NOTE *Parasitology*

Henneguya doneci (Myxosporea: Bivalvulida) in the gill filaments of Prussian carp *Carassius gibelio* (Bloch) from the upper Yellow River running through Inner Mongolia, China

Ying-Chun LI[1)#], Yu ZHANG[2)#], SIRIGULENG[2)] and Hiroshi SATO[1,3)*]

[1)]*Department of Clinical Veterinary Science, United Graduate School of Veterinary Science, Yamaguchi University, 1677–1 Yoshida, Yamaguchi 753–8515, Japan*
[2)]*Division of Aquaculture, Faculty of Animal Science and Medicine, Inner Mongolia Agricultural University, 306 Zhaowuda Road, Hohhot, Inner Mongolia, People's Republic of China*
[3)]*Laboratory of Parasitology, Joint Faculty of Veterinary Medicine, Yamaguchi University, 1677–1 Yoshida, Yamaguchi 753–8515, Japan*

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ABSTRACT. We examined 11 Prussian carp, *Carassius gibelio* (Bloch), from the upper Yellow River running through Inner Mongolia (Wuhai City) to record myxosporean species. Between 6 and 15 elongated cysts of *Henneguya doneci* were located at the basal part of the gill filaments of 3 carp (27.3%); no more myxosporean plasmodia were found in other organs. Although the morphology and morphometric values of the spores (average measurements of 14 spores in μm: 11.4 long by 9.2 wide with 7.5 in thickness; 2 polar capsules, equal, 5.5 long by 3.2 wide; and a bifurcated caudal process, 51.6 long) with an evident intercapsular appendix were basically coincident with the species, the dimensions of the spore bodies were marginally larger, and the length of the caudal processes was distinctly longer than previously reported values for *H. doneci* (44.2–59.2 μm vs. 26.8–42.6 μm, respectively). Genetic analysis of the 18S ribosomal RNA gene (rDNA) found few nucleotide substitutions when compared with 3 deposited sequences of *H. doneci* collected around the Yangtze River (Sichuan and Hubei Provinces), China, indicating that the uniqueness of some of the morphological features exhibited by the present Wuhai isolate should be ascribed to intraspecific variation.

KEY WORDS: *Carassius gibelio*, China, *Henneguya doneci*, Myxozoa, Yellow River

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Genera *Myxobolus* Bütschli, 1882 and *Henneguya* Thélohan, 1892 (Myxosporea: Bivalvulida: Myxobolidae) are speciose, occupying almost a half of nominal species of the phylum Myxozoa; approximately 860 and 200 species, respectively [3–5, 11, 17]. Morphologically, myxobolids of these 2 genera have basically similar bivalvulid spores which are oval or pisiform in shape. However, for morphological taxonomy, the presence of a bifurcated caudal process distinguishes the genus *Henneguya* from *Myxobolus* [17].

Prussian carp, *Carassius gibelio* (Bloch), have a wide geographical distribution on the Eurasian continent from central Europe to the Far East. Many myxobolid species have been recorded from this fish host, including 23 *Myxobolus* spp. (*M. acutus*, *M. alacaudatus*, *M. ampullicapsulatus*, *M. artus*, *M. bilis*, *M. carassii*, *M. divergens carassii*, *M. gibelio*, *M. gibelioi*, *M. hearti*, *M. honghuensis*, *M. koi*, *M. kubanicus*, *M. oralis*, *M. orientalis*, *M. platyrostris*, *M. pseudoparvus*, *M. pyramidis*, *M. sacchalinensis*, *M. solidus*, *M. sphaericus*, *M. turpisrotundus* and *M. wulii*), *Henneguya doneci* Schulman, 1962, and 6 *Thelohanellus* spp. (*T. carassii*, *T. dogieli*, *T. oliviformis*, *T. testudineus*, *T. wangi* and *T. wuhanensis*) [2–5, 13–15, 21–28]. The third genus mentioned above, *Thelohanellus* Kudo, 1933, has a tear-shaped bivalvulid spore like some *Myxobolus* spores, but with a single polar capsule [17]. These 3 major genera in the family Myxobolidae resemble one another regarding fundamental spore morphology and closely relate to each other in molecular phylogeny [7, 8, 14, 17, 24].

