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Lifecycle of an introduced *Dollfustrema* (Bucephalidae) trematode in the Tone River system, Japan

Y. Saito[1], S. Iwata[1], M. Hayashi[1], M. Nitta[2], T. Ishikawa[3], T. Hagiwara[4], H. Ikezawa[5], N. Mano[3] and T. Waki[1]

[1]Toho University, Faculty of Science, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan; [2]Pathology Division, Nansei Field Station, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, 422 1, Nakatsuhamaura, Minami Ise, Watarai, Mie 516 0193, Japan; [3]College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa, 252-0880, Japan; [4]Global Environmental Forum, 8th Floor Kuramae Intelligent Bldg., 3-17-3 Kuramae, Taito, Tokyo, 111-0051, Japan and [5]Ibaraki Nature Museum, 700, Osaki, Bando, Ibaraki 306-0622, Japan

Research Paper

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Corresponding author:
T. Waki;
Email: tsukasa.waki@sci.toho-u.ac.jp

Abstract

During 2021 through 2023, the golden mussel *Limnoperna fortunei* and freshwater fishes were sampled from 28 sites in the Tone River system, Japan, and adult trematodes of *Dollfustrema* were found in the fishes. Molecular and morphological analyses based on 28S rDNA and the ITS1−5.8S−ITS2 region revealed the trematode as '*Dollfustrema hefeiense*', previously reported in Mainland China and likely introduced to Japan. Given that its scientific name was considered invalid, we re-described the species as *Dollfustrema invadens* n. sp. Additionally, the DNA-based survey helped clarify the trematode's life cycle in the river system. A sporocyst and metacercariae were detected in the golden mussel's visceral mass and in the muscles of two small freshwater fish species, respectively. The channel catfish *Ictalurus punctatus* harboured mature trematodes in its intestine, and adult trematodes were also found in the muscles of fishes infected with metacercariae, suggesting direct metacercariae development in fish muscle. Furthermore, another introduced bucephalid trematode, *Prosorhynchoides ozakii*, previously reported in the river system, was detected in the mussels and fishes. Moreover, co-infection of both bucephalid trematodes was observed in certain fishes.

Introduction

Bucephalidae Poche, 1907 is a trematode group that typically uses three hosts in its lifecycle: molluscs, fishes, and piscivorous fishes (Baba and Urabe 2015; Overstreet and Curran 2002; Shimazu 2014; Yamaguti 1975). Piscivorous fishes, the definitive hosts, harbour adult worms and shed trematode eggs through faeces. When molluscs ingest the eggs, the larvae develop into sporocysts in the host tissues. The sporocyst-infected molluscs, acting as the first intermediate host, release cercariae. The cercariae directly penetrate fishes, the second intermediate host, and develop into metacercariae. The metacercariae mature into adult worms when piscivorous fishes consume the metacercaria-infected fish. Intense bucephalid infections (e.g., from 650 to 9700 worms per fish host (Ogawa *et al.* 2004)), especially by metacercariae, have been reported to negatively affect host fish, causing haemorrhages (e.g., in fins and head skin) and abnormal swimming, sometimes leading to the host's death (Baba and Urabe 2011; Bullard and Overstreet 2008; Hoffmann *et al.* 1990; MacKenzie 1991; Ogawa *et al.* 2004).

The bucephalid trematode '*Dollfustrema hefeiense*' (an invalid name for reasons described later; the specific name was changed from '*Dollfustrema hefeiensis*' for grammatical agreement with the epithet of Gibson (2020)) is found in Mainland China (Chen 2007; Chen *et al.* 2007). Adults have been detected in the intestines of freshwater piscivorous fishes of Bagridae, Cyprinidae, and Sinipercidae, such as an oriental perch *Coreoperca whiteheadi* Boulenger, 1900; a folktaled bullhead *Tachysurus sinensis* Lacepède, 1803; the mandarin fish *Siniperca chuatsi* (Basilewsky, 1855); the big-eye mandarin fish *Siniperca kneri* Garman, 1912; a Chinese perch *Siniperca obscura* Nichols, 1930; the Leopard mandarin fish *Siniperca scherzeri* Steindachner, 1892; and the gills of the Chinese false gudgeon *Abbottina rivularis* (Basilewsky, 1852) (Chen *et al.* 2007). Although '*D. hefeiense*' has been reported from Mainland China (e.g., Chen *et al.* 2007; Zhang *et al.* 1999), its scientific name is considered invalid for the following reasons. Chen *et al.* (2007) cited Zhang *et al.* (1999) as providing the original description of '*D. hefeiense*'; however, Zhang *et al.* (1999) used 'Liu' as the authority and only provided brief information regarding the trematode's host and sampling locality without citations, morphological details, or type series. Therefore, based on ICZN13.1.1, 13.1.2 and 16.1 (ICZN 1999), Zhang *et al.* (1999) did not provide the original description, making '*D. hefeiense* Liu in Zhang, Qiu & Ding, 1999' a nomen nudum.

Zhang *et al.* (1999) and Li (2019) used 'Liu' and 'Liu, 1985' for the authority and description date for this trematode, respectively. Furthermore, Chen (2007), a doctoral thesis, cited Liu (1985) and 'Wang (1995)' regarding the morphological features of this trematode and *Dollfustrema vaneyi* (Tseng, 1930). This suggests that Liu (1985) or 'Wang (1995)' was the original description of the trematode. Liu (1985) proposed a description of '*D. hefeiensis*' as a new species, and there was no information of *D. vaneyi*. However, Liu (1985) was a handwritten manuscript and/or its copies. The remaining candidate 'Wang (1995)' was a doctoral thesis which is not open to the public and we could not obtain, indicating that these publications cannot be considered the original descriptions under ICZN 8.1 and 9.1 (ICZN 1999). Moreover, later publications by the author of 'Wang (1995)' (Wang and Wang 1998a, b, c) do not mention '*D. hefeiense*' although *D. vaneyi* was only provided (Wang *et al.* 1998b), suggesting that 'Wang (1995)' was not the original description. Chen (2007) and Chen *et al.* (2007) briefly described the trematode's morphology without providing figures, stating that '*D. hefeiense*' is morphologically similar to *D. vaneyi* but distinguishable by genital pore position and DNA barcodes. Li (2019) provided a detailed description and illustration of the trematode, which matched information provided by Liu (1985). However, because none of these publications (Chen 2007; Chen *et al.* 2007; Li 2019) explicitly intended to describe a new species, they do not qualify as original descriptions of the parasite based on IZCN 16.1 (ICZN 1999). Consequently '*D. hefeiense* Liu, 1985' is considered an invalid name as per ICZN 10.1 (ICZN 1999). Moreover, Nolan and Cribb (2010) and Nolan *et al.* (2015) reported that the original description for this species was unavailable. Given that no valid synonym exists (refer to the synonyms listed below), '*D. hefeiense*' must be described as a new species.

Bucephalid trematodes were not detected in Japanese freshwater until 1998 (Shimazu 2003; Urabe *et al.* 2001). However, in 1999, a heavy infestation of bucephalid metacercariae was suddenly discovered in cyprinid fishes from the Uji River, central Japan (Urabe *et al.* 2001). The metacercariae were later identified as two species: *Prosorhynchoides ozakii* (Nagaty, 1937) and *Parabucephalopsis parasiluri* Wang, 1985, both found across a wide area of the Uji River system (Baba and Urabe 2011; Ogawa *et al.* 2004; Urabe *et al.* 2007). In 2019, *Pr. ozakii* was detected in freshwater fish from the Tone River system, east Japan (Hayashi *et al.* 2022). These two species are thought to have been introduced to Japan along with the golden mussel *Limnoperna fortunei* (Dunker, 1857), which serves as the first intermediate host for both bucephalid trematodes in Japan (Baba and Urabe 2011; Hayashi *et al.* 2022). It is thought that the golden mussel was introduced to Japan around 1990 along with the Asian clam *Corbicula fluminea* (Muller, 1774), imported from Mainland China for freshwater aquaculture (Magara *et al.* 2001; Nishimura and Habe 1987). An unidentified bucephalid sporocyst was also detected in golden mussels from the Yodo River system, Japan (Hayakawa *et al.* 2019).

Following the introduction of *Pr. ozakii* to the Tone River system (Hayahshi *et al.* 2022), we conducted surveys of molluscs and fishes to monitor bucephalid infections in this system. In 2021, we occasionally found adult trematodes of the genus *Dollfustrema* in freshwater fishes in the water system. Molecular and morphological analyses confirmed that the *Dollfustrema* species was identified as '*D. hefeiense*', as reported in previous studies (Chen 2007; Chen *et al.* 2007; Li 2019). Additionally, a DNA-based survey allowed us to trace the larval stages of the trematode in the water system. The objectives of the present study are to describe this species, document its introduction to Japan, and determine its life cycle in the water system.

Materials and methods

Mussel and fish survey

Golden mussels were sampled from seven sites in the Tone River system from 2021 to 2023 (Figure 1). The mussels were transported to the laboratory, identified following Masuda and Uchiyama (2004), and then killed with knives and subsequently dissected to search for sporocysts under a stereomicroscope. When sporocysts were found, they were fixed and preserved in 70% or 99% ethanol. Sporocyst tissues from randomly selected mussels at each site were used for polymerase chain reaction (PCR) to identify species, as described later.

