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New Japanese Record of *Henneguya postexilis* (Cnidaria: Myxobolidae) from Gills of Alien Channel Catfish *Ictalurus punctatus* (Siluriformes: Ictaluridae) in Japan

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Henneguya postexilis Minchew, 1977 (Cnidaria: Myxobolidae) is described as a novel record for Japan. It was found in the gills of non-native *Ictalurus punctatus* (Rafinesque, 1818) (Siluriformes: Ictaluridae), which were collected from the Omoi River, a tributary of the Tone River system in Tochigi Prefecture, central Honshu. This myxozoan species is native to North America and its discovery from Japan in this study is the second case reported from a non-native region. Until now, *H. postexilis* has only been observed in *I. punctatus*, suggesting that it is an introduced alien species in Japan, likely accompanying its host.

Key Words: Myxozoa, alien species, new country record, molecular identification.

Introduction

Alien fish species can inadvertently facilitate the co-invasion or establishment of parasites associated with them (Bauer and Hoffman 1976; Bauer 1991; Lymbery et al. 2014). A case in point is the channel catfish *Ictalurus punctatus* (Rafinesque, 1818) (Siluriformes: Ictaluridae), native to North America, which was deliberately introduced into various habitats for aquaculture purposes. This introduction resulted in the co-invasion of accompanying parasites such as monogeneans, ciliates, and myxozoans (see Nitta and Nagasawa 2015; Feng et al. 2023; Zhang et al. 2023). Specifically, in Japan, the alien monogenean *Ligictaluridus pricei* (Mueller, 1936) (Dactylogyridae) was reported to co-establish with *I. punctatus* in Lake Kasumigaura in the lower reaches of the Tone River system in central Honshu (Nitta and Nagasawa 2015). During a parasitological examination of alien fish species within the Tone River system, not only *L. pricei* but also gill-infesting myxozoans were discovered in *I. punctatus*. Moreover, through morphological and molecular examination, these myxozoans were identified as *Henneguya postexilis* Minchew, 1977 (Myxobolidae), which had not been previously reported in Japan. Hence, this finding represents a novel record of *H. postexilis* infection in Japan.

Species of *Henneguya* Thélohan, 1892 are fish parasitic myxospores, of which more than 250 species have been described (Rangel et al. 2023). This genus is distinguished from the other member of Myxobolidae by the morphology of its symmetrical spores along with the presence of paired polar capsules and two caudal projections (Lom and Dyková 1992). Although the presence of two caudal projections is one of the important characters of this genus, the previous molecular phylogenetic analyses indicated that *Henneguya* is polyphyletic and the character has independently arisen several times in the Myxobolidae (Kent et al. 2001; Liu et al. 2019). In addition to the phylogenetic analysis results, Liu et al. (2019) posited that the taxonomic status of the genus should be maintained until comprehensive molecular data becomes available for the type species of all genera encompassed within the Myxobolidae. In Japan, a total of 14 nominal *Henneguya* species have been reported from 10 fish species (Table 1).

Materials and Methods

Two specimens of *I. punctatus* with standard lengths of 82.6 mm and 65.2 mm, respectively, were captured by angling in the Omoi River (36°17′18.1″N, 139°46′18.7″E), a tributary of the Tone River system located in Oyama City, Tochigi Prefecture, Japan, on 23 August 2015. The captured fish were stored in a freezer until parasitological examination. Using forceps under a dissecting microscope, each plasmodium of the myxosporean was carefully extracted from the gills and placed on a glass slide, and approximately half of the spores were fixed in 99% ethanol in a 1.5 mL tube. The remaining fresh spores were wet-mounted, and microphotographs were taken using a CANON EOS Kiss X7i digital camera (Canon, Tokyo, Japan) attached to an Olympus BX51 light microscope (Olympus, Tokyo, Japan).

Table 1. Records of nominal species of *Henneguya* from Japanese fishes.