On April 18, 2014, 11 Prussian carp were collected using a fishing net from the upper part of the Yellow River in Wuhai City, Inner Mongolia (Fig. 1). The fish were transported alive in water to the laboratory at the Inner Mongolia Agricultural University, Hohhot. They were 10.0–14.5 (average, 12.1) cm in total length and 18.3–43.2 (31.1) g in body weight. All organs of the fish, including the skin, gills, viscera and trunk muscles, were examined by the naked eye and under a dissection microscope. For the latter observations, fragments or slices of organs were pressed between 2 glass plates. In 3 fish (27.3%), 6, 7 and 15 elongated myxosporean cysts, white in color, were located in the mucosa at the basal part of the gill filaments of individual hosts (Fig. 2). Their dimensions, expressed as range with mean and standard deviation in parentheses (n=7), were 1.1–1.7 (1.3 ± 0.2) mm by 0.22–0.37 (0.31 ± 0.06) mm. No more myxosporean plasmodia in either cysts or pseudocysts were detected in the other organs examined.

*CORRESPONDENCE TO: SATO, H., Laboratory of Parasitology, Joint Faculty of Veterinary Medicine, Yamaguchi University, 1677–1 Yoshida, Yamaguchi 753–8515, Japan.
e-mail: sato7dp4@yamaguchi-u.ac.jp
#These authors contributed equally to this work.

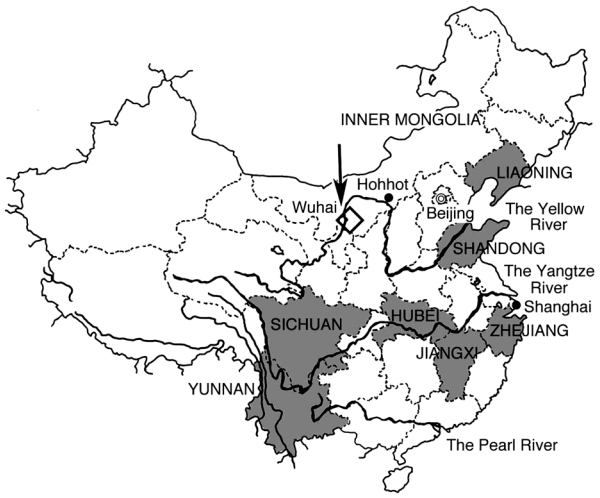

Fig. 1. The collection site of *C. gibelio* for myxosporean species in the present study (the upper Yellow River running through Wuhai, Inner Mongolia, China), indicated by an arrow. Seven provinces where *H. doneci* has been recorded are shown by gray shading.

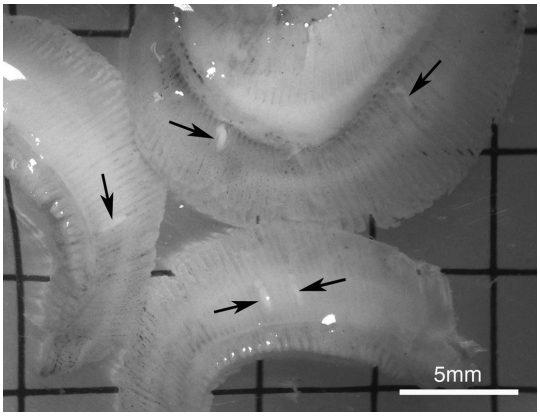

Fig. 2. Plasmodia of *H. doneci* (arrows) at the basal part of gill filaments of a *C. gibelio* under a dissection microscope.

Elongated plasmodia were histozoic and highly polysporous, and the spores in each plasmodium were synchronous in development. The spore body was almost round in frontal view and lemon-shaped in sutural view, with 2 polar capsules and a long bifurcated caudal process (tails), typical for the genus *Henneguya* (Fig. 3). The surface of spores was smooth without mucous envelopes. Spores preserved in 10% neutral-buffered formalin were observed using a microscope equipped with differential interference contrast imaging and processed for detailed measurements as described previously [11, 16]. Measurements, expressed in micrometers (μm) as range with mean and standard deviation in parentheses, were performed on 14 spores chosen arbitrarily. Spore body length, 10.4–12.5 (11.4 ± 0.7); spore body width, 8.8–10.0 (9.2 ± 0.4); spore body thickness, 6.8–7.7 (7.5 ± 0.3); length of 2 tails, almost equal, 44.2–59.2 (51.6 ± 4.9); total length of spore, 54.6–70.4 (63.1 ± 5.1); 2 polar capsules, equal in