Freshwater fishes were either sampled or purchased from fisheries in 28 sites between 2021 and 2023 (Figure 1). They were transported to the laboratory and identified following Nakabo (2013) and Fukuchi *et al.* (2018), after which they were dissected to examine the fins, gills, muscles, and internal organs both with the naked eye and under a stereomicroscope. When metacercariae and adult bucephalid trematodes were detected, the worms were fixed for detailed morphological observations and PCR as described later. The scientific names of the fishes used in this study follow those of Froese and Pauly (2024), and common names basically follow Froese and Pauly (2024) and Hosoya (2019).

Morphological observations

From fish analyses, we detected bucephalid adults and metacercariae of two trematode species: *Pr. ozakii*, the only species previously reported in the Tone River system (Hayashi *et al.* 2022), and '*D. hefeiense*'. Notably, '*D. hefeiense*' and *Pr. ozakii* were easily distinguished at both adult and metacercaria stages, including under the stereomicroscope, as they differ in body colour (yellow or brown in '*D. hefeiense*' vs. white in *Pr. ozakii*), body shape (elongate oval or pear-shaped vs. oval or ellipsoid), and rhynchus shape (truncate with spines vs. rounded without spines) (Figure 2b). Species identifications were confirmed through molecular analyses and detailed morphological observations of slide-mounted specimens.

For the morphological observations, selected adults and metacercariae were fixed in 70% or 99% ethanol between cover slips and glass slides. These specimens were stained with alum carmine or Heidenhein's iron hematoxylin, dehydrated in an ethanol series, cleared in xylene or creosote, and mounted on slides with Canada balsam. Some adults and metacercariae were identified as *Pr. ozakii* through comparisons with morphological data reported by Hayashi *et al.* (2022). The remaining adults and metacercariae were morphologically and molecularly identified as '*D. hefeiense*', as described below. Selected specimens (type series and additional specimens) were measured for body and organ size using a light microscope (BX53, Olympus) and a digital camera unit (AdvanCam-U3II, Advan Vision) to provide morphological descriptions. Observations were made ventrally, and line drawings were created using a camera lucida (U-DA, Olympus) attached to the microscope.

Cercariae from a fixed sporocyst molecularly identified as '*D. hefeiense*' (see below) were mounted on slides with Hoyer's medium for several hours and observed under the microscope (as described above). The type series and sporocyst were deposited

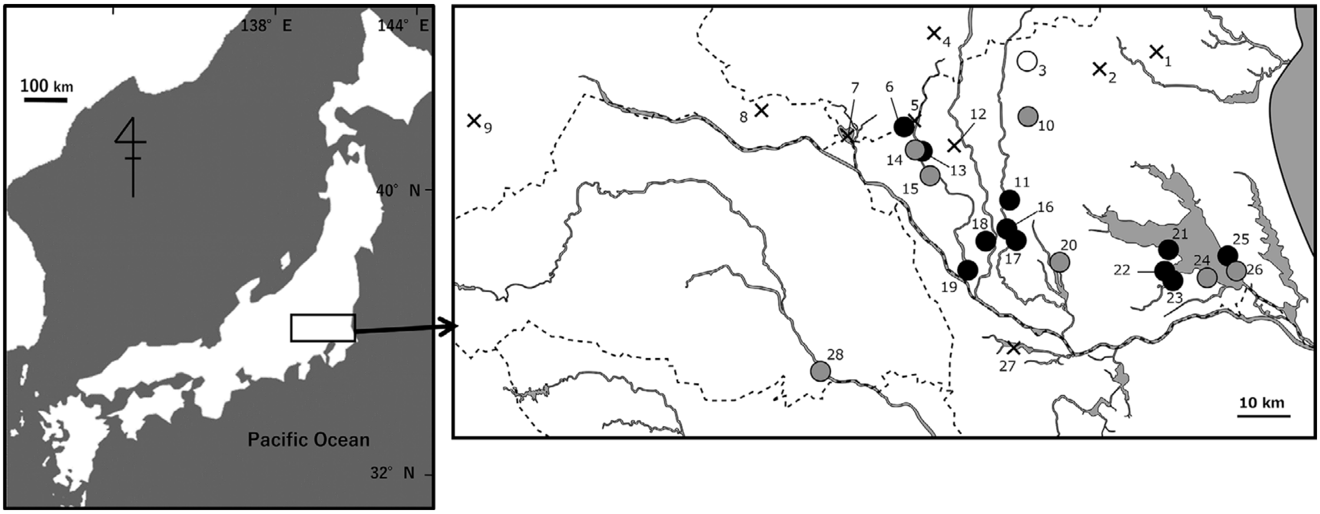

Figure 1. Sampling sites from the present study, numbered from 1 to 28. Site numbers correspond to those given in Table 1. Black circles: only *Prosorhynchoides ozakii* detected. White circle: only *Dollfustrema invadens* n. sp. detected. Grey circles: both species detected. Crosses: neither species detected.

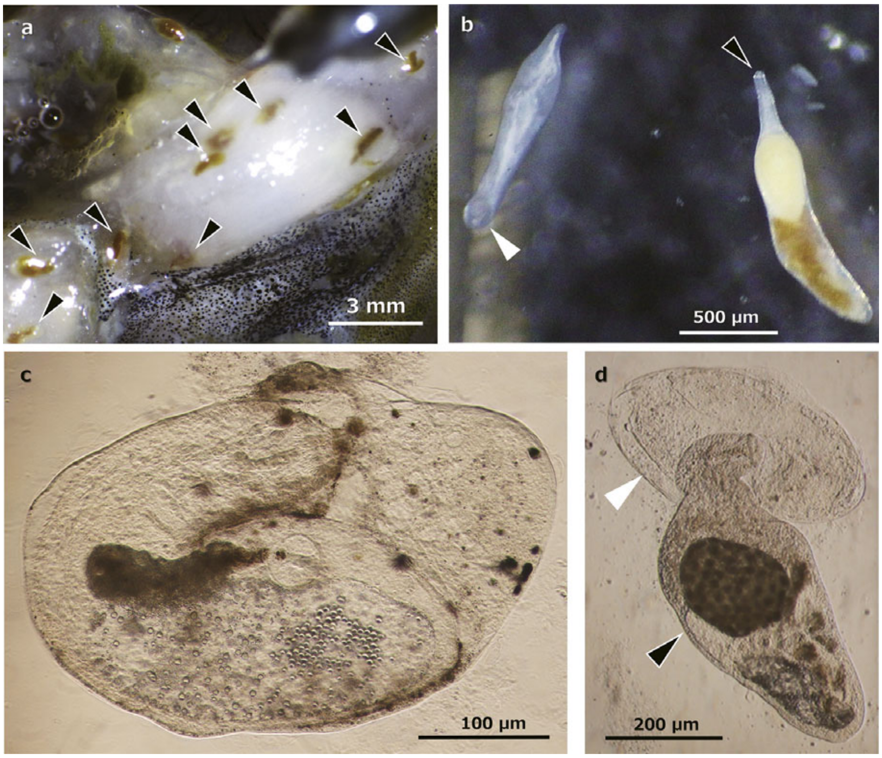

Figure 2. *Dollfustrema invadens* n. sp. from the present study. (a) Pectoral fin base of the short-spined Japanese trident goby *Tridentiger brevispinis.* Black arrowhead: *D. invadens* n. sp. adults in muscle tissue. (b) *D. invadens* n. sp. adult (black arrowhead) and *Prosorhynchoides ozakii* metacercariae (white arrowhead) under a stereomicroscope. Arrowhead position indicates the rhynchus in each species. (c, d) *D. invadens* n. sp. metacercariae from the bluegill *Lepomis macrochirus.* c, encysted metacercaria; d, metacercaria and its cyst wall. Black and white arrowheads indicate the metacercaria and cyst wall, respectively.

at Ibaraki Nature Museum, Japan. Selected *Pr. ozakii* adults and metacercariae were also stained and mounted for museum deposition.

Molecular analysis

Alkaline lysates of sporocysts, metacercariae, and small tissue pieces of adults were used as templates for PCR, targeting nuclear 28S ribosomal RNA (rDNA) and the ITS1–5.8S–ITS2 region. The PCR primer sets were digl2 and LSU1500R (Snyder and Tkach 2001; Tkach *et al.* 2016) for 28S rDNA and BD1 and BD2 (Chen *et al.* 2007) for the ITS1–5.8S–ITS2 region. DNA amplification and sequencing were performed as described by Nakao *et al.* (2017). Alignment datasets were prepared using BioEdit Sequence Alignment Editor (Hall 1999) for data comparison. Sequences from related species were retrieved from the International Nucleotide Sequence Database Collaboration (INSDC: DDBJ/ENA/GenBank). The 28S rDNA and ITS1–5.8S–ITS2 datasets were employed for comparisons between the sporocyst, metacercariae, and adult sequences to identify species via pairwise divergence values (*p*-distance) using MEGA X (Kumar *et al.* 2018). Phylogenetic analyses were performed based on ITS1−5.8S−ITS2 and 28S rDNA sequences of the new species and related species retrieved from INSDC in MEGA X, using the maximum likelihood method with 1,000 bootstrap replicates. MEGA X selected the Kimura 2-parameter model (Kimura 1980) and the HKY model (Hasegawa *et al.* 1985) for ITS1−5.8S−ITS2 and 28S rDNA trees, respectively.