Species	Host	Host family	Locality		Reference
			Prefecture	Site	
H. carassii Fujita, 1924*	*Carassius* sp. (as *C. vulgaris* von Nordmann, 1840)	Cyprinidae	Okayama	—	Fujita (1924)
H. cartilaginis Yokoyama, Urawa, Grabner, and Shirakashi, 2012	*Oncorhynchus masou* (Brevoort, 1856)	Salmonidae	Hokkaido	—	Yokoyama et al. (2012)
H. lateolabracis Yokoyama, Kawakami, Yasuda, and Tanaka, 2003	*Lateolabrax maculatus* (McClelland, 1844) (as Chinese sea bass *Lateolabrax* sp.)	Lateolabracidae	Mie	—	Yokoyama et al. (2003)
H. mogurndae Fujita, 1936**	*Odontobutis obscura* (Temminck and Schlegel, 1845) (as *Mogrunda obscura*)	Odontobutidae	Kyoto	Ogura Pond	Fujita (1936)
H. miyairii Kudo, 1920	*Carassius* sp. [as *Carassius auratus* (Linnaeus, 1758)]	Cyprinidae	Fukuoka	—	Kudo (1920)
H. miyazakii Hoshina, 1952	*Chaenogobius annularis* Gill, 1859	Gobiidae	Kanagawa	Kanazawa Bay	Hoshina (1952)
H. ogawai Li, Sato, Kamata, Ohnishi, and Sugita-Konishi, 2012	*Acanthopagrus schlegelii* (Bleeker, 1854)	Sparidae	Yamaguchi	Seto Inland Sea off Hofu City	Li et al. (2012)
H. pagri Yokoyama, Itoh, and Tanaka, 2005	*Pagrus major* (Temminck and Schlegel, 1843)	Sparidae	Mie	—	Yokoyama et al. (2005)
H. postexilis Minchew, 1977	*Ictalurus punctatus* (Rafinesque, 1818)	Ictaluridae	Tochigi	Omoi River	This study
H. spatulata Fujita, 1924*	*Carassius* sp. (as *C. vulgaris*)	Cyprinidae	Fukuoka, Shimane	—	Fujita (1924)
H. preintestinalis Ozaki and Isizaki, 1941	*Tridentiger obscurus* (Temminck and Schlegel, 1845)	Gobiidae	Hiroshima; Yamaguchi	Ota River; Iwakuni River	Ozaki and Isizaki (1941)
H. pseudorhinogobii Kageyama, Yanagida, Ohara, and Yokoyama, 2009	*Rhinogobius* sp.	Gobiidae	Gifu	Nagara River	Kageyama et al. (2009)
H. rhinogobii Lee and Nie in Chen, 1973	*Rhinogobius* sp.	Gobiidae	Gifu	Nagara River	Kageyama et al. (2009)
H. tridentigeri Ozaki and Isizaki, 1941	*Tridentiger obscurus*	Gobiidae	Hiroshima; Yamaguchi	Ota River; Iwakuni River	Ozaki and Isizaki (1941)
	Acanthogobius flavimanus (Temminck and Schlegel, 1845)	Gobiidae	Kanagawa	Kanazawa Bay	Hoshina (1952)
H. yokoyamai Li, Sato, Kamata, Ohnishi, and Sugita-Konishi, 2012	*Acanthopagrus schlegelii*	Sparidae	Yamaguchi	Seto inland Sea off Hofu City	Li et al. (2012)

* These species were pointed out as not conforming to the morphological traits defined by Lom and Dyková (1992) and were excluded from the synopsis of *Henneguya* (see Eiras 2002).
** This species is not listed in synopses or checklists of *Henneguya* (Erias 2002; Eiras and Adriano 2012; Rangel et al. 2023).

Subsequently, the spores were fixed in 70% ethanol, air-dried, stained with Giemsa, and mounted in Canada balsam. Meanwhile, some plasmodia were fixed in 5% formalin with the gill tissues and preserved in 70% ethanol in a vial. The specimens were deposited at the Meguro Parasitological Museum (MPM) in Tokyo, Japan. Measurements were acquired from these images using the ImageJ software (version 1.53t; https://imagej.net/ij/index.html), as described by Lom and Dyková (1992). Plasmodia were measured on the broad surface of the gill filament, with the vertical axis of the gill filament representing length and the horizontal axis representing width. Measurements, all in micrometers, were expressed as the range followed by the mean ± SD and number (n) of structures measured in parentheses.