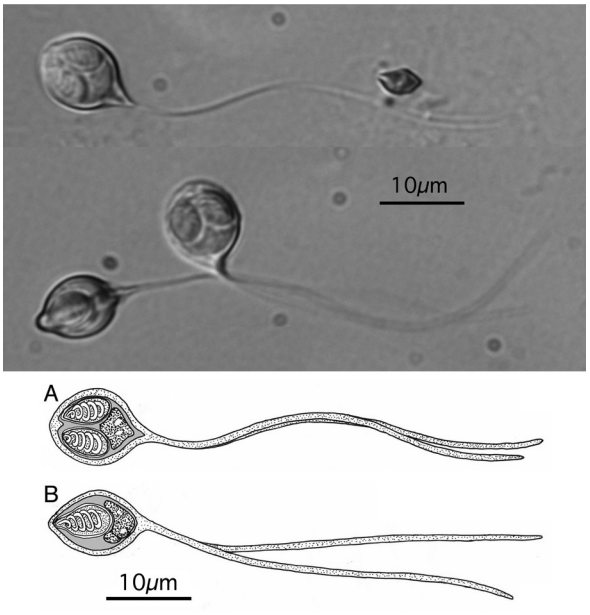

Fig. 3. Photographs and stylized drawings in frontal (A) and lateral (B) views of the Wuhai isolate of *H. doneci.*

dimensions, containing a spiral polar filament with 5 or 6 turns; polar capsule length, 5.4–5.8 (5.5 ± 0.1); polar capsule width, 2.9–3.5 (3.2 ± 0.2); and an evident intercapsular appendix. Specimens were deposited in the Meguro Parasitological Museum, Tokyo, Japan (MPM Coll. No. 20958).

As shown in Table 1, the spore body dimensions of the Wuhai isolate were marginally larger than those of *H. doneci* isolates. The length of the bifurcated caudal processes was clearly different between *H. doneci* isolates from the Basin of Amur River, Russia and the southern part of China, e.g. 49.0–50.0 μm vs. 26.8–42.6 μm, respectively. As the length of the Wuhai isolate's bifurcated caudal processes was 44.2–59.2 μm, it was closer to the original description of *H. doneci* in *C. gibelio* from the Basin of Amur River, Russia, than the isolates collected around the Yangtze River (Sichuan and Hubei Provinces) and other provinces in southern China (Table 1). Molecular phylogenetic analyses were then applied to the isolates to clarify their genetic relationship.

Parasite DNA was extracted from 70% alcohol-preserved spores using an Illustra^{TM} tissue and cells genomicPrep Mini Spin Kit (GE Healthcare UK, Buckinghamshire, U.K.) according to the instructions of the manufacturer. PCR amplification, purification of PCR products and nucleotide sequencing of 2 overlapping fragments of the 18S ribosomal RNA gene (rDNA) were performed as described previously [11]. Consequently, a 2,017-bp sequence of the 18S rDNA, excluding primer aligning 38-bp regions, was obtained and deposited in the DDBJ/EMBL/GenBank databases (accession no. LC011456). The Basic Local Alignment Search Tool (BLAST), available at the DDBJ homepage (http://ddbj.nig.ac.jp/blast/), found the highest nucleotide identity (99.8% [2,007/2,011]–99.9% [1,522/1,524 or 1,607/1,609]) with the

Table 1. Morphometrics of *H. doneci* in the gills of *Carassius* spp. at different localities[a)]

Host	Locality	Cyst sizes	No. of spores examined	Spore length	LSB	WSB	TSB	LPC	WPC	LT	Reference
C. gibelio	Yellow River in Wuhai, Inner Mongolia, China	1.06–1.72 (1.34) mm × 0.22–0.37 (0.31) mm	n=14	54.6–70.4 (63.1 ± 5.1)	10.4–12.5 (11.4 ± 0.7)	8.8–10.0 (9.2 ± 0.4)	6.8–7.7 (7.5 ± 0.3)	5.4–5.8 (5.5 ± 0.1)	2.9–3.5 (3.2 ± 0.2)	44.2–59.2 (51.6 ± 4.9)	The present study
C. gibelio	Basin of Amur River, Russia	3.0 mm in diameter	?	—[b)]	8.5–9.5	9.1	ca. 7.0	5.5–6.7	3.0–4.0	49.0–50.0	[19]
C. auratus	Southern China[b)]	2.0–2.8 mm × 1.0–2.0 mm	?	32.4–48.2 (41.9)	8.4–9.8 (9.2)	7.2–8.8 (7.8)	6.7–7.2 (7.0)	3.6–4.1 (3.7)	2.3–2.8 (2.4)	24.0–38.4 (32.7)	[2]
C. auratus	Hubei Province, China	0.6–4.5 mm (seasonality)	n=25	—[b)]	9.2–11.5 (10.1)	7.5–8.5 (8.0)	7–8 (7.5)	4.0–5.5 (4.7)	2.5–4.0 (3.3)	26.8–42.6 (31.5)	[22]
C. auratus	Southern China[b)]	1.5–2.5 mm	?	—[b)]	9.8–11.4 (10.2)	8.5–9.5 (8.9)	6.1–7.2 (7.0)	4.9–5.6 (5.4)	3.2–3.4 (3.2)	25.6–41.5 (34.7)	[18]

a) LSB, length of spore body; WSB, width of spore body; TSB, thickness of spore body; LPC, length of polar capsule; WPC, width of polar capsule; and LT, length of tails. b) Details not indicated.