Results

Mussel survey

In total, 730 mussels were sampled from six sites during the survey (Table 1; Figure 1). Golden mussels with sporocysts were found at five of the six sites, with 77 infected mussels in total. From these, sporocysts from randomly selected mussels at each site (12 sporocysts in total) were used for PCR analysis. Results revealed that only one sporocyst was '*D. hefeiense*', whereas the remaining 11 were confirmed as *Pr. ozakii* through later molecular analysis (Table 1).

Fish survey

In total, 1,237 fish, representing 38 species, were collected from 28 sampling sites (Figure 1; Table 2). Adults of '*D. hefeiense*' were found in eight fish species (Table 2): yoshinobori goby *Rhinogobius* sp., the river sleeper *Odontobutis potamophila*, the short-spined Japanese trident goby *Tridentiger brevispinis*, the swamp moroko gudgeon *Gnathopogon elongatus*, the sugo moroko gudgeon *Squalidus chankaensis*, the Chinese false gudgeon *Abbottina rivularis*, the channel catfish *Ictalurus punctatus*, and the bluegill *Lepomis macrochirus.* Among the species, only the *T. brevispinis* and *Rhinogobius* sp. are native to the survey area (Hosoya 2019). Three species of gudgeon are introduced species from western Japan (Hosoya 2019), whereas the river sleeper likely originated from East Asia (Fukuchi *et al.* 2018). The channel catfish and the bluegill were introduced to Japan from North America (Hosoya 2019). Adults were found in the intestines of the channel catfish, the gill tissues of the river sleeper, and the muscles and fin tissues of the other six host fishes. A few encysted metacercariae of '*D. hefeiense*' were found in the fin tissues and muscles of the swamp moroko gudgeon and the bluegill (Figure 2c, d).

Regarding *Pr. ozakii*, metacercariae were detected in the fins and epidermis of 504 individuals from 21 fish species across 18 sites (Table 2). Adults of *Pr. ozakii* were found only in the intestines of the channel catfish, as noted by Hayashi *et al.* (2022). In addition to the 13 second intermediate host species reported by Hayashi *et al.* (2002), 10 fish species were newly identified as metacercariae hosts in the present study: *Cyprinus rubrofuscus*, *Carassius langsdorfii*, *Carassius auratus*, *Zacco platypus*, *Squalidus chankaensis*, *Micropterus salmoides*, *Odontobutis potamophila*, *Gymnogobius castaneus*, *Gymnogobius urotaenia*, and *Tachysurus sinensis.* Metacercariae were found in the fins and fin base tissues in all hosts, except *Tachysurus sinensis*, in which they were found only in the liver. Slide specimens of *Pr. ozakii* were deposited in the Ibaraki Nature Museum, Japan, as follows: adults (slide specimens), INM-1-123584; metacercariae (slide specimens), INM-1-123585; sporocysts (molecularly identified and preserved in 99% ethanol), INM-1-123586.

Molecular analysis

DNA fragments of the ITS1−5.8S−ITS2, amplified by one primer set, from 10 '*D. hefeiense*' adults and three *Dollfustrema* metacercariae were identical (766 bp). The sequence alignment (733 bp) of ITS1−5.8S−ITS2 from the database showed intraspecific divergence values of 0.000−0.016 and interspecific variations of 0.011−0.075 within the genus *Dollfustrema* (Supplementary Table S1). Divergence between our sequences and those of '*D. hefeiense*' reported by Chen *et al.* (2007) was 0.000−0.005 (Supplementary Table S1), indicating that the adults and metacercariae in both studies belong to the same species: '*D. hefeiense*'. In the ITS1−5.8S−ITS2 phylogenetic tree, our sequences formed a single clade with the '*D. hefeiense*' sequences from the previous study (Chen *et al.* 2007) (Figure 3a), supporting this identification.

For 28S rDNA, DNA fragments (829−1174 bp) from five adults, one metacercaria, and one sporocyst were also identical. Sequence comparisons from online databases (745 bp) revealed intraspecific divergence values of 0.000−0.004 and interspecific variations of 0.033−0.060 within the genus *Dollfustrema* (Supplementary Table S2). Given the divergence values of zero between our sequences, the sporocyst, metacercariae, and adults were confirmed as a single species. In the 28S rDNA phylogenetic tree, the sporocyst sequence formed a single clade with the other '*D. hefeiense*' sequences, supporting our molecular identification. Additionally, this new species occupied an independent phylogenetic position, even in this conserved gene.

The remaining 11 sporocysts could not be identified via our analysis, as their 28S rDNA sequences (884−1253 bp) differed from those of *Dollfustrema* spp. (0.123−0.140; Supplementary Table S2).

Table 1. Detection of sporocysts of *Dollfustrema invadens* n. sp. and *Prosorhynchoides ozakii* from the golden mussels. Site numbers correspond to those given in Figure 1 (only sites where mussels were sampled are shown). Pref.: Prefecture

Locality	Date (s)	No. examined	No. infected	No. used for PCR	Prevalence of sporocysts
Kawasakimachi, Joso City, Ibaraki Pref.	Jan. 21, 2022	177	50	1	28.2%
Kitayama, Tsukubamirai City, Ibaraki Pref.	Dec. 14, 2021–Dec. 10, 2022	169	11	1	6.5%
Sugaomachi, Joso City, Ibaraki Pref.	Jan. 21, 2022	38	2	2	5.3%
Takasaki, Tsukuba City, Ibaraki Pref.	Jun. 19, 2021	1	1	1*	100%
	Jun. 24, 2022	4	0	-	0%
Magaki, Mihomura, Inashiki-gun, Ibaraki Pref.	Apr. 14, 2023	11	3	3	27.3%
Aso, Namegata City, Ibaraki Pref.	Jun. 16–Jul. 22, 2023	330	10	4	3.0%

*Identified as *D. invadens* n. sp. (the other sporocysts were *Pr. ozakii*)

Table 2. Detection of adults and metacercariae of *Dollfustrema invadens* n. sp. and *Prosorhynchoides ozakii* from fish. Site numbers correspond to those given in Figure 1. Pref.: Prefecture

					Dollfustrema invadens n. sp.		*Prosorhynchoides ozakii*	
Site No.	Locarity	Date (s)	Fish species	No. examined	No. infected (%)	Intensity (mean±SD) [ind./host] No. of adults unless otherwise stated	No. infected (%)	Intensity (mean±SD) [ind./host] No. of metacercariae unless otherwise stated
1	Obara, Kasama City, Ibaraki Pref.	Sep. 10, 2023	*Tridentiger brevispinis*	3	0(0)	0	0(0)	0
			Pseudorasbora parva	1	0(0)	0	0(0)	0
			Rhinogobius sp.	2	0(0)	0	0(0)	0
			Tachysurus tokiensis	1	0(0)	0	0(0)	0
			Lepomis macrochirus	2	0(0)	0	0(0)	0
2	Fukuhara, Kasama City, Ibaraki Pref.	Sep. 17, 2023	*Tachysurus tokiensis*	8	0(0)	0	0(0)	0
			Nipponocypris temminckii	6	0(0)	0	0(0)	0
			Zacco platypus	1	0(0)	0	0(0)	0
			Gnathopogon elongatus	2	0(0)	0	0(0)	0
3	Nakaizumi, Sakuragawa City, Ibaraki	Aug. 13, 2023	*Misgurnus anguillicaudatus*	4	0(0)	0	0(0)	0
			Micropterus nigricans	1	0(0)	0	0(0)	0
			Tridentiger brevispinis	3	1(33.3)	7	0(0)	0
			Rhinogobius sp.	3	2(66.7)	17.5±9.2 (11–24)	0(0)	0
			Gnathopogon elongatus	1	1(100)	3	0(0)	0
4	Nakadairyou, Shimotsuke City, Tochigi Pref.	Oct. 14, 2023	*Zacco platypus*	4	0(0)	0	0(0)	0
			Pungtungia herzi	2	0(0)	0	0(0)	0
			Pseudogobio polystictus	2	0(0)	0	0(0)	0
			Pseudorasbora parva	2	0(0)	0	0(0)	0
			Gnathopogon elongatus	26	0(0)	0	0(0)	0
			Silurus asotus	2	0(0)	0	0(0)	0
			Carassius langsdorfii	2	0(0)	0	0(0)	0
5	Takei, Oyama City, Tochigi Pref.	Sep. 30, 2023	*Misgurnus anguillicaudatus*	1	0(0)	0	0(0)	0
			Tridentiger brevispinis	1	0(0)	0	0(0)	0
			Pseudorasbora parva	2	0(0)	0	0(0)	0
			Gnathopogon elongatus	1	0(0)	0	0(0)	0
6	Higashinoda, Oyama City, Tochigi Pref.	Sep. 30, 2023	*Gnathopogon elongatus*	1	0(0)	0	0(0)	0
			Rhodeus ocellatus	7	0(0)	0	2(28.6)	14.5±12.0 (6–23)
			Rhinogobius sp.	4	0(0)	0	0(0)	0