DNA was extracted from the spores fixed in 99% ethanol using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) and subsequently eluted in 50 μL of AE buffer (Qiagen). To amplify the partial sequence of 18S rDNA, two sets of primers were used: ERIB1 (5′-ACC TGG TTG ATC CTG CCA G-3′: Barta et al. 1997) or MyxospecF (5′-TTC TGC CCT ATC AAC TTG TTG-3′: Fiala 2006) in conjunction with ACT1r (5′-AAT TTC ACC TCT CGC TGC CA-3′: Hallett and Diamant 2001), and Myxgen4F (5′-GTG CCT TGA ATA AAT CAG AG-3′: Diamant et al. 2004) paired with 18gM (5′-CTT CCG CTG GTT CAC CTA CG-3′: Freeman et al. 2008). The PCR reaction was conducted in a 15.0 μL volume, comprising 7.5 μL of KOD One PCR Master Mix (Toyobo, Osaka, Japan), 0.45 μL of each 10 μM primer, 1.0 μL of extracted DNA, and 5.6 μL of distilled water. The PCR cycling protocol involved an initial denaturation step at 98°C for 10 s, followed by 35 cycles of denaturation at 98°C for 10 s, annealing at 54°C for 5 s, extension at 68°C for 10 s, and a final extension step at 68°C for 60 s. Subsequently, the PCR products obtained were purified using ExoSAP-IT (USB Corporation, Cleveland, USA), and commercial sequencing services were employed (Azenta Life Sciences, Tokyo, Japan) using the aforementioned PCR primers. The obtained sequences and electropherograms were manually reviewed and edited utilizing MEGA11 (Tamura et al. 2021). The resulting sequences were submitted to the DNA Data Bank of Japan and compared with existing sequences in the International Nucleotide Sequence Databases (INSD) through a BLASTn search (http://www.ncbi.nlm.nih.gov/) performed on 15 October 2023.

The phylogenetic tree was reconstructed with the following method. The analyzed dataset was comprised of the newly obtained sequence of 18S rDNA, and 17 sequences that retrieved high similarity to the new sequence by the

Table 2. List of taxa included in the phylogenetic analysis with INSD accession numbers for 18S rDNA sequence.

Species	Host	Locality	Accession No.	Reference
Henneguya adiposa Minchew, 1977	*Ictalurus punctatus* (Rafinesque, 1818)	USA	EU492929	Griffin et al. (2009)
	I. punctatus	USA	MK253077	Stilwell et al. (2019)
	I. punctatus	USA	MZ905348	Woodyard et al. (2022)
Henneguya exilis Kudo, 1929	*I. punctatus*	USA	AF021881	Lin et al. (1999)
	I. punctatus	USA	MZ905345	Woodyard et al. (2022)
Henneguya gurleyi Kudo, 1920*	*Ameiurus nebulosus* (Lesueur, 1819)	USA	DQ673465	Iwanowicz et al. (2008)
Henneguya ictalurid Pote, Hanson, and Shivji, 2000	*Dero digitata* (Müller, 1774) and/or *I. punctatus*	USA	AF195510	Pote et al. (2000)
Henneguya laseeae Leis, Rosser, Baumgartner, and Griffin, 2017	*Pylodictis olivaris* (Rafinesque, 1818)	USA	KX354352	Leis et al. (2017)
Henneguya mississippiensis Rosser, Griffin, Quiniou, Khoo, Greenway, Wise, and Pote, 2015	*D. digitata*	USA	AF021878**	Hanson et al. (2001)
	I. punctatus	USA	KP404438	Rosser et al. (2015)
Henneguya postexilis Minchew, 1977	*I. punctatus*	Japan	LC781947	This study
	I. punctatus	USA	MZ905344	Woodyard et al. (2022)
	I. punctatus	China	MK811030	Zhang et al. (2023)
Henneguya sutherlandi Griffin, Pote, Wise, Greenway, Mauel, and Camus, 2008	*I. punctatus*	USA	EF191200	Griffin et al. (2008)
	I. punctatus	USA	MZ905346	Woodyard et al. (2022)
Henneguya sp.	*Lates calcarifer* (Bloch, 1790)	India	MT437047	Unpublished
Helioactinomyxon sp.	*D. digitata*	USA	KF263537**	Rosser et al. (2014)
Raabeia sp.	*D. digitata*	USA	KF263539**	Rosser et al. (2014)
Myxobolus lepomis Rosser, Baumgartner, Barger, and Griffin, 2017	*Lepomis marginatus* (Holbrook, 1855)	USA	KY203391	Rosser et al. (2017)

