in 1955. The round spores of *M. turpisrotundus* are similar in morphology to *Myxobolus rotundus* Nemeczek 1911. Chen and Ma (1998) did not realize the importance of the minor differences in shape and size and identified this species as *M. rotundus*. Subsequently, Wu and Wang (2000, 2003) studied *M.*

turpisrotundus by optical and electron microscope and accepted the identification by Chen and Ma (1998). Later, Lu and Nie (2004) obtained the DNA sequence data of *M. turpisrotundus*. Owing to the absence of comparative sequence data from *M. rotundus* from common bream *Abramis brama* (L.), they also accepted the former identification. Subsequently, Molnár et al. (2009) obtained a small subunit (SSU) ribosomal (rRNA) gene sequence from spores from the type host common bream. Zhang et al. (unpublished data) observed a species of *Myxobolus* from skin of allogynogenetic gibel carp and identified it as *M. turpisrotundus*. However, they did not provide enough evidence to distinguish these two species.

The objective of this paper is to clarify the differences between *M. turpisrotundus* and *M. rotundus* using morphological and molecular biological methods, and support the validity of *M. turpisrotundus*.

Materials and methods

Thirty-two specimens of allogynogenetic gibel carp weighting 300-500 g with visible cysts were obtained from Yezhi Lake in Wuhan, Hubei Province, China (30° 27′ 5″ N, 114° 21′ 2″ E). All fish were transported to the laboratory alive and held in aquaria.

Morphological methods

Cysts were collected from the skin and fins of allogynogenetic gibel carp. Myxozoan identification was performed from the fresh spores according to Lom and Noble (1984) and Lom and Arthur (1989). For the visualization of the iodophilic vacuole, the spores were fixed in a 10% buffered formalin solution and stained by Lugol's solution. The presence of a mucous envelope was observed by adding a drop of iodine solution to spores under a coverslip. The maturity of spores was checked by placing them into a 0.4% urea solution. Spores of a given plasmodium were regarded as mature when at least 90% of them extruded polar filaments in this solution. Descriptions and measurements of spores were performed using a light microscope equipped with an ocular micrometer and image analysis software (Motic images 3.2). Mean and standard deviations of each spore characteristics were obtained from 60 fresh mature spores.

For scanning electron microscope, isolated spores removed from mature plasmodia in the fins were rapidly rinsed twice in 0.2 M sodium cacodylate buffer (pH 7.4) and fixed in a solution of 3% glutaraldehyde in the same buffer for 4 h. It was dried in an atmosphere saturated with absolute ethanol and then dehydrated with acetone and dried with CO_2 using the critical point method. The samples were finally sputter-coated with gold and observed with a JSM-6390 scanning electron microscope.

Molecular methods

The SSU rRNA gene of myxosporea from one allogynogenetic gibel carp was sequenced. Samples fixed using 95-100% 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 SSU rRNA gene of myxosporea, the primer pairs MyxoF (5′-CGCGGTAATTCCAGCTCCAGTAG-3′) and MyxoR (5′-ACCAGGTAAGTTTTCCCGTGTTGA-3′) were used (Lu and Nie 2004). 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 PCT-100 DNA Reproducer (MJ Research Inc.) was used for amplification. Amplification conditions were: 95°C for 50 s, 56°C for 50 s, and 72°C for 60 s for 35 cycles with a terminal extension at 72°C for 10 min. The PCR products obtained were purified for sequencing using the High-Pure PCR Product Purification Kit (Omega, USA). Purified products were sequenced in both directions using the ABI PRISM® 3730 DNA sequencer (Applied Biosystems, USA). Forward and reverse sequence segments were aligned in BioEdit software (Hall 1999) and a consensus sequence was deposited in GenBank under the accession number: GU188285. A standard nucleotide-nucleotide BLAST (blastn) search was conducted.