(*Continued*)

Table 2. (*Continued*)

					Dollfustrema invadens n. sp.		*Prosorhynchoides ozakii*	
Site No.	Locarity	Date (s)	Fish species	No. examined	No. infected (%)	Intensity (mean±SD) [ind./host] No. of adults unless otherwise stated	No. infected (%)	Intensity (mean±SD) [ind./host] No. of metacercariae unless otherwise stated
			Misgurnus anguillicaudatus	1	0(0)	0	0(0)	0
			Carassius langsdorfii	1	0(0)	0	0(0)	0
			Cyprinus rubrofuscus	3	0(0)	0	0(0)	0
7	Fujiokamachi, Tochigi City, Tochigi Pref.	Aug. 28, 2023	*Ictalurus punctatus*	2	0(0)	0	0(0)	0
			Silurus asotus	23	0(0)	0	0(0)	0
			Tachysurus sinensis	12	0(0)	0	0(0)	0
			Hemibarbus labeo	5	0(0)	0	0(0)	0
			Anguilla japonica	4	0(0)	0	0(0)	0
8	Hinagacho, Tatebayashi City, Gumma Pref.	Oct. 8, 2023	*Rhodeus ocellatus*	24	0(0)	0	0(0)	0
			Abbottina rivularis	3	0(0)	0	0(0)	0
			Oryzias latipes	2	0(0)	0	0(0)	0
			Rhinogobius nagoyae	8	0(0)	0	0(0)	0
9	Okamoto, Tomioka City, Gumma Pref.	Apr. 22, 2023	*Pseudorasbora parva*	8	0(0)	0	0(0)	0
			Rhinogobius fluviatilis	1	0(0)	0	0(0)	0
			Rhinogobius sp.	23	0(0)	0	0(0)	0
			Lefua echigonia	1	0(0)	0	0(0)	0
			Rhynchocypris lagowskii	12	0(0)	0	0(0)	0
10	Oshibi, Tikusei City, Ibaraki Pref.	Aug. 13, 2023	*Zacco platypus*	7	0(0)	0	0(0)	0
			Carassius langsdorfii	2	0(0)	0	0(0)	0
			Channa argus	2	0(0)	0	0(0)	0
			Cyprinus rubrofuscus	1	0(0)	0	1(100)	1
			Abbottina rivularis	2	2(100)	5.0±5.7 (1–9)	1(50)	4
			Pseudorasbora parva	2	0(0)	0	0(0)	0
			Misgurnus anguillicaudatus	1	0(0)	0	0(0)	0
11	Kamigo, Tsukuba City, Ibaraki Pref.	Dec. 14, 2021	*Micropterus nigricans*	1	0(0)	0	1(100)	396
12	Setoi, Yachitomachi, Ibaraki Pref.	Sep. 17, 2023	*Carassius buergeri*	18	0(0)	0	0(0)	0
			Abbottina rivularis	2	0(0)	0	0(0)	0
			Rhinogobius sp.	1	0(0)	0	0(0)	0

(*Continued*)

Table 2. (*Continued*)

					Dollfustrema invadens n. sp.		*Prosorhynchoides ozakii*	
Site No.	Locarity	Date (s)	Fish species	No. examined	No. infected (%)	Intensity (mean±SD) [ind./host] No. of adults unless otherwise stated	No. infected (%)	Intensity (mean±SD) [ind./host] No. of metacercariae unless otherwise stated
13	Eguchi, Koga City, Ibaraki Pref.	Sep. 30, 2023	*Gymnogobius urotaenia*	4	0(0)	0	4(100)	7.5±2.4(5–10)
			Rhinogobius sp.	2	0(0)	0	2(100)	8.5±10.6(1–16)
			Abbottina rivularis	1	0(0)	0	0(0)	0
			Pseudorasbora parva	1	0(0)	0	0(0)	0
14	Nirei, Koga City, Ibaraki Pref.	Sep. 30, 2023	*Rhinogobius* sp.	2	2(100)	13.0±14.1 (3–23)	2(100)	36.5±9.2(30–43)
			Tridentiger brevispinis	3	1(33.3)	4	3(100)	131.3±221.4(2–387)
			Abbottina rivularis	9	6(66.7)	18.7±33.7 (1–87)	8(88.9)	50.5±42.8(6–142)
			Misgurnus anguillicaudatus	1	0(0)	0	0(0)	0
15	Sakasai, Bandou City, Ibaraki Pref.	Sep. 2, 2023	*Odontobutis potamophila*	5	5(100)	13.0±8.9 (3–25)	5(100)	192.8±395.5(4–900)
			Hemibarbus labeo	2	0(0)	0	0(0)	0
			Gnathopogon elongatus	1	1(100)	95	1(100)	5839
			Abbottina rivularis	2	2(100)	3.5±3.5 (1–6)	2(100)	128.5±106.8(53–204)
			Tridentiger brevispinis	2	2(100)	3.5±2.1 (2–5)	2(100)	79.5±27.6(60–99)
			Carassius langsdorfii	1	0(0)	0	1(100)	58
			Lepomis macrochirus	1	0(0)	0	0(0)	0
16	Kawasakimachi, Joso City, Ibaraki Pref.	Jul. 29, 2022	*Carassius langsdorfii*	1	0(0)	0	0(0)	0
			Cyprinus rubrofuscus	11	0(0)	0	6(54.5)	12.8±12.6(3–31)
			Gnathopogon elongatus	2	0(0)	0	1(50)	2
17	Kitayama, Tsukubamirai City, Ibaraki Pref.	Dec. 10, 2022	*Gymnogobius urotaenia*	1	0(0)	0	1(100)	1541
18	Toyookamachi, Joso City, Ibaraki Pref.	Dec. 11, 2021	*Abbottina rivularis*	2	0(0)	0	1(100)	1
			Lepomis macrochirus	8	0(0)	0	0(0)	0
19	Sugaomachi, Joso City, Ibaraki Pref.	Jan. 21, 2022	*Zacco platypus*	41	0(0)	0	36(87.8)	192.5±264.1(3–969)
			Carassius cuvieri	1	0(0)	0	0(0)	0
20	Takasaki, Tsukuba City, Ibaraki Pref.	Apr. 14, 2022–Jun. 24, 2023	*Rhinogobius* sp.	9	1(11.1)	12	1(11.1)	26
			Hemibarbus labeo	2	0(0)	0	2(100)	15.5±3.5(13–18)
			Zacco platypus	2	0(0)	0	1(50)	80
			Gymnogobius urotaenia	3	0(0)	0	1(33.3)	1969

(*Continued*)

Table 2. (*Continued*)

					Dollfustrema invadens n. sp.		*Prosorhynchoides ozakii*	
Site No.	Locarity	Date (s)	Fish species	No. examined	No. infected (%)	Intensity (mean±SD) [ind./host] No. of adults unless otherwise stated	No. infected (%)	Intensity (mean±SD) [ind./host] No. of metacercariae unless otherwise stated
21	Magaki, Mihomura, Inashiki-gun, Ibaraki Pref.	Jul. 18, 2022–Mar. 5, 2023	*Pseudorasbora parva*	108	0(0)	0	56(51.9)	97.3±120.7(2–524)
			Rhodeus ocellatus	273	0(0)	0	182(66.7)	34.1±46.8(1–301)
			Tridentiger brevispinis	10	0(0)	0	1(10)	1
			Gnathopogon elongatus	9	0(0)	0	0(0)	0
			Carassius langsdorfii	2	0(0)	0	1(50)	16
			Carassius buergeri	3	0(0)	0	3(100)	39.3±29.7(17–73)
			Lepomis macrochirus	2	0(0)	0	0(0)	0
			Abbottina rivularis	22	0(0)	0	2(9.1)	25.5±26.2(7–44)
			Gymnogobius castaneus	1	0(0)	0	1(100)	1
			Tachysurus sinensis	1	0(0)	0	0(0)	0
			Gymnogobius urotaenia	2	0(0)	0	2(100)	105.0±56.6(65–145)
22	Shidafutto, Inashiki City, Ibaraki Pref.	Jun. 18–Nov. 6, 2022	*Tachysurus fulvidraco*	1	0(0)	0	1(100)	Not counted***
			Opsariichthys uncirostris	14	0(0)	0	2(14.3)	2.5±2.1(1–4)
			Hyporhamphus intermedius	1	0(0)	0	0(0)	0
			Acheilognathus macropterus	63	0(0)	0	53(84.1)	266.8±377.9(3–1706)
			Pseudorasbora parva	45	0(0)	0	29(64.4)	231.6±210.8(2–664)
			Rhodeus ocellatus	1	0(0)	0	1(100)	151
			Tridentiger brevispinis	26	0(0)	0	7(26.9)	405.6±594.3(2–1622)
			Gnathopogon elongatus	21	0(0)	0	17(81)	37.4±36.3(1–132)
			Gymnogobius urotaenia	115	0(0)	0	3(2.6)	3.3±3.2(1–7)
			Rhinogobius sp.	24	0(0)	0	0(0)	0
			Ischikauia steenackeri	4	0(0)	0	0(0)	0
			Carassius langsdorfii	1	0(0)	0	0(0)	0
			Abbottina rivularis	2	0(0)	0	1(50)	35
			Lepomis macrochirus	9	0(0)	0	6(66.7)	55.7±66.3(5–183)
			Squalidus chankaensis	1	0(0)	0	0(0)	0
			Zacco platypus	1	0(0)	0	1(100)	8