* This species was recorded as *Henneguya gurlei* [sic] (Iwanowicz et al. 2008).
** Sequences were obtained from actinospores (Hanson et al. 2001; Rosser et al. 2014).

BLAST search. In addition, an 18S sequence of *Myxobolus lepomis* Rosser, Baumgartner, Barger, and Griffin, 2017 was included in the dataset as an outgroup taxon following Woodyard et al. (2022) from INSD (Table 2). The dataset was aligned using MAFFT version 7 (Katoh et al. 2019) using the Q-INS-i strategy. Ambiguous sites in the aligned dataset were removed with Gblocks version 0.91b (Castresana 2000) using the "Allow gap positions within the final blocks" option. The best-fit models were determined based on the Bayesian information criterion using IQ-TREE version 2.2.0. (Kalyaanamoorthy et al. 2017; Minh et al. 2020). The maximum likelihood trees were constructed under the TN+F+I+G4 model using IQ-TREE version 2.2.0 with 1000 bootstrap repeats.

Henneguya postexilis Minchew, 1977
[New Japanese name: Amerika-namazu-uchiwa-mushi]
(Figs 1, 2)

Henneguya postexilis Minchew, 1977: 219, figs 49–64, 73, 74; Lom and Dyková 1992: 298; Hoffman 1999: 65; Eiras 2002: 46; Wagner 2016: 76; Woodyard et al. 2022: 47–48, figs 1–4, 7–10; Zhang et al. 2023: 5–6, fig. 5.

Specimens. MPM Coll.-No. 25259.

Description. Plasmodium oblong, 82.8–115.7 (94.3±13.8, n=5) long, 121.8–200. 5 (169.1±29.3, n=5) wide. Total length of myxospore including caudal processes 45.7–69.9 (56.2±6.3, n=51). Spore body lanceolate, 12.9–17.9 (14.9±1.1, n=137) long, 3.2–4.4 (3.8±0.3, n=83) wide, length : width ratio 1 : 0.20–0.34 (0.26±0.03, n=83), thickness 2.8–3.8 (3.4±0.2, n=55). Pair of caudal process, equal in length, 29.4–54.4 (41.3±6.4, n=51). Pair of polar capsules pyriform, occupying anterior half of spore body, mostly unequal in length, with 6–9 (7.2±0.8, n=67) polar tubule turns. Longer polar capsule 5.3–7.5 (6.4±0.4, n=80) long, 0.9–1.6 (1.2±0.2, n=80) wide; shorter polar capsule 4.9–6.8 (5.9±0.5, n=80) long, 0.8–1.7 (1.2±0.2, n=80) wide; length ratio of longer and shorter polar capsule 1 : 0.75–0.99 (0.93±0.05, n=80).

Locality. Omoi River (36°17′18.1″N, 139°46′18.7″E), Tone River system, in Oyama City, Tochigi Prefecture, Japan.

Host. *Ictalurus punctatus* (Rafinesque, 1818) (Siluriformes: Ictaluridae).

Site of infection. Gill filaments.

Japanese name. The new Japanese name, "amerika-namazu" refers to the host, *Ictalurus* Rafinesque, 1820, in Japanese, and "uchiwa-mushi" means the genus *Henneguya*.