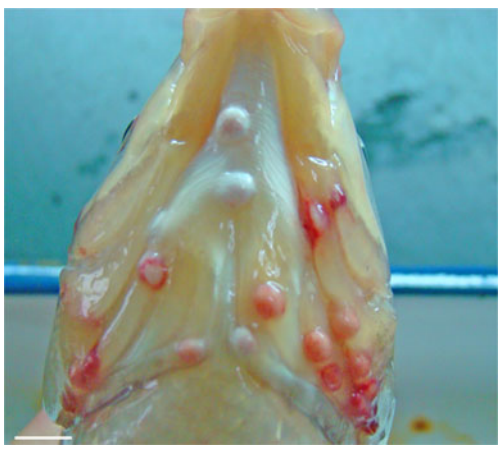

Fig. 1 Morphological aspects of some prominent cysts containing plasmodia of *M. turpisrotundus*. *Scale bar* 10 mm

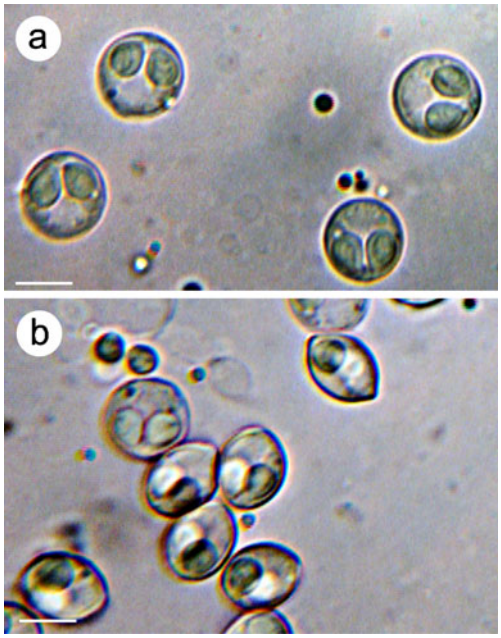

Fig. 2 Photomicrograph of fresh spores of *M. turpisrotundus* observed in frontal view (**a**) and in lateral view (**b**). *Scale bar* 5 μm

Phylogenetic analysis

Myxobolus species nucleotide sequences from GenBank were subjected to phylogenetic analysis; they consist of *M. turpisrotundus* 2 (EF690299), *M. rotundus* 1 (AY165179), *M. rotundus* 2 (FJ851449), *M. rotundus* 3 (FJ851448), *M. rotundus* 4 (FJ851447), *M. rotundus* 5 (EU710583), *Myxobolus alburni* (EU567313), *M. cycloides* (DQ439810), *Myxobolus gayerae* (DQ439809), *Myxobolus leuciscini* (DQ439811), *Myxobolus pavlovskii* (AF507973), *Myxobolus dogieli* 1 (EU003977), *M. dogieli* 2 (EU003978), *Myxobolus basilamellaris* (AF507971), *Myxobolus ellipsoides* (DQ439813), *Myxobolus macrocapsularis* 1 (FJ716095), *M. macrocapsularis* 2 (AF507969), *Myxobolus cyprinicola* (DQ439805), *Myxobolus margitae* (EU598803), and *Ceratomyxa shasta* (AF001579) as an outgroup.

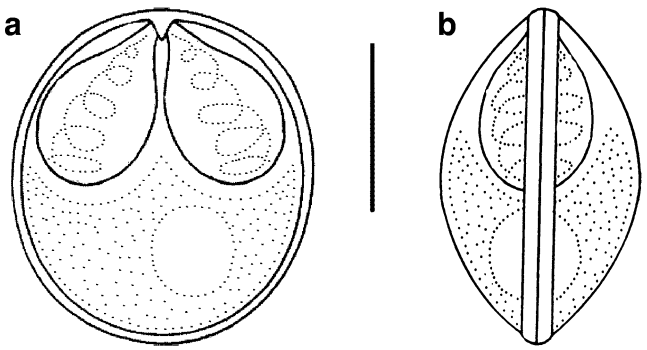

Fig. 3 Line drawing of fresh spores of *M. turpisrotundus* observed in frontal view (**a**) and in lateral view (**b**). *Scale bar* 5 μm