(*Continued*)

Table 2. (*Continued*)

					Dollfustrema invadens n. sp.		*Prosorhynchoides ozakii*	
Site No.	Locarity	Date (s)	Fish species	No. examined	No. infected (%)	Intensity (mean±SD) [ind./host] No. of adults unless otherwise stated	No. infected (%)	Intensity (mean±SD) [ind./host] No. of metacercariae unless otherwise stated
23	Futto, Inashiki City, Ibaraki Pref.	Sep. 29–Oct. 20, 2023	*Ictalurus punctatus*	11	0(0)	0	1(9.1)	10****
			Acheilognathus macropterus	2	0(0)	0	2(100)	104.0±113.1(24–184)
			Pseudorasbora parva	1	0(0)	0	1(100)	188
24	Ukishima, Inashiki City, Ibaraki Pref.	Aug. 25–Dec. 10, 2022	*Squalidus chankaensis*	9	1(11)	3	9(100)	179.1±222.5(4–707)
			Acheilognathus macropterus	13	0(0)	0	13(100)	406.2±436.1(10–1569)
			Pseudorasbora parva	9	0(0)	0	8(88.9)	78.6±102.2(2–291)
			Gnathopogon elongatus	16	11(69)	4.4±3.5 (2–14)*	10(62.5)	31.0±37.4(4–125)
			Pseudaspius hakonensis	1	0(0)	0	1(100)	1102
			Ischikauia steenackeri	2	0(0)	0	0(0)	0
			Rhinogobius sp.	17	0(0)	0	0(0)	0
25	Shimanami, Namegata City, Ibaraki Pref.	Jul. 22, 2023	*Ictalurus punctatus*	1	0(0)	0	1(100)	61****
26	Aso, Namegata City, Ibaraki Pref.	Jun. 16, 2023	*Tridentiger brevispinis*	1	1(100)	62	1(100)	235
27	Teganuma, Kashiwa City, Chiba Pref.	Jun. 24, 2022	*Channa argus*	1	0(0)	0	0(0)	0
28	Dai, Asaka City, Saitama Pref.	May 27, 2023	*Ictalurus punctatus*	3	2(66.7)	116±160 (2–229)	2(66.7)	7 (7–7)****
			Lepomis macrochirus	1	1(100)	175**	1(100)	268

*Four metacercariae were included.
**Twenty-five metacercariae were included.
***Metacercariae infection was found in liver.
****Adults were only detected.

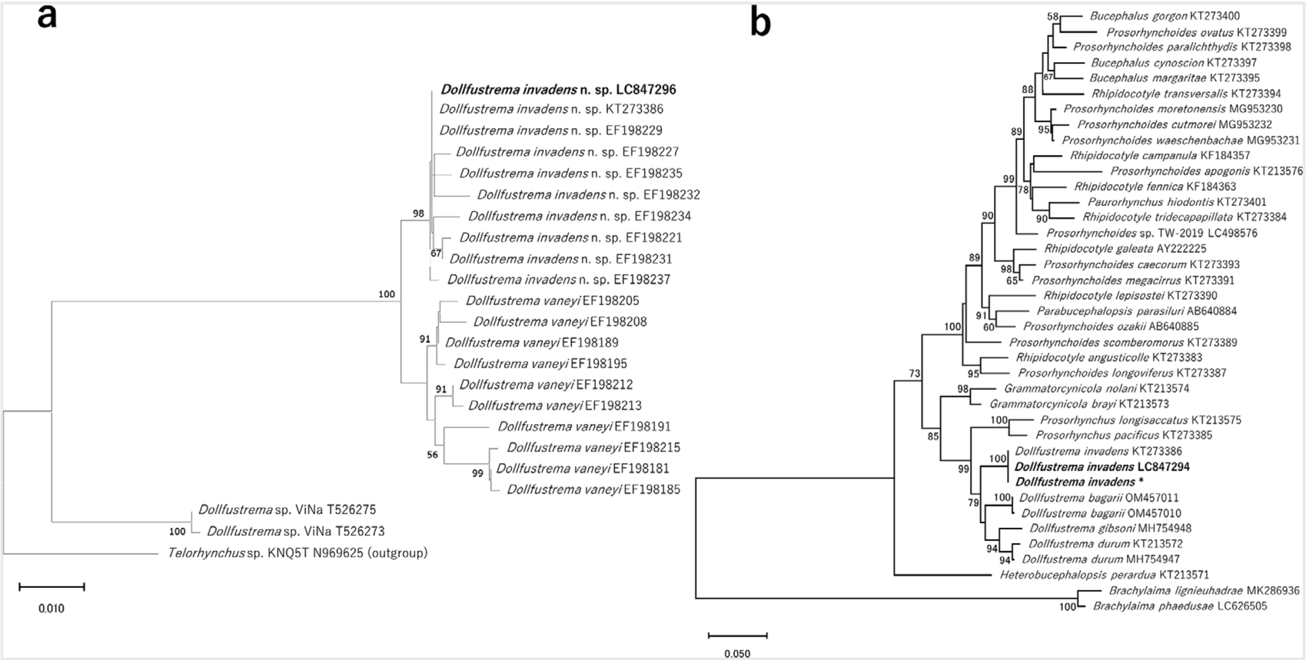

Figure 3. Maximum-likelihood phylogenetic tree for trematodes of the genus *Dollfustrema* inferred from the partial nucleotide sequences of ITS1−5.8S−ITS2 (763 bp) (a) and 28S rDNA (909 bp) (b). The trematode isolates from the present study are shown in bold. Bootstrap values >50% are shown, and INSDC accession numbers are provided after scientific names. *Telorhynchus* sp. KNQ5T (N969625) and *Brachylaima* spp. (MK286936 and LC626505) comprised the outgroups. Asterisk indicates a sequence identical to LC847294.

These unidentified sporocysts were suspected to be *Pr. ozakii*, so we compared their sequences with those of *Prosorhynchoides* spp. from online databases. Based on 28S rDNA sequences (784 bp), intraspecific divergence in *Prosorhynchoides* was zero, whereas interspecific variation was 0.006−0.112 (Supplementary Table S3). Our sporocyst sequences were identical to those of *Pr. ozakii* from previous studies, confirming that these 11 sporocysts belong to *Pr. ozakii.*

The sequences of '*D. hefeiense*' and *Pr. ozakii* obtained in this study were deposited in the INSDC through DNA Data Bank of Japan under the following accession numbers: new species LC847294 (28S rDNA: 1174 bp) and LC847296−847297 (ITS1−5.8S−ITS2: 763 bp); *Pr. ozakii* LC847295 (28S rDNA: 1253 bp).

Morphological descriptions of *Dollfustrema invadens* Saito, Iwata, Nitta & Waki n. sp.

The specimens used for morphological descriptions included five gravid adults (holotype and four paratypes) and one metacercaria (paratype) from tissue of the swamp moroko gudgeon *Gnathopogon elongatus*, as well as five cercariae from a sporocyst in the golden mussel. For data comparison, morphological characters of four gravid adults from an intestine of the channel catfish *Ictalurus punctatus* were reported as additional materials. All measurements are presented at averages, with ranges in parentheses, and are in micrometres unless otherwise stated.

Adult (based on holotype and three paratypes, Figure 4a–c, Table 3)

Body elongated oval or pear-shaped, 888 (783–945) in length and 215 (194–215) in width at widest point. Body yellow or brown in living condition. Tegmental spines on body surface. Suckers absent. Rhynchus small, truncate, 75 (56–95) in length and 67 (54–77) in width, with muscular apical disc. Three rows of rhynchal spines circling the anterior portion of rhynchus. Rhynchal spines larger in middle row, 6.3 (5.4–7.0) in length, and shorter in anterior and posterior rows, 3.6 (2.3–4.5) in length. Mouth opening ventral surface, posterior to anterior margin of anterior testis, from middle to two-third of body. Pharynx globular, 56 (50–62) in length and 51 (43–59) in width. Esophagus short, 21 (16–30) in length, extending anteriorly from pharynx. Intestinal cecum oblong, 182 (173–191) in length and 89 (60–99) in width. Testes two, ovoid, slightly lobed, placed obliquely. Anterior testis 118 (99–155) in length and 68 (47–88) in width. Posterior testis 106 (77–135) in length and 71 (59–81) in width. Cirrus pouch cylindrical, 229 (159–253) in length and 59 (53–64) in width. Seminal vesicle ovoid, in proximal part of cirrus pouch. Seminal duct, proximal part of prostatic duct in Bucephalidae (Overstreet and Curran 2002), from distal portion of seminal vesicle. Boundaries of seminal vesicle and seminal duct, and seminal duct and pars prostatica unclear. Pars prostatica well developed, looped at the level of cirrus pouch, surrounded by prostate gland cells. Genital lobe wide but its detail indistinct. Genital atrium posterior to ejaculatory duct. Genital pore opening just posterior to posterior margin of genital atrium. Ovary elliptic, situated between testes, 94 (91–100) in length and 70 (42–87) in width. Uterus extends from anterior fourth of body to nearly posterior end of cirrus pouch. Uterus wall indistinct. Metraterm lateral to cirrus pouch, opening into genital atrium. Eggs elongated oval, 32 (29–36) in length and 15 (13–18) in width. Vitelline follicles distributed dome-shape, extending forward beyond anterior terminal of caecum. Excretory pore at posterior terminal of body.