Molecular data comparison. A BLASTn analysis of the newly obtained 18S rDNA fragment (1894 bp; LC781947) returned a 100% match of *H. postexilis* [MZ905344, Woodyard et al. (2022) from USA; MK811030, Zhang et al. (2023) from China] with 100% and 99% coverage, respectively.

Phylogenetic analysis. The newly obtained sequence was placed in a clade with the other *H. postexilis* sequences (MZ905344, MK811030). This species formed a sister clade with a clade of *H. ictalurid* Pote, Hanson, and Shivji, 2000, *H. mississippiensis* Rosser, Griffin, Quiniou, Khoo, Green-

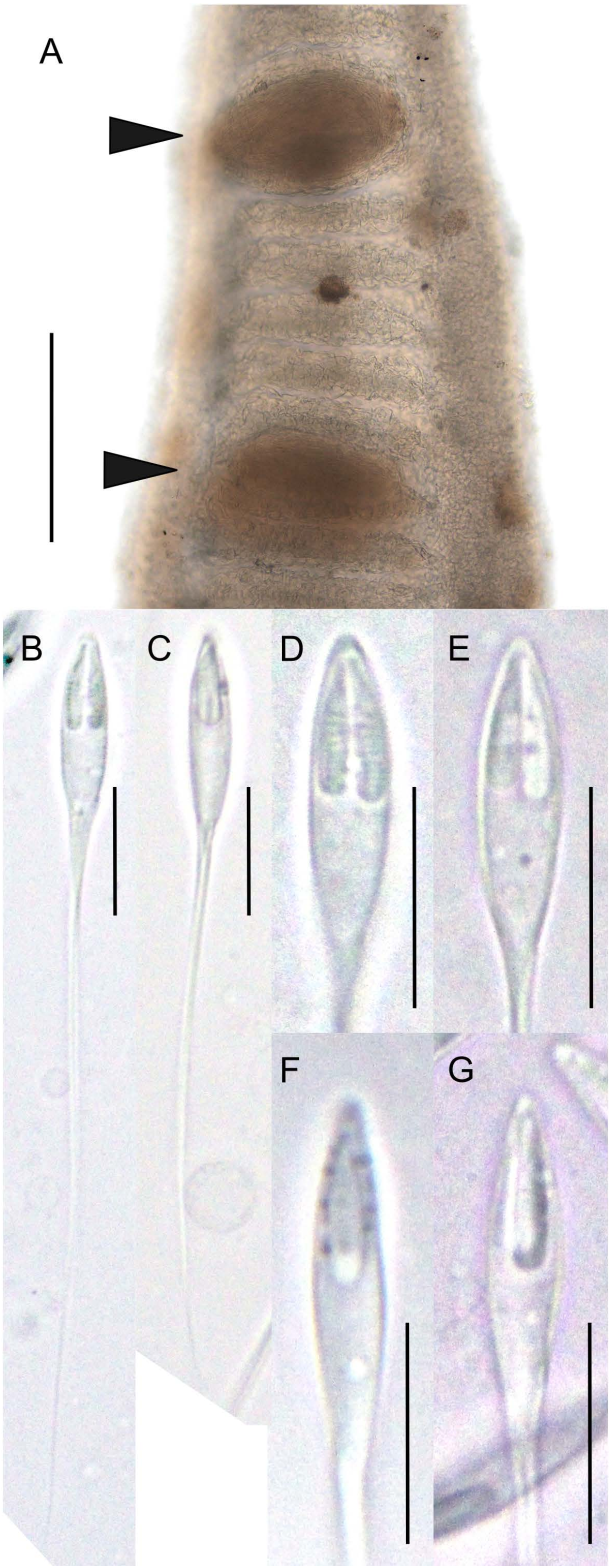

Fig. 1. Light photomicrograph of two fixed plasmodia with a gill filament of *Ictalurus punctatus* (A) and fresh myxospores (B–G) of *Henneguya postexilis*. Arrowheads indicate each plasmodium. B, D, E, Valvular view; C, F, G, sutural view. Scale bars: A, 200 µm; B–G, 10 µm.

way, Wise, and Pote, 2015, and *Helioactinomyxon* sp.