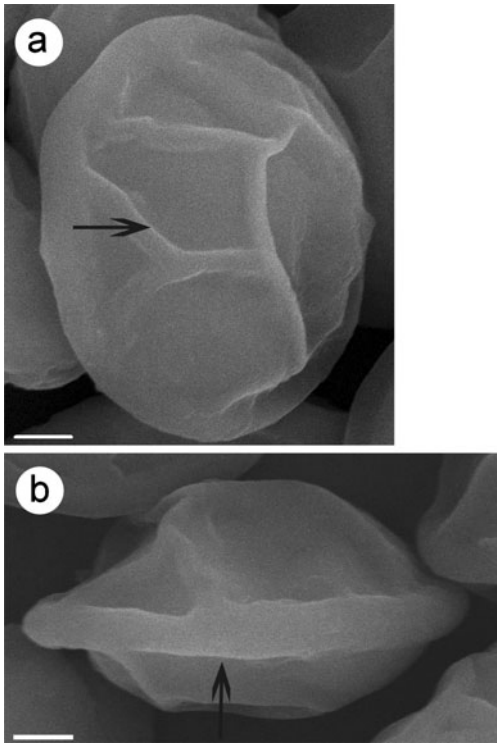

Fig. 4 Mature spores of *M. turpisrotundus* observed in scanning electron microscope **a** note the anomalistic ridges in frontal view (*arrow*), **b** note the sutural line in lateral view (*arrow*). *Scale bar* 1 μm

Nucleotide sequences were aligned using ClustalX (Thompson et al. 1997) with default settings. Manual adjustments were done by eye to correct the alignment (mainly the long sequences of *M. turpisrotundus* 2, *M. rotundus* 2, *M. rotundus* 3, and *M. rotundus* 4) using the BioEdit sequence alignment editor (Hall 1999). Ambiguous positions were selected and then removed from the dataset. Phylogenetic trees were constructed by the neighbor-joining (NJ) method and the maximum parsimony (MP) method, which were implemented in the MEGA3 computer package (Kumar et al. 2004) and PAUP* computer package (Swofford 2003), respectively. The NJ tree was constructed using the Kimura two-parameter distance and pairwise deletion for gaps, and MP tree were generated using a heuristic search with tree bisection-reconnection branch swapping. Gaps were treated as missing data. To estimate the relative branch support of NJ and MP trees, bootstrap analysis with 1,000 replicates was conducted.

Results

Morphological results

Vegetative stages The cysts (Fig. 1) containing plasmodia were found in skin, jaws, gills and fins of allogynogenetic gibel carp. These plasmodia are whitish, round, or

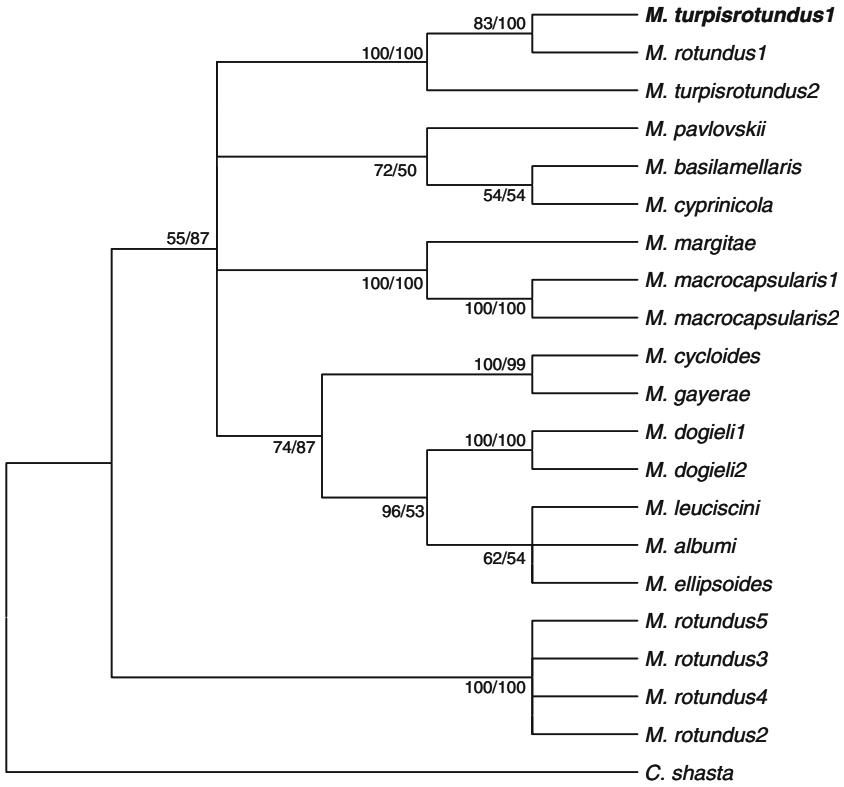

Fig. 5 Phylogenetic tree of Myxosporea based on the SSU rRNA gene sequence using neighbor-joining analysis with 1,000 bootstrap replicates. *Ceratomyxa shasta* is used as an outgroup. Bootstrap values are shown for the neighbor-joining/maximum parsimony analysis