Metacercaria (based on one paratype, Figure 4d)

Excysted body elongated oval, 533 and 134 in length and greatest width, respectively. Body yellow-brown in living condition. Tiny tegmental spines on surface of body. Suckers absent. Rhynchus with muscular apical disc. Rhynchus small and truncate, 58 in length and 54 in width at widest point, with three rows of tiny rhynchal spines.

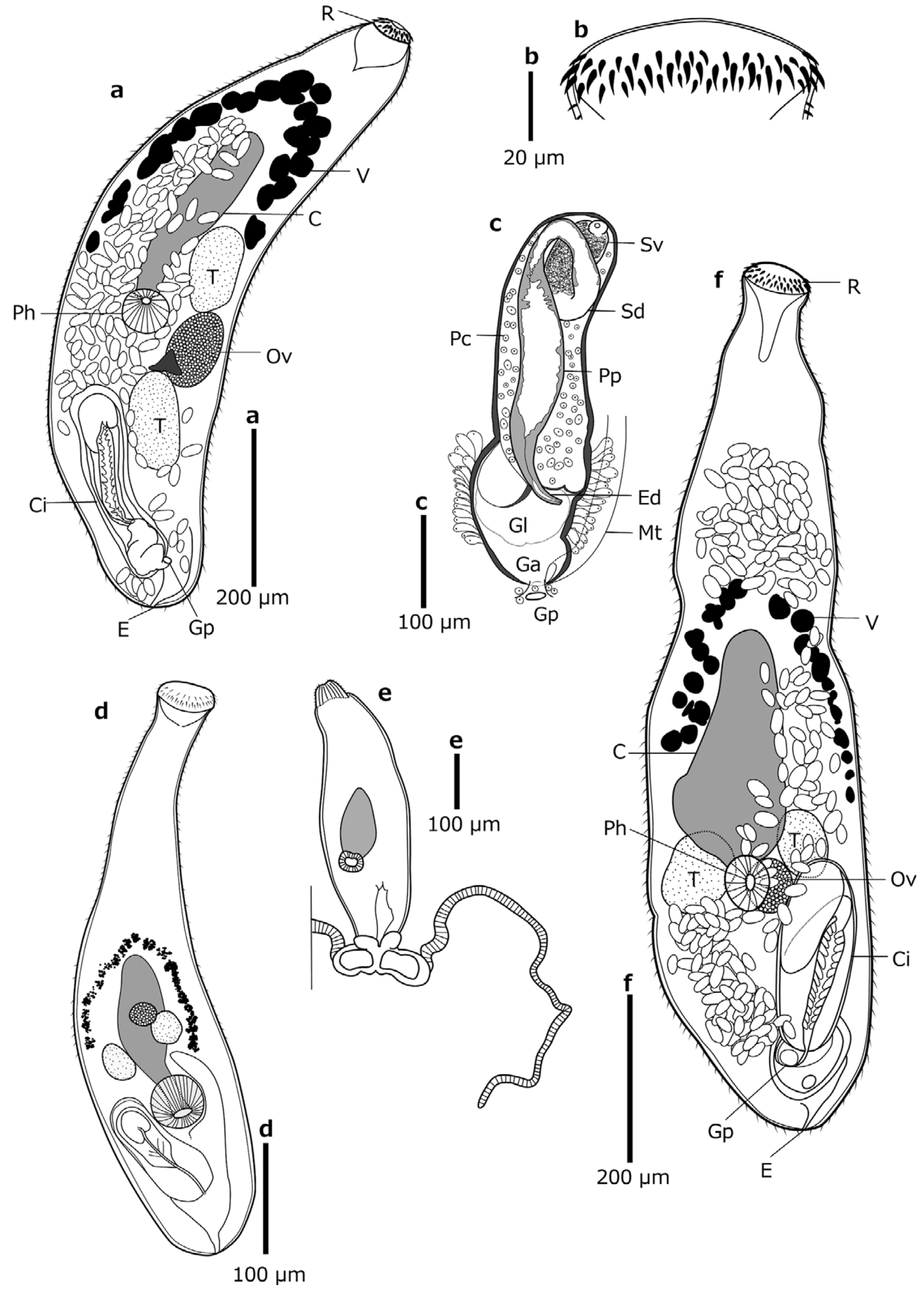

Figure 4. *Dollfustrema invadens* n. sp. (a–c) Adult, ventral view. (a) Entire body, holotype. (b) Rhynchal spines, holotype. (c) Cirrus pouch, paratype. (d) Metacercaria, paratype. (e) Cercaria (an additional material). (f) adult (an additional material). Abbreviations: c: caecum; ci, cirrus pouch; e, excretory pore; ed, ejaculatory duct; ga, genital atrium; gl, genital lobe; gp, genital pore; mt, metraterm; oe, oesophagus; ov, ovary; pc, prostatic gland cells; pp, pars prostatica; r, rhynchus; sd, seminal duct; sv, seminal vesicle; t, testis; v, vitellarium.

Mouth opening ventral surface at two-third of body. Pharynx globular, 28 in length and 21 in width. Esophagus 32 in length, extending anteriorly from pharynx. Intestinal caecum oblong and elongated, 83 in length and 29 in width. Testes two, ovoid and slightly lobed. Anterior testis 41 in length and 26 in width. Posterior testis 48 in length and 21 in width. Ovary oblong, between two testes, 26 in length and 23 in width. Cirrus pouch cylindrical, 119 in length and 38 in width. Seminal vesicle oval and lobed, situated in anterior part of cirrus pouch. Prostate gland cells indistinct. Genital pore at nearly posterior end of cirrus pouch. Vitelline follicles immature, distributed dome-shape, extending forward beyond anterior terminal of caecum. Excretory pore at posterior terminal of body. Anterior extent of excretory bladder at level of posterior testis.

Cercaria (based on five additional materials Figure 4e)

Body elliptic, 205 (165–238) in length and 54 (20–79) in width. Suckers absent. Rhynchus with tiny spines, situated anterior

Table 3. Comparison of morphological measurements of *Dollfustrema invadens* n. sp. in different references. All measurements, unless indicated otherwise, are in micrometres. Dash represents no description in the cited reference

	D. invadens n. sp.			*D. vaneyi*			*D. bengalense*	*D. gibsoni*	*D. bagarii*
Citation	This study (type series)	This study	Liu (1985), Chen (2007), Chen *et al.* (2007), Li (2019)	Tseng (1930), Skrjabin and Guschanskaja (1962)	Wang (1985)	Wu *et al.* (1991)	Madhavi (1974)	Nolan and Cribb (2010)	Moravec and Sey (1989)
Body [size]	888×215 (783–945×194–215)	1041×300 (980–1148×292–308)	630–970×210–330	580–840×162–220	560–880×240–256	956–2128×211–390	1920–2430×830–990	1178×514 (1152–1203×512–515)	612–802×190–258
Rhynchus	75×67 (56–95×54–77)	118×92 (92–141×83–99)	80–106×66–69	90×57	80×96	124–162×82–122	230–350×310–410	146×192 (144–147×189–195)	42–75×42–63
Rows on rhynchus	3	3	2 or 3*	3	-	3	5	4–5	3
Long rhynchal spine position and length	Middle row, 6.3 (5.4–7.0)	Middle row, 7.2 (6.6–7.6)	4–5	Middle row, 6	-	7–13**	-	-	Middle and posterior rows, 6–9
Short rhynchal spine position and length	Anterior and posterior rows, 3.6 (2.3–4.5)	Anterior and posterior rows, 5.2 (4.8–5.9)	3–4	Anterior and posterior rows, 3	-		-	-	Anterior row, 3–5
Pharynx [size]	56×51 (50–62×43–59)	64×65 (59–70×61–70)	40–66	60 (diameter)	-	60–86×51–81	116–167 (diameter)	98×123 (96–99×122–125)	30–54×39–63
Esophagus [length]	21 (16–30)	28 (26–30)	66–160	30	-	-	-	80 (64–96)	-
Intestinal caecum [size]	182×89 (173–191×60–99)	274×97 (145–365×68–125)	170–290×100–170	105×81	-	175–205×105–125	360–440×200–270	245×195 (234–256×192–198)	-
Anterior (left) testis [size]	118×68 (99–155×47–88)	87×80 (63–110×62–99)	100–150×70–100	-	-	78–125×89–118	160–240×172–240***	179×150 (163–195×128–173)	57–75×51–90
Posterior (right) testis [size]	106×71 (77–135×59–81)	88×77 (75–101×67–87)	100–170×60–90	-	-	110–138×75–124		152×165 (138–166×160–170)	45–78×57–105
Position of ovary	Between two testes	Between two testes	Between two testes	Anterior to anterior testis or ventral to anterior testis	At the level of anterior testis	Anterior to anterior testis	Between two testes	Anterior to posterior margin of anterior testis	Anterior to anterior testis
Ovary [size]	94×70 (91–100×42–87)	86×77 (67–105×63–92)	86–116×66–89	63×48	-	88–100×63–100	156–175×167–200	174×176 (170–179×176)	45–75×48–75
Cirrus pouch	229×59 (159–253×53–64)	263×78 (241–274×63–92)	180–250×60–180	186×45	-	290–298×73–88	400–520×156–170	341×109 (333–349×102–115)	195–261×45–99
Egg [size]	32×15 (29–36×13–18)	33×18 (31–35×16–19)	30–37×14–17		24–26×18	18–23×11–13	23×19	27×18 (22–32×14–21)	21–24×12–15
Position of genital pore	Posterior to genital atrium	Posterior to genital atrium	Close to posterior terminal of cirrus pouch	Anterior to posterior margin of body	-	Close to posterior margin of body	-	Close to posterior margin of body	-
Host	*Gnathopogon elongatus*	*Ictalurus punctatus*	*Abbottina rivularis*	*Siniperca scherzeri*	*Hemibarbus maculatus*	*Siniperca chuatsi*, *Parasilurus asotus*	*Gymnothorax undulatus*	*Gymnothorax woodwardi*	*Bagarius bagarius*
Locality	Japan (introduced)	Japan (introduced)	Mainland China	Mainland China	Mainland China	Mainland China	Bay of Bengal	Australia	Vietnam