Remarks. *Henneguya postexilis* was originally described based on the observation of specimens retrieved from the gill filaments of *I. punctatus* reared in a fish farm in Missouri, USA (Minchew 1977). However, the species was not identified until Woodyard et al. (2022) provided a new and more complete molecular description based on specimens obtained from the same host in Mississippi, USA. Notably, morphology and molecular data for *H. postexilis* have been reported in China (Zhang et al. 2023).

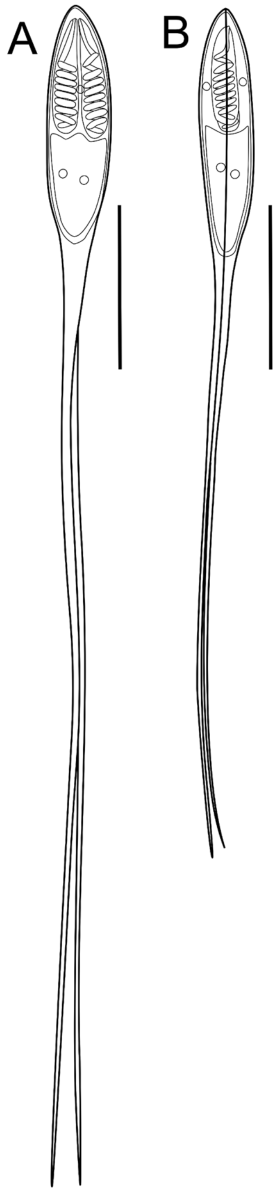

Fig. 2. Line drawings (composite) of fresh myxospores of *Henneguya postexilis*. A, Valvular view; B, sutural view. Scale bars: 10 µm.

The morphology and measurements of the spores examined in this study (Table 3) align with those reported by Minchew (1977), Woodyard et al. (2022), and Zhang et al. (2023) for *H. postexilis*. While the size of the plasmodia observed in this study surpasses prior records (see Table 3), this is probably due to the degree of maturation of the plasmodia. The plasmodia of our specimens contained many mature spores, whereas previously observed diminutive plasmodia were without mature spores (Minchew 1977). Considering that our specimens were collected from small

Table 3. Measurements and counts of *Henneguya postexilis*.

Locality Reference		Japan: Tochigi This study	USA: Missouri Minchew (1977)	USA: Mississippi Woodyard et al. (2022)	China: Jiangsu Zhang et al. (2023)
Plasmodium	length	82.8–115.7	12–80	63.1–68.8	—
	width	121.8–200. 5	12–75	56.1–57.0	—
Total myxospore	length	45.7–69.9 (56.2)	42.0–62.0 (52.0)	42.7–49.1 (45.9)	49.4–74.2 (57.9)
Spore body	length	12.9–17.9 (14.9)	13.5–17.0 (15.0)	12.1–17.2 (15.5)	13.6–18.9 (15.6)
	width	3.1–4.4 (3.8)	3.5–4.0 (3.4)	3.6–4.8 (4.1)	3.4–4.6 (4)
	thickness	2.8–3.8 (3.4)	3.5–4.0 (3.0)	2.9–3.8 (3.5)	3.1–4.4 (3.7)
Longer polar capsule	length	5.3–7.5 (6.4)	6.0–8.0 (7.0)	4.4–6.7 (5.9)	6.1–8 (7)
	width	0.9–1.6 (1.2)	1.2–2.0 (1.5)	1.1–1.6 (1.4)	1.2–1.8 (1.5)
Shorter polar capsule	length	4.9–6.8 (5.9)	5.9–7.2 (6.6)	4.4–6.4 (5.6)	5.4–7.5 (6.5)
	width	0.8–1.7 (1.2)	1.2–2.0 (1.5)	1.1–1.6 (1.4)	1.1–1.7 (1.4)
Caudal process	length	29.4–54.4 (41.4)	28.0–49.0 (37.0)	25.7–38.1 (31.2)	32.6–58 (42.7)
Number of polar tubule coil		6–9 (7.2)	6–8	6–8 (7.3)	8–10

