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Rediscovery After Almost 120 Years: Morphological and Genetic Evidence Supporting the Validity of *Daphnia mitsukuri* (Crustacea: Cladocera)

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We examined the morphology of *Daphnia* individuals maintained in our laboratory for several years, originally collected in Lake Inbanuma, Chiba, Japan. We determined partial sequences of the mitochondrial cytochrome c oxidase subunit I and 12S rRNA genes from specimens in the cultured material. These animals are morphologically similar to *D. obtusa* Kurz, 1874, but genetically distinct from this species. Our detailed observation shows that the morphological characteristics in the female and male individuals of our material are highly congruent with those of *D. mitsukuri* Ishikawa, 1896, which has not been identified positively for more than 120 years since its original description, with its taxonomic identity having been questioned for almost 90 years. Based on our morphological and genetic data, we conclude that *D. mitsukuri* should be regarded as a taxonomically valid species. A search among public DNA sequence databases suggests *D. mitsukuri* is also distributed in China, although these Chinese sequences have been labeled as *'Daphnia pulex'*, representing misidentification.

Key words: 12S rRNA, COI, *Daphnia*, DNA barcode, genetic analysis, morphology, taxonomy, zooplankton

INTRODUCTION

Daphniids are zooplankters that play crucial roles, both functionally and structurally, in the pelagic communities of lakes and ponds (Brooks and Dodson, 1965; Lampert and Sommer, 2007). Based on specimens collected at small ponds and pools in Tokyo, Ishikawa (1895a, b, 1896) described three species of *Daphnia*; *D. morsei* Ishikawa, 1895a, *D. whitmani* Ishikawa, 1895b, and *D. mitsukuri* Ishikawa, 1896. Although his descriptions of these species were detailed and covered most key morphological features used for species identification in *Daphnia*, he cited only limited studies and thus did not sufficiently compare these three with other known species in the world. Ishikawa's work was 30 years later referenced by Uéno (1926, 1933, 1934a, b) in his intensive ecological and taxonomical studies of *Daphnia* species in Japan. Uéno (1926) regarded *D. whitmani* as synonymous with *D. pulex* var. *obtusa* Kurz, 1874. As to *D. morsei*, Uéno (1926) commented that it might also represent a variety of *D. pulex* Leydig, 1860. In addition, Uéno (1926) stated that he never found any *Daphnia* having morphological characters similar to *D. mitsukuri* in the vicinity of Kyoto and questioned its validity.

Since some *Daphnia* species exhibit large morphological variations depending on nutritional and environmental conditions, the taxonomy has long been confused (Korovchinsky, 1996; Kotov, 2015). Recently, however, owing to the development of molecular techniques, species identification by use of genetic information has allowed for more precision (Colbourne et al., 1996, 1998). Indeed, use of these new techniques has enabled the identification of *Daphnia* species in Japan (Urabe et al., 2003; Ishida et al., 2006, 2007a, b, 2011; So et al., 2015). Among these, *D. pulex* was confirmed to be commonly found in small lakes and ponds of lowland areas of Japan, supporting Uéno's (1926) observation, although *D. pulex* is not native to Japan (So et al., 2015).

Other than *D. pulex*, Tanaka and Shigaki (1987) and Tanaka (1997) suggested that *D. obtusa* Kurz, 1874—morphologically similar to *D. pulex*—is distributed in Japan. Although Uéno (1926) treated this as a variety of *D. pulex*, *D. obtusa* is morphologically distinguishable from *D. pulex* by the existence of a row of long plumose setae on the inner lip of the ventral margin of the carapace (Scourfield, 1942; Schwarz et al., 1985). However, since *D. obtusa* was originally recorded in Europe, it is debatable whether this species is naturally or natively distributed in Japan. Recent studies with molecular data showed that *D. obtusa*-like species are found in various regions around the world and phylogenetically regarded as a rich species complex (Penton et al., 2004; Adamowicz et al., 2004, 2009; Kotov and Taylor, 2010). Among these, Kotov and Taylor (2010) found two Japanese specimens that were genetically different from other known species in *D. obtusa* complex. They suggested that these might include *Daphnia* species described by Ishikawa (1895a, b, 1896). However, Kotov and Taylor (2010) prudently suspended identification of these specimens as

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they had only female specimens; the morphology of both female and male individuals is often crucial for precise identification of *Daphnia* species.