(*Continued*)

Table 3. (*Continued*)

	D. invadens n. sp.			*D. vaneyi*			*D. bengalense*	*D. gibsoni*	*D. bagarii*
Citation	This study (type series)	This study	Liu (1985), Chen (2007), Chen *et al.* (2007), Li (2019)	Tseng (1930), Skrjabin and Guschanskaja (1962)	Wang (1985)	Wu *et al.* (1991)	Madhavi (1974)	Nolan and Cribb (2010)	Moravec and Sey (1989)
Name in citations	*D. invadens*	*D. invadens*	In Liu (1985), Chen (2007) and Chen *et al.* (2007): *D. hefeiensis.* In Li (2019): *Neodollfustrema hefeiensis*	*Prosorhynchus vaneyi*	*D. vaneyi*	*D. vaneyi*	*D. bengalense*	*D. ginsoni*	*D. bagarii*

*Variation described in Chen *et al.* (2007)
**Length of all spines
***Sizes of two testes

terminal of body, 21 (15–26) in length and 27 (26–35) in width at widest point. Mouth opening ventral surface at two-third of body. Pharynx globular, 19 (18–21) in length and 18 (16–22) in width. Caecum oval, extending anteriorly from pharynx, length 40 (26–62) and width 21 (14–30). Tail stem oblong, connected to posterior terminal of body, 57 (29–80) in length and 61 (34–93) in width. Furcae paired, long, very elastic, 352 (194–435) in length and 14 (10–20) in width.

Adult (based on three additional materials, Figure 4f, Table 3)

Shape as in type series. Body slightly larger than the type series, 1041 (980–1148) in length and 300 (292–308) in width at widest point. Rhynchus 118 (92–141) in length and 92 (83–99) in width. Rhynchal spines larger in middle row, 7.2 (6.6–7.6) in length, and shorter in anterior and posterior rows, 5.2 (4.8–5.9) in length. Pharynx 64 (59–70) in length and 65 (61–70) in width. Esophagus 28 (26–30) in length, intestinal cecum 274 (145–365) in length and 97 (68–125) in width. Anterior testis 87 (63–110) in length and 80 (62–99) in width. Posterior testis 88 (75–101) in length and 77 (67–87) in width. Cirrus pouch 263 (241–274) in length and 78 (67–87) in width. Ovary 86 (67–105) in length and 87 (63–110) in width. Eggs elongated oval, 33 (31–35) in length and 18 (16–19) in width.

Remarks

The adults in this study are considered members of the genus *Dollfustrema* based on the following morphological characters: a rhynchus with a muscular apical disc, a small and truncate rhynchus with three rows of rhynchal spines, and a proximal part of pars prostatica looped in anterior part of cirrus pouch (Overstreet and Curran 2002). Our adult specimens can be distinguished from other members of genus *Dollfustrema* by the following morphological characters (Table 3): a small, truncate rhynchus with three rows of rhynchal spines (with the middle row having longer spines), a mouth opening located posterior to the anterior margin of the anterior testis, genital openings positioned at the level of the posterior margin of the cirrus pouch, the ovary between the testes, and a dome-shaped vitellarium. The new species resembles *D. bagarii*, *D. bengalense*, *D. gibsoni*, and *D. vaneyi*, as these species also have a truncate rhynchus and dome-shaped vitellarium. However, *D. bagarii* differs from our specimens in the position of the mouth opening (anterior to anterior testis in *D. bagarii* vs. not anterior to anterior testis in our specimens) and the location of the longest rhynchal spines (anterior row in *D. bagarii* vs. middle row in our specimens). Notably, *D. gibsoni* has a rhynchus with 4–5 rows of rhynchal spines (vs. 3 rows in our specimens) and genital pores near the posterior margin of the body (vs. the posterior terminal of the cirrus pouch in our specimens), further distinguishing it from the new species. Additionally, *D. bengalense* differed from our specimens by having 5 rows of spines on the anterior rhynchus (vs. 3 rows in our specimens). Although *D. vaneyi* has been reported to show morphological variations in body size, rhynchal spine length, and ovary position, our specimens can be distinguished from this species based on the position of the genital opening (at the level of the posterior margin of the cirrus pouch in *D. hefeiense* vs. the posterior margin of the ventral body in *D. vaneyi*). The morphological characters of '*D. hefeiense*' reported by Liu (1985), Zhang *et al.* (1999), Chen (2007), and Li (2019) closely resemble those of our specimens except numbers of rows of rhynchal spines. However, Chen *et al.* (2007) mentioned that two or three rows of rhynchal spines were found both in '*D. hefeiense*' and *D. vaneyi*. Moreover, the ITS1–5.8S–ITS2 and 28S rDNA sequences of '*D. hefeiense*' reported by Chen *et al.* (2007) were identical to those of our specimens as mentioned above. In addition, the distinct of the sampling localities in Chen *et al.* (2007) were adjacent to that of Liu (1985). These findings confirm that the trematodes from both our study and prior studies belong to the same species – namely, *D. invadens* n. sp. The additional materials are slightly larger than the type series but identified as *D. invadens* n. sp. as described above. Additionally, Li (2019) classified this species under the genus *Neodollfustrema* Long & Lee, 1964. However, the distinction between *Neodollfustrema* and *Dollfustrema* was based on the position of the ovary (anterior to the testes in *Neodollfustrema* vs. not anterior in *Dollfustrema*) (Li 2019). However, *Neodollfustrema* had already been synonymised with *Dollfustrema* (Liu *et al.* 2010). Moreover, molecular analyses conducted by Chen *et al.* (2007) and that in the present study revealed that *D. invadens* n. sp. and *D. vaneyi*, which had been placed in the genera *Neodollfustrema* and *Dollfustrema* by Li (2019), respectively, were closely related, supporting the synonymising of *Neodollfustrema* with *Dollfustrema*.

Taxonomical summary

Family Bucephalidae Poche, 1907

Genus *Dollfustrema* Eckmann, 1934

Species *Dollfustrema invadens* Saito, Iwata, Nitta & Waki n. sp.

Synonyms

Dollfustrema hefeiensis Liu, 1985: 1–6, fig. 1 (invalidly described as new).

Neodollfustrema hefeiensis (Liu, 1985): Li, 2019: 195–196, fig 132 (invalid).

Dollfustrema hefeiensis Liu in Zhang, Qiu & Ding, 1999: 306; Nolan & Cribb, 2010: 85; Nolan et al., 2015: 563–567; Anglade & Randhawa, 2018: 190; Corner *et al.*, 2020: 458, 462; Atopkin et al., 2022: 783 (nomen nudum).

Dollfustrema hefeiense Liu in Zhang, Qiu & Ding, 1999: de Oliveira *et al.*, 2022: 3 (nomen nudum).

Dollfustrema hefeiensis Zhang, Qiu & Ding, 1999: Chen *et al.*, 2007: 791–799 (nomen nudum).

Dollfustrema hefeiensis (without attribution): Chen, 2007: 16, 98, 107–109; Bott *et al.*, 2013: 2564; Cremonte *et al.*, 2013: 86; Choudhary *et al.*, 2015: 169; Cremonte *et al.*, 2015: 203; Hammond *et al.*, 2018: 455; Hammond *et al.*, 2020: 5; Shirakashi *et al.*, 2020: 98; Malsawmtluangi, & Lalramliana, 2023: 2–5; Galaktionov *et al.*, 2024: 336, 338.