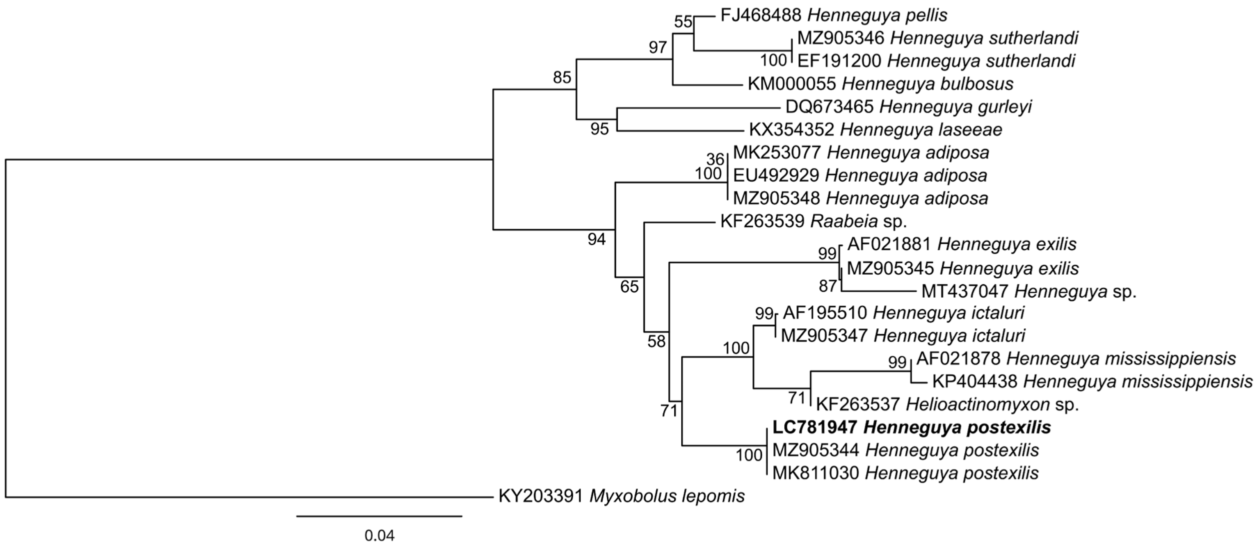

Fig. 3. Maximum likelihood phylogenetic tree based on 18S rDNA data (1513 bp including gaps) from *Henneguya postexilis* and other related myxozoans using *Myxobolus lepomis* as an outgroup. The accession number and scientific name of the newly sequenced species in this study are indicated in bold. The corresponding ISND accession numbers are shown. Arabic numerals at nodes indicate bootstrap values.

hosts during the summer, it is also possible that the variation in the plasmodium size is related to host development and/or seasonal factors. However, further comparison is unavailable since the previous reports lacked information concerning the collection date and host size (Minchew 1977; Woodyard et al. 2022).

Additionally, the 18S rDNA sequence is consistent with previous findings (Woodyard et al. 2022; Zhang et al. 2023), and consequently forms a clade with the other sequences of *H. postexilis* from USA and China in the phylogenetic tree (Fig. 3). Both morphological and molecular analysis results indicated that all the newly collected specimens were *H. postexilis*, representing a novel country record for the species.

Discussion

Henneguya postexilis has been reported in North America, China, and Japan, and all the observed host specimens were *I. punctatus*, suggesting that *H. postexilis* likely exhibits host specificity for this species (Minchew 1977; Woodyard et al. 2022; Zhang et al. 2023; this study). While our understanding of the *Henneguya* fauna parasitizing freshwater fish in Japan remains limited (see Table 1), it is a plausible conjecture that *H. postexilis* was an introduced species that coexisted and became established alongside *I. punctatus*. This hypothesis is supported by the absence of *H. postexilis* in parasite surveys conducted on other freshwater fish in the region and neighboring water systems (M. Nitta and T. Ishikawa, unpublished data).