ellipsoidal measuring 600-6,200 μm in diameter and delimited by a single layer of connective tissue derived from the host.

Spore characteristics Mature spores (Fig. 2a, b) were round in frontal view, lemon-shaped in lateral view, measuring 8.0-9.3 (8.7±0.4) μm in length, 7.5-8.9 (8.2±0.3) μm in width, and 6.0-7.0 (6.2±0.3) μm in thickness. Some spores had five to six "V" shaped edge markings distributed in the posterior end of valves. The two polar capsules were pyriform and of equal size with 3.8-5.0 (4.4±0.4) μm in length and 2.9-4.0 (3.3±0.4) μm in breadth, situating on the anterior extremity. Intercapsular appendix was present. Polar filaments coiled, with five to six turns. A single binucleated sporoplasm with a round iodinophilous vacuole was present. Mucous envelope was not found. Drawings of spores (Fig. 3a, b) were made based on the fresh wet mounts with the help of a Nikon FDX-35 camera.

Type host Allogynogenetic gibel carp *C. gibelio* (Bloch).

Locality Yezhi Lake, Wuhan, Hubei Province, China (30° 27′ 5″ N, 114° 21′ 2″ E).

Site of infection Skin, jaws, gills, and fins.

Date of sampling December 2008.

Host size 13-20 cm (prevalence 100%)

Type material Syntype spores on slide had been deposited in the Laboratory of Fish Diseases, College of Fisheries, Huazhong Agricultural University, accession no. MTR20091228.

Scanning electron microscope revealed that the surface of mature spores of *M. turpisrotundus* was generally pitted and there were a number of anomalistic ridges on the surface. The mucous envelope was not observed. Its sutural line was straight and distinctive, running near the middle of the valves (Fig. 4a, b).

Sequence analysis

The SSU rRNA gene of *M. turpisrotundus* was determined to be 742 bp long, not inclusive of primers MyxoF and MyxoR, and the consensus sequence was deposited in GenBank (acc. no. GU188285). A BLAST search revealed that the SSU rRNA gene sequence of *M. turpisrotundus* was identical to the previously published sequence of *M. turpisrotundus* from allogynogenetic gibel carp, but did not

Table 1 Morphometric data of fresh spores of *Myxobolus turpisrotundus* and *M. rotundus*

Parasite Source	*M. turpisrotundus* Present study	*M. turpisrotundus* Wu and Wang (2003)	*M. rotundus* Nemeczek (1911)	*M. rotundus* Molnár et al. (2009)
Spore shape	Roundish	Roundish	Roundish	Roundish
Spore length	8.0-9.3 (8.7±0.4)[a]	9.32 (8.06-9.89)[b]	10	10.6 (9-12)
Spore width	7.5-8.9 (8.2 ± 0.3)	8.31 (7.56-8.64)	9.8	9.5 (8-11)
Spore thickness	6.0-7.0 (6.2±0.3)	5.67 (5.17-6.44)	3	6.2 (5.5-6.5)
Polar capsule length	3.8-5.0 (4.4±0.4)	4.65 (3.81-5.53)	3.8-5[c]	4.9 (4.5-5.0)
Polar capsule width	2.9-4.0 (3.3±0.4)	3.01 (2.72-3.34)	-[d]	3.2 (3-3.5)
No. filament turns	5-6	7	-	6
Ratio spore length to polar capsule length	1.98	2.00	2.00-2.63	2.16
Ratio spore width to polar capsule width	1.86	1.79	-	1.94

All measurements are in micrometers

[a] Minimum–maximum (Mean±SD)

[b] Mean (minimum–maximum)

[c] Minimun–maximum

[d] Data not available

match any published sequence of *M. rotundus* (EU710583, 85% over 742 bp; FJ851447, 85% over 742 bp, FJ851448, 85% over 742 bp; FJ851449, 85% over 742 bp).