We have collected and cultured the *D. obtusa*-like species that is genetically identical to one of the two unidentified forms of Kotov and Taylor (2010). Also, we have been able to examine the morphology of both female and male individuals. In this study, we describe their morphology, provide genetic information, and show that their morphological characteristics are closely congruent with those of *D. mitsukuri*. Along with the two other species described by Ishikawa (1895a, b), *D. mitsukuri* has been listed as *species inquirenda* in Kotov et al.'s (2013) world checklist of freshwater Cladocera. Based on the evidence in this study, we conclude that *D. mitsukuri* should be regarded as a taxonomically valid species.

MATERIALS AND METHODS

Animals

The animals were collected in May 2014 from Lake Inbanuma (35°48′0″N, 140°15′0″E), Japan, and cultured extensively in tap water, fed green algae and maintained at ambient room conditions for ca. a year. Thereafter, we cultured these animals under a strict condition in the COMBO medium (Kilham, 1998), feeding with green algae of the genus *Scenedesmus*, at a constant room temperature (20°C). For these cultures, we routinely replaced the medium and algal food, observing several individuals under a microscope each time. During this procedure, in addition to females producing parthenogenetic eggs, we found males and ephippial females with resting eggs enveloped by the ephippia. These, together with parthenogenetic females, were individually isolated and fixed with 99% ethanol for observation. In addition, two other animals were randomly taken from the culture and used for genetic analysis. Although we started the culture with ~10 individuals, it is likely that our *Daphnia* specimens were not monoclonal individuals, since resting eggs produced by sexual reproduction were often found during culture. Samples of these specimens were deposited in the Tohoku University Museum (TUMC No. 111765: parthenogenetic females, No. 111766: ephippial females, No. 111767: males, and No. 111768: various individuals including neonates).

Morphological analysis

We observed parthenogenetic female, ephippial female, and male individuals under microscopes at ×60–400 magnification and examined their morphology. Eight adult females and ten adult

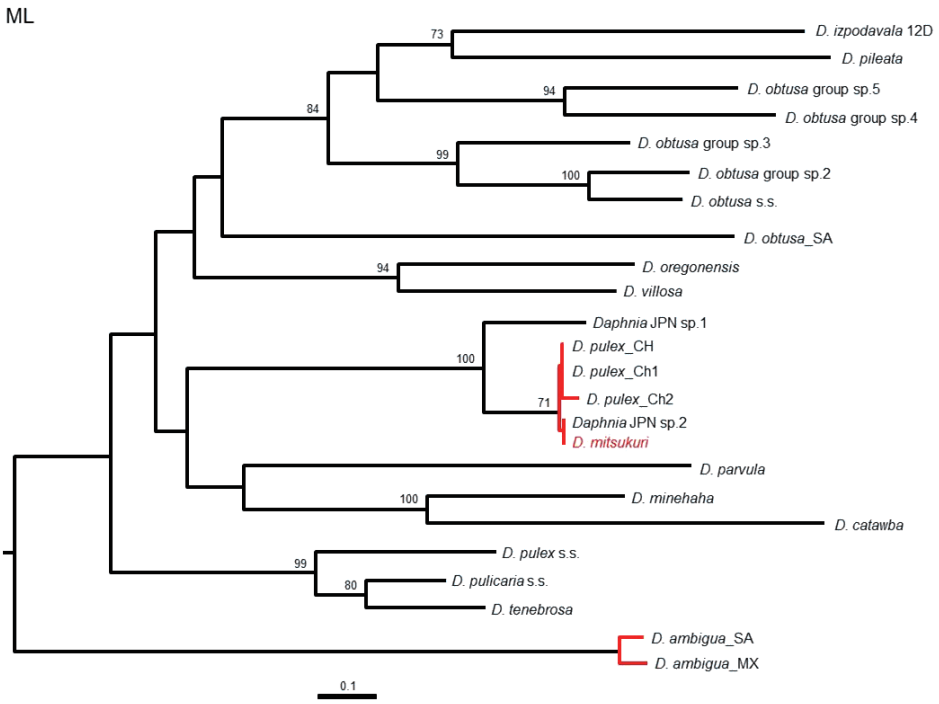

Fig. 1. Phylogenetic tree of *Daphnia* species constructed by a maximum likelihood (ML) analysis based on sequences of the COI (469 bp) and 12S (420 bp). Bootstrap values greater than 50 % are shown above branches. *Daphnia mitsukuri* (the same sequence with *Daphnia* JPN sp.2) is indicated by red color. Groups of the same species supported by results of PTP analyses are figured by red-colored branch. The sequence source information of each species is shown in Supplementary Table S1.