Dollfustrema hefeiense (without attribution): Curran *et al.*, 2022: 85.

Japanese name: Dorufusu-fukkou-kyuchu (bucephalid trematode of Dollfus)

Type host: The swamp moroko gudgeon *Gnathopogon elongatus* (Temminck & Schlegel, 1846)

Infection site: Holotype and adult paratypes, fin and fin-base tissues. Metacercaria paratype, fin.

Type locality: Kasumigaura lake, Ibaraki Prefecture, Japan

Date of collection: August 25, 2022

Additional material: Three adults from an intestine of the channel catfish *Ictalurus punctatus* (Rafinesque, 1818). A sporocyst from the golden mussel *Limnoperna fortune* (Dunker, 1857)

Deposition: Ibaraki Nature Museum, Ibaraki Prefecture, Japan. Collection Nos. INM-1-123580 (holotype, adult), INM-1-123581 (paratypes, adults), INM-1-123582 (paratype, metacercaria), INM-1-XXXXXX (additional material, adults), INM-1-123583 (additional material, sporocyst).

Etymology: The new species is named after 'invasive' species in Latin because it is an introduced species in Japan, type locality.

DNA markers: LC847294 (28S rDNA, 1174 bp) and LC847296–847297 (ITS1–5.8S–ITS2, 763 bp)

ZooBank identifier: urn:lsid:zoobank.org:act:A2169C5F-35A7-4283-A5EC-2CD67B22BA6A

ZooBank identifer (reference): urn:lsid:zoobank.org:pub:7F83C0D0-56FE-44FB-9C11-6FEB506BFF37

Discussion

In the present study, we described a *Dollfustrema* species previously reported in Mainland China, the origin of this trematode (Chen 2007; Chen *et al.* 2007; Li 2019; Liu 1985; Zhang *et al.* 1999). Although the exact introduction route is unclear, our hypothesis is that *D. hefeiense* n. sp. was possibly introduced directly to the Tone River system. From 2019 to 2021, Hayashi *et al.* (2022) detected *Pr. ozakii* in golden mussels and freshwater fishes in the same water system, but they did not find *D. invadens* n. sp. In contrast, we first detected *D. invadens* n. sp. in golden mussels and freshwater fishes in 2021 and 2022, respectively. These findings suggest that *D. invadens* n. sp. was introduced around 2020 and is now expanding its population in the water system.

The golden mussel, the first intermediate host of this new species, is thought to have been introduced to the Tone River system in 2005, likely with Asian clam seeds imported for aquaculture from East Asia (Ito 2007), although no official records of these clam imports exist. Multiple haplotypes of golden mussels in this water system suggest repeated introductions (Tominaga *et al.* 2009), and it is possible that that *D. invadens* n. sp. arrived through such repeated introductions. Additionally, we detected the new species in *Odontobutis potamophila*, which was likely introduced directly from the East Asia continent to the Tone River system in 2017 (Fukuchi *et al.* 2018). Therefore, the trematode may have been introduced to Japan from Mainland China along with specific fishes, including the *O. potamophila*.

Bucephalid trematodes typically have three hosts in their life cycle (e.g., Hayashi *et al.* (2022)). In the current study, mature adult worms were found in the intestine of channel catfish, indicating that the catfish consumed small fishes infected with metacercariae. However, adult worms with eggs were also found in the fins, fin bases, and gills of small fishes, where both adults and metacercariae co-occurred. This suggests that metacercariae can develop directly into adults within the tissues of second intermediate hosts. Moreover, the adults may reproduce via self-fertilisation within the host tissues, as movement between tissues to find mates is unlikely. Consequently, *D. invadens* n. sp. appears to use a two- or three-host life cycle (Figure 5). The golden mussel serves as the first intermediate host, harbouring sporocysts that release cercariae, which infect small freshwater fishes, such as the Tamoroko, serving as second intermediate hosts. In these fish, the cercariae develop into encysted metacercariae, which may further develop into adults within the fish tissues or intestines of definitive hosts, such as the channel catfish, after ingestion. Adult worms in the muscles of small fishes may survive in the channel catfish's intestines when it consumes its prey.

In the studied water system, the three life stages of *D. invadens* n. sp. – namely, sporocysts, metacercariae, and adults – primarily use introduced species as hosts. The golden mussel, the first

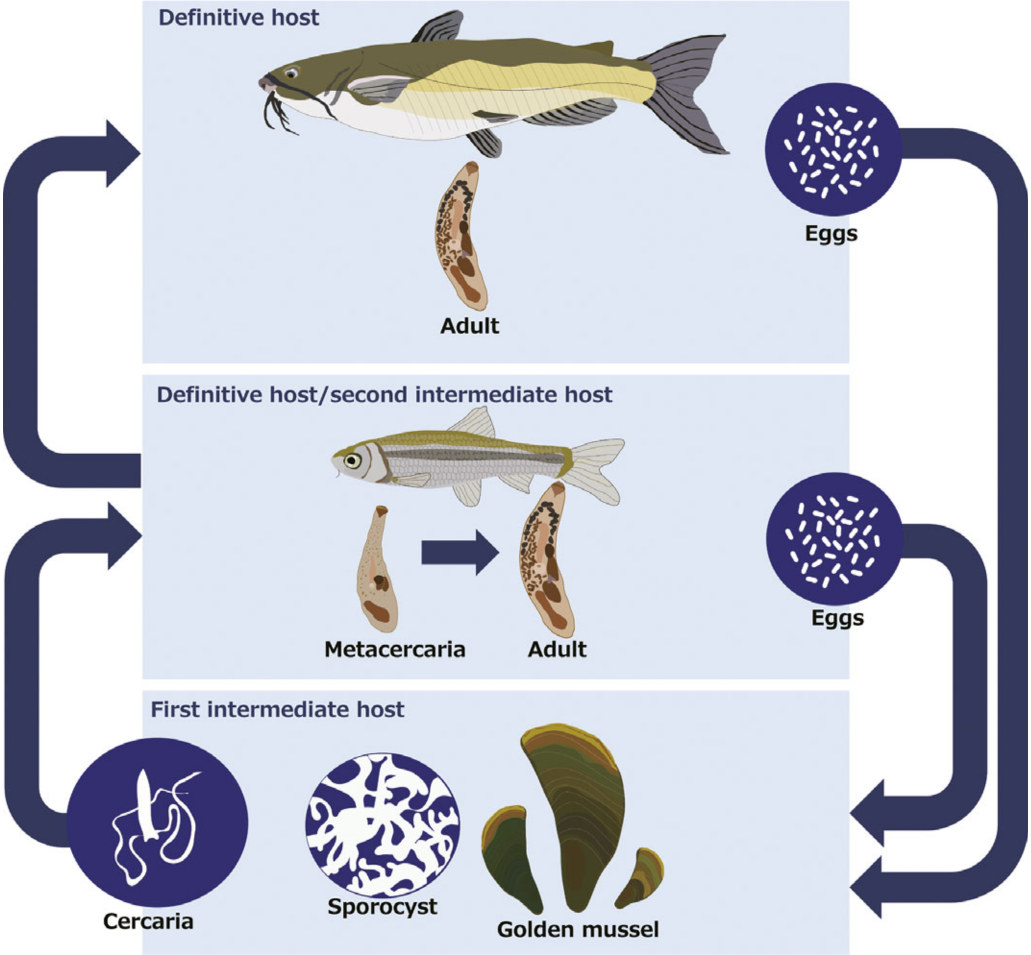

Figure 5. Life cycle of *Dollfustrema invadens* n. sp. in the Tone River system, Japan.

intermediate host, is native to East Asia (Boltovskoy 2015). Regarding metacercariae and adults, six of the eight host fish species were introduced from western Japan, Mainland China, and North America (Hosoya 2019; NatureServe 2013). Notably, the parasite itself is an alien species, and its life cycle is maintained primarily by introduced hosts. The channel catfish and bluegill, both introduced from North America, are common in the water system (Japan Wild Research Center 2008; Ozaki and Miyabe 2007; Seno and Matsuzawa 2008) and exhibited heavy infections (approximately 200 worms per host), suggesting that they may be major spreaders of the new species.

The potential negative effects of *D. invadens* n. sp. infection on fishes, especially native species in Japan, remain unclear. However, the visible brown or yellow worms in fish muscle lower the commercial value of freshwater fish. As the golden mussel expands its distribution, this mussels are currently found in at least eight river systems (Nakano *et al.* 2015). The potential fish hosts of *D. invadens* n. sp., such as gobies, perches, and catfishes, are also becoming more widespread in these river systems (Hosoya 2019; Tsuji *et al.* 2024). Given that a single adult likely generates eggs through self-fertilisation, the population of *D. invadens* n. sp. can expand rapidly when newly introduced into water systems. To prevent further spread of the species, the introduction of potential hosts from the Tone River system into other water systems should be strictly avoided.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/S0022149X24000932.

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Competing interest. The authors declare none.

Ethical standard. Approval from research ethics committees was not for this study, as the experimental work involved unregulated fish and invertebrate species.

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