Phylogenetic analysis

Phylogenetic analysis was based on the final edited alignment which was 737 bp in length and contained 21 taxa. The neighbor-joining analysis confirmed the clustering patterns of the maximum parsimony analysis in several cases with different bootstrap values (Fig. 5). In the neighbor-joining tree, *M. turpisrotundus* 1 was placed within the cluster of species from allogynogenetic gibel carp with highest bootstrap values (100% NJ, 100% MP). However, *M. rotundus* 2, *M. rotundus* 3, *M. rotundus* 4, and *M. rotundus* 5 composed a new cluster with higher bootstrap values (100% NJ, 100% MP) and *M. rotundus* 1 from allogynogenetic gibel carp did not cluster with *M. rotundus* 2, 3, 4, 5 from common bream.

Discussion

In China, approximately 270 *Myxobolus* species have been described and more than 60 *Myxobolus* species are found from different tissues and organs of crucian carp (Chen and Ma 1998). With simple, round spores, *M. turpisrotundus* morphologically resembles a great number of *Myxobolus* spp., but it shows closest affinity with *M. rotundus*, being similar in shape. However, their measurements appear different with careful scrutiny. The morphometric data of *M. turpisrotundus* compared to that of *M. rotundus* given by Nemeczek (1911) and Molnár et al. (2009) are summarized in Table 1. The comparison shows *M. turpisrotundus* and *M. rotundus* differ in the number of polar filament turns (*M. turpisrotundus*=5-6, *M. rotundus*=6) and in the larger size of *M. rotundus* spores, as demonstrated by the bigger ratios (Table 1) of the length and width of the spore to the length and width of the polar capsule of *M. rotundus*. In addition, the plasmodium size of *M. turpisrotundus* is 600-6,200 μm in diameter and that of *M. rotundus* is 60-180 μm in diameter. Plasmodia of *M. turpisrotundus* were found in skin, jaws, gills, and fins of allogynogenetic gibel carp, while Molnár et al. (2009) and Székely et al. (2009a) pointed out that *M. rotundus* was a specific gill parasite of the common bream. Moreover, *M. turpisrotundus* was previously described from allogynogenetic gibel carp, which is taxonomically distant from common bream. Molnár et al. (2009) proposed that the host range of *M. rotundus* might include leuciscine fishes, such as *Abramis sapa*, *Abramis ballerus*, or *Blicca bjoerkna*. Eszterbauer (2004) previously suggested that when parasite species came into contact with new fish hosts and adapted to infect them, in most cases, their location within the new fish hosts (organ and/or tissue tropism) remained unchanged.

Molnár et al. (2009) have shown that the surface of *M. rotundus* is smooth. Yet our scanning observation revealed the spore surface of *M. turpisrotundus* was generally pitted. Differences in ultrastructure suggest these are distinct species, despite the similarity in morphology (Shen 1999). Wu and Wang (2003) observed the surface of *M. turpisrotundus* was smooth. However, we suggest that the spores observed by Wu and Wang (2003) might be not mature as the surface of spores change during development, becoming stable when mature. On the other hand, there was

a dense, fuzzy material adhering to the surface of the shell valves of *Myxosoma cerebralis* and *Myxosoma* sp., respectively (Lom and Hoffman 1971; Desser and Paterson 1978). But we did not observe the same structure on the spore surface of *M. turpisrotundus* under the light and scanning electron microscope, which is in accordance with Wu and Wang (2003).

As pointed out by a number of researchers (Eszterbauer et al. 2001; Molnár et al. 2002, 2009; Adriano et al. 2009), molecular biological methods have become important taxonomic tools for differentiating between morphologically similar myxosporean species. The BLAST search revealed that the SSU rRNA gene sequence of *M. turpisrotundus* was identical to the previously published sequence of *M. turpisrotundus*, but did not match any published sequence of *M. rotundus* (EU710583, 85% over 742 bp; FJ851447, 85% over 742 bp, FJ851448, 85% over 742 bp; and FJ851449, 85% over 742 bp). This result is consistent with Molnár et al. (2009). Moreover, phylogenetic analysis in present study showed *M. turpisrotundus* clustered with the species from allogynogenetic gibel carp with high bootstrap values (100% NJ, 100% MP) and *M. rotundus* from common bream composed a new cluster with high bootstrap values (100% NJ, 100% MP).