The protozoan parasite checklist by Lom and Dyková (1992) included the silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) (Cypriniformes: Xenocyprididae) as a host for *H. postexilis*. However, the *Henneguya* species listed under silver carp were consistent with those found in *I. punctatus* in the adjacent column, except for *H. petrotschenkoi* Achmerov, 1960 (now *H. cutanea* Dogiel and Petruschewsky, 1933). Moreover, due to the absence of original data on infections by *Henneguya* species in silver carp, the *Henneguya* record associated with silver carp in Lom and

Dyková (1992) is likely a typographical error.

Myxozoans, including *Henneguya* species, rely on invertebrates and vertebrates as definitive and intermediate hosts, respectively, to complete their lifecycle (see El-Matbouli and Hoffman 1998; El-Matbouli et al. 1998; Eszterbauer et al. 2015; Okamura et al. 2015). Although the definitive host of *H. postexilis* remains unidentified, five *Henneguya* species infecting *Dero digitata* (Müller, 1773) (Clitellata: Naididae) have been documented through surveys conducted in ictalurid aquaculture ponds (Lin et al. 1999; Pote et al. 2000; Rosser et al. 2015). Furthermore, actinospores of an unidentified species have been found in *Amphichaeta* sp. (as *Amphicheta* [sic] sp.) (Naididae) (see Rosser et al. 2014), suggesting a potential association between *H. postexilis* and these clitellate oligochaetes (Woodyard et al. 2022). Both *D. digitata* and unidentified *Amphichaeta* species have been observed in the lower reaches of the Tone River system in Lake Kasumigaura (Ohtaka 2018), indicating that *H. postexilis* may have established itself in Japan by utilizing these clitellate oligochaetes as hosts.

This record highlights the potential establishment of myxozoans in Japan through the introduction of non-native fish. Importantly, certain myxozoans that parasitize freshwater fish can induce diseases in fish. For example, *Myxobolus cerebralis* (Hofer, 1903) causes whirling disease in farmed salmon, trout, and wild fish populations (see Halliday 1976). In turn, *Sphaerospora dykovae* Gunter and Adlard, 2010 (better known under its synonym "*S. renicola* Dyková and Lom 1982") (Sphaerosporidae) is a highly pathogenic and important parasite of *Cyprinus carpio* Linnaeus, 1758 (Cypriniformes: Cyprinidae) (Dyková and Lom 1982; Gunter and Adlard 2010). Furthermore, *M. cerebralis* is globally distributed as a non-native parasite and is a major cause of diseases in salmonids (see Halliday 1976; Hoffman 1990), and a fish disease caused by *S. dykovae*, which was previously known in Central Europe, has recently broken out in Taiwan (Chang et al. 2016). These myxozoan species infect *Tubifex tubifex* (Müller, 1774) (Naididae) as one of the definitive hosts, which is also present in Japan (El-Matbouli and Hoffman 1998; Molnár et al. 1999; Ohtaka 2018). Thus, if these myxozoans are introduced, they are likely to establish themselves in Japan, similar to *H. postexilis*. Moreover, because both hosts are widely distributed in natural waters in Japan, it will not be easy to prevent the spread of the disease if native individuals become infected. Another potential pathway for the invasion of these parasites is through imported live 'tubifex' oligochaetes for ornamental fish feed, as several species of myxozoans have been detected in such imports (Yoshikawa 1999; Hallett et al. 2006). It is likely to pose a significant risk to fisheries and ecosystems in Japan and making the management of the risk associated with imported invertebrates crucial to the prevention of new parasitic diseases in aquatic species.

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Authors Contributions

Masato Nitta: Conceptualization; Resources; Investigation; Visualization; Writing – original draft; Writing – review & editing; Project administration; Funding acquisition. Takanori Ishikawa: Resources.

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Declarations

Competing interests. The authors declare no conflicts of interest.

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