Table 1. Pairwise comparisons of DNA sequences in *Daphnia* species showing uncorrected p-distances of COI (469 bp: above diagonal) and 12S (420 bp: below diagonal), respectively.

	Area	*D. mitsukuri*	*Daphnia* sp.2	*D. pulex*_CH	*Daphnia* sp.1	*D. pulex* s.s.	*D. obtusa*	*D. obtusa* sp.2	*D. catawba*
D. mitsukuri *	JPN	–	0.000	0.000	0.090	0.203	0.203	0.205	0.211
Daphnia JPN sp.2 *	JPN	0.000		0.000	0.090	0.203	0.203	0.205	0.211
*D. pulex*_CH *	CHN	0.007	0.007		0.090	0.203	0.203	0.205	0.211
Daphnia JPN sp.1	JPN	0.045	0.045	0.048		0.203	0.209	0.194	0.200
D. pulex s.s.	EU	0.124	0.124	0.124	0.133		0.203	0.207	0.220
D. obtusa s.s.	EU	0.129	0.129	0.126	0.126	0.131		0.098	0.190
D. obtusa group sp.2	EU	0.129	0.129	0.136	0.114	0.138	0.040		0.186
D. catawba	MEX	0.140	0.140	0.140	0.143	0.140	0.162	0.167	–

Specimens belonging to the same species as *D. mitsukuri* by the result of PTP analysis (Fig. 1) are denoted by asterisks.
Abbreviations for collection areas of each specimen are given as follows: CHN, China; EU, Europe; JPN, Japan; MEX, Mexico.
Accession numbers of the sequences are shown in Supplementary Table S1.

males were dissected for observation of limb I appendage. We applied a system of setal enumeration according to Ishida et al. (2006).

DNA sequencing

DNA extraction from individual animals was performed using 50 μl of the QuickExtract™ DNA Extraction solution (Epicentre) and incubation at 65°C for 2 h followed by heating at 95°C for 10 min. Using 1.0 μl of this DNA extraction, we amplified the partial sequences of the mitochondrial cytochrome *c* oxidase subunit I (COI) and 12S rRNA (12S) genes. PCR to amplify COI with a primer pair LCO1490 and HCO2198 (Folmer et al., 1994) was performed under the same reaction conditions as described in So et al. (2015). For amplification of 12S, 10 μl of reaction solution consisted of 0.2 units of KOD FX Neo (TOYOBO) and primers reported in Taylor et al. (1996) for 12S rRNA. The following temperature profile was used for 12S: 94°C for 2 min, followed by 30 cycles of 98°C for 10 s, 50°C for 30 min, and 68°C for 30 s. The PCR product was refined by ExoSAP-IT(R) Kit (Affymetrix) and sequenced using BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The sequenced product was refined by BigDye(R) XTerminator™ Purification Kit (Applied Biosystems) and analyzed using ABI PRISM(R) 3100 Avant Genetic Analyzer (Applied Biosystems). The mitochondrial COI and 12S sequences obtained in this study were deposited in DNA Data Bank of Japan (Accession # LC223743 and LC325497, respectively).

Molecular phylogenetic analysis

To examine the genetic distance of the examined animals from related taxa, a BLAST search was performed using the Web BLAST tool (Nucleotide BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) with default parameters and the obtained COI and 12S sequence as the query to find its reference sequences. Sequences of COI and 12S in *D. pulex*, *D. obtusa* and related taxa were obtained from Genbank (Supplementary Table S1) and used for construction of phylogenetic trees with those of the present study. For this, the sequences were first aligned by MAFFT version 7.308 (Katoh et al., 2013) and edited visually using BioEdit version 7.0.5.3 (Hall, 1999). All positions containing gaps were eliminated from the aligned 12S dataset. The final datasets of COI and 12S sequences consisted of total of 469 bp and 420 bp, respectively. Then, pairwise comparisons of the COI and 12S sequences were done with uncorrected p-distances with MEGA6 software (Tamura et al., 2013).

Substitution model selection for phylogenetic analysis was carried out by Kakusan4 (Tanabe, 2011). For some specimens having sequences of COI alone, sequence data for 12S were treated as missing data. A model with the lowest corrected Akaike Information Criterion (AICc) values was chosen as the best model for construction of most likelihood (ML) phylogenetic tree (EqualRate_CodonEqualRate model). The tree was constructed by RAxML version 8.2.9 (Stamatakis, 2014). The data set was partitioned by gene (COI and 12S) and by codon position (1st, 2nd and 3rd codon position of COI). The ML analysis with 100 replicates of bootstrap analysis was performed using GTRGAMMAX model with some options (--no-seq-check --no-bfgs -f d). Using the resulting trees, a species delimitation analysis was carried out with Poisson-tree-processes (PTP) model (Zhang et al., 2013). The analysis was conducted on the web server of the Exelixis Lab (bPTP web server; http://species.h-its.org/ptp/) with default settings (No. MCMC generations: 10,000, Thinning: 100, Burn-in: 0.1).