In conclusion, morphological and molecular biological studies in the present study have demonstrated that *M. turpisrotundus* from the skin of allogynogenetic gibel carp is not the same species as *M. rotundus* from common bream, and supported the validity of *M. turpisrotundus*. Further study on life cycles will provide more information about the differences between these two species.

Acknowledgments The authors thank Prof. Molnár, Prof. Bahri, and Dr. Li for their valuable suggestions and critical reading of this manuscript. This study was supported by grant from Natural Science Foundation of Hubei Province (2008CDB078).

References

Adriano EA, Arana S, Alves AL, Silva MR, Ceccarelli PS, Henrique-Silva F, Maia AA (2009) *Myxobolus cordeiroi* n. sp., a parasite of *Zungaro jahu* (Siluriformes: Pimelodiade) from Brazilian Pantanal: morphology, phylogeny and histopathology. Vet Parasitol 162:221–229

Ali MA, AL-Rasheid K, Sakran T, Abdel-Baki A, Abdel Ghaffar F (2002) Some species of the genus *Myxobolus* (Myxozoa: Myxosporea) infecting freshwater fish of the River Nile, Egypt, and the impact on their hosts. Parasitol Res 88:9–15

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

Chen QL, Ma CL (1998) Myxozoa: myxosporea (in Chinese with English summary). Science Press, Beijing

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

Desser SS, Paterson WB (1978) Ultrastructural and cytochemical observations on sporogenesis of *Myxobolus* sp. (Myxosporida: myxobolidae) from the common shiner *Notropis cornutus*. J Protozool 25:314–326

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

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 goldfish (*Carassius auratus auratus*). Acta Vet Hung 52:469–478

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

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

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:50–163

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

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, Hoffman GL (1971) Morphology of the spores of *Myxosoma cerebralis* (Hofer, 1903) and *Myxobolus cartilaginis* (Hoffman, Putz, and Dunbar, 1965). J Parasitol 57:1302–1308

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

Lu YS, Nie P (2004) Phylogenetic analysis of the myxosporeans parasitic in freshwater fish of China based on 18 S ribosomal DNA (in Chinese with English summary). Acta Hydrobiol Sin 28:587–591

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, 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, 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

Munoz P, Sitjà-Bobadilla A, Álvarez-Pellitero P (2000) Antigenic characterization of *Sphaerospora dicentrarchi* (Myxospora: Bivalvulida), a parasite from European sea bass *Dicentrarchus labrax* (Teleostei: Serranidae). Dis Aquat Org 40:117–124

Nemeczek A (1911) Beiträge zur Kenntnis der Myxo- und Microsporidien der Fische. Arch Protistenkd 22:143–169

Shen YF (1999) Protozoology (in Chinese with English summary). Science Press, Beijing

Swofford DL (2003) PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer, Sunderland, MA

Székely C, Hallett SL, Atkinson SD, Molnár K (2009a) Complete life cycle of *Myxobolus rotundus* (Myxosporea: Myxobolidae), a gill myxozoan of common bream *Abramis brama*. Dis Aquat Org 85:147–155

Székely C, Shaharom-Harrison F, Cech G, Ostoros G, Molnár K (2009b) Myxozoan infections in fishes of the Tasik Kenyir Water Reservoir, Terengganu, Malaysia. Dis Aquat Org 83:37–48

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

Wu YS, Wang JG (2000) Studies on the ultrastructure of the sporogensis of *Myxobolus rotundus* Nemeczek, 1911 (in Chinese with English summary). Acta Hydrobiol Sin 25:61–69

Wu YS, Wang JG (2003) Structure of the spore of *Myxobolus rotundus* and *Myxobolus guanqiaoensis* under optical and scanning electron microscope (in Chinese with English summary). Acta Hydrobiol Sin 27:264–268