Table 2. Variations in the 18S rDNA nucleotide sequences of *H. doneci* in the gills of *Carassius* spp. at different localities

DDBJ/EMBL/GenBank accession no.	No. of available nucleotides (bp)	Host	Locality in China	Reference	18S rDNA[a)]																Nucleotide identity[b)]
					444	1,446	1,462	1,463	1,531	1,534	1,550	1,565	1,607	1,608	1,609	1,610	1,620	1,621	1,623	1,957	
LC011456	2,017	*C. gibelio*	Wuhai, Inner Mongolia	The present study	A	A	R	G	T	C	A	C	A	C	T	A	T	T	T	T	—
HM146129	2,011	*C. gibelio*	Hubei Prov.	[22]	G	•	A	•	•	•	•	T	•	•	•	•	•	•	•	C	99.8% (2,007 / 2,011)
KJ725083	1,609	*C. gibelio*	Hubei Prov.	Liu *et al.* (unpublished)[c)]	•	•	A	A	•	•	•	•	•	•	•	•	•	•	•		99.9% (1,607 / 1,609)
EU344898	1,524	*C. auratus*	Sichuan Prov.	Huang *et al.* (unpublished)[c)]	•	•	A	•	•	T	•	•	•	•	•	•	•	•	•		99.9% (1,522 / 1,524)
EU344899	1,550	*C. auratus*	Sichuan Prov.	Huang *et al.* (unpublished)[c)]	•	C	A	A	G	T	C	•	C	A	G	•	G	A	C		99.2% (1,538 / 1,550)
EU344900	900	*C. auratus*	Sichuan Prov.	Huang *et al.* (unpublished)[c)]		C	A	A	•	T	C	•	C	T	•	—	G	A	C		98.8% (889 / 900)

a) Nucleotide position is expressed relative to the 5′-terminus of the Wuhai *H. doneci* isolate (DDBJ/EMBL/GenBank accession no. LC011456). Dots denote an identical base to that of the uppermost sequence, and '—' and blank indicate a gap and no data, respectively. b) Nucleotide identity to the Wuhai isolate of *H. doneci* (accession no. LC011456). c) Only direct submission of sequences to the DDBJ/EMBL/GenBank databases. No morphological characterization of spores is available at present.

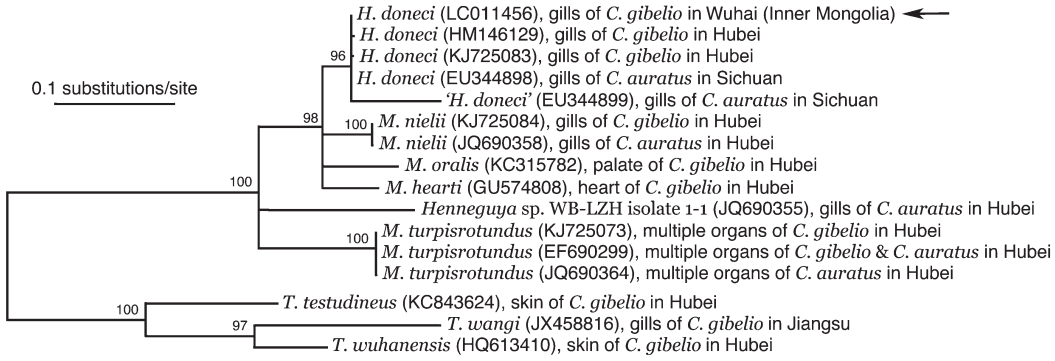

Fig. 4. An ML phylogenetic tree based on the 18S rDNA sequences of representative myxobolid species from *Carassius* spp. in China. The Wuhai isolate of *H. doneci* examined in the present study is indicated by an arrow. Each sequence defined by its DDBJ/EMBL/GenBank accession no. is followed by plasmodium localization in the host, host name and collection site (province name).