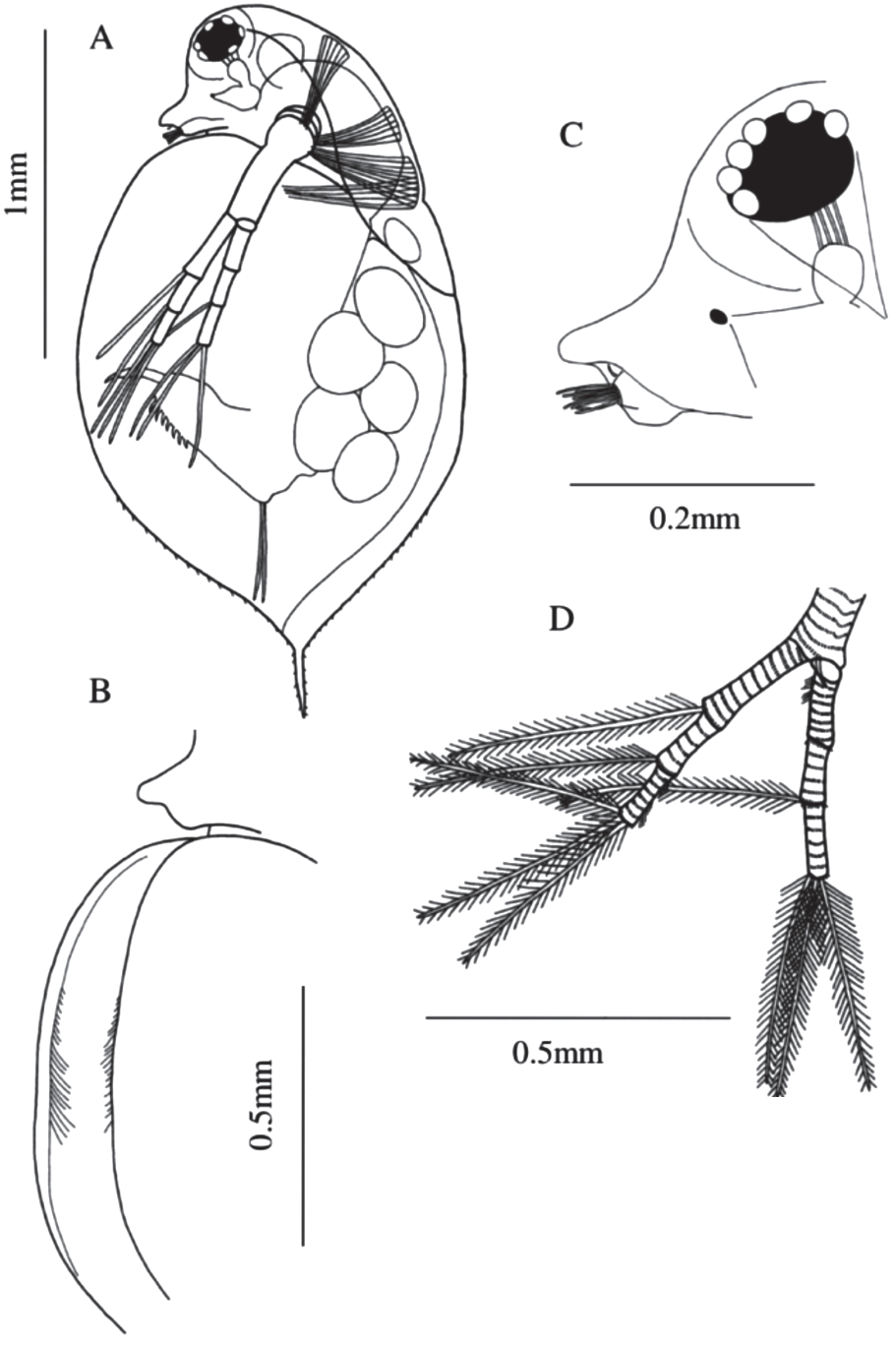

Fig. 2. Continued.