18S rDNA sequences of *H. doneci*(DDBJ/EMBL/GenBank accession nos. HM146129, KJ725083 and EU344898), followed by *M. nielii* (JQ690358) with a 97.5% (1,967/2,017) identity and *M. hearti* (GU574808) at a 96.5% (1,882/1,951) identity. Upon comparison of the 18S rDNA sequences of the present Wuhai isolate of *H. doneci* with 3 other *H. doneci* isolates from Sichuan and Hubei Provinces (accession nos. HM146129, KJ725083 and EU344898), no consistent nucleotide substitutions were found between our northern isolate and the southern isolates, albeit a few random nucleotide substitutions occurred (Table 2). Two 18S rDNA sequences (accession nos. EU344899 and EU344900) of a *Henneguya* isolate from the gills of *C. auratus* in Sichuan Province, labeled as '*H. doneci*' at present (December 2014), showed rather lower nucleotide identities with those of *H. doneci*, i.e. 98.8–99.2% (see Table 2).

For phylogenetic analysis, the 18S rDNA sequences of *H. doneci* and some representatives of closely related myxobolids recorded in *C. gibelio* and its congener in China, *C. auratus*, were retrieved from the DDBJ/EMBL/GenBank databases and aligned using the CLUSTAL W multiple alignment program [20] with subsequent manual adjustment. The accession numbers, sites of parasitism, hosts and collection sites of analyzed sequences are given in the figure (Fig. 4) showing the phylogenetic tree. Regions judged to be poorly aligned and characters with a gap in any sequences were excluded from subsequent analyses; 1,281 characters, of which 357 were variable, remained for subsequent analysis in the present study. Maximum likelihood (ML) analysis was performed as described previously [11].

An ML phylogenetic tree based on the 18S rDNA showed genetic relationships among representative spp. of the *Henneguya*/*Myxobolus*/*Thelohanellus* recorded from *Carassius* spp. in China (Fig. 4). The Wuhai isolate of *H. doneci* clustered robustly with 3 other *H. doneci* isolates from Hubei and Sichuan Provinces, with additional close genetic relationships with *M. nielii* from gills and *M. hearti* from the heart or *M. oralis* from the palate of *Carassius* spp. in China.

Carassius spp. are popular freshwater fish on the Eurasian continent, increasingly expanding their geographical distribution in the world over the last several decades by artificial introduction. *C. gibelio* and *C. auratus* are 2 representatives of the genus in China. It has recently been reported that fish in natural water and aquaculture are being increasingly consumed by the Chinese people [14, 21]. At present, *H. doneci* is a single myxobolid species parasitic solely to the gill filaments of *Carassius* spp. in China. From *C. auratus*, however, 4 more *Henneguya* spp. have been recorded as follows: *H. chongqingensis* Ma, 1998 and *H. rhomboideus* Ma, Dong et Wang, 1982 from the ureter and urinary bladder; *H. miyairii* Kudo, 1919 from the subcutaneous tissue around the head; and *H. zikawiensis* Sikama, 1938 from the gills, gallbladder, intestine, heart and kidneys [3]. All these *Henneguya* spp. have distinct morphology and organ or tissue tropism (tissue specificity) from those of *H. doneci*. As mentioned above, the Sichuan isolate of *Henneguya* sp., currently labeled as '*H. doneci* in the DDBJ/EMBL/GenBank databases (accession nos. EU344899 and EU344900; see also Table 2 and Fig. 4), from *C. auratus* should be characterized morphologically to clarify its precise taxonomic position.

Myxosporeans showing the same organ or tissue tropism, or those taking the same host species or groups, rather than those with an identical spore morphology, appear to cluster in the phylogenetic tree [1, 6, 7, 9, 13, 23]. Furthermore, it is currently hypothesized that the bifurcated caudal process of the genus '*Henneguya*' arose on separate occasions during the evolution of ancient myxobolids in the world's water [7, 10, 17]. Liu *et al.* [12] observed a single myxobolid species, *M. turpisrotundus*, showing both *Myxobolus*-type and *Henneguya*-type spores (albeit the latter type occupied about 10%). Similarly, the appearance of atypical spores within a single myxosporean plasmodium of multiple species has been described in detail by Shulman [19]. The Wuhai isolate of *H. doneci* had marginally larger spore bodies and distinctly longer caudal processes than other isolates of the species in southern and eastern China (Table 1), although the host and plasmodium localization were identical. The 18S rDNA sequencing detected few nucleotide substitutions between the Wuhai isolate and the other isolates from Hubei and Sichuan (Table 2). Based on this genetic characteriza-

tion, we identify the present Wuhai isolate as *H. doneci* and ascribe its genetic and morphological uniqueness to an intraspecific variation. In other words, genetic analyses have a substantial importance for a taxonomic study for myxosporean species, particularly for speciose myxobolid genera, such as *Myxobolus*, *Henneguya* and *Thelohanellus*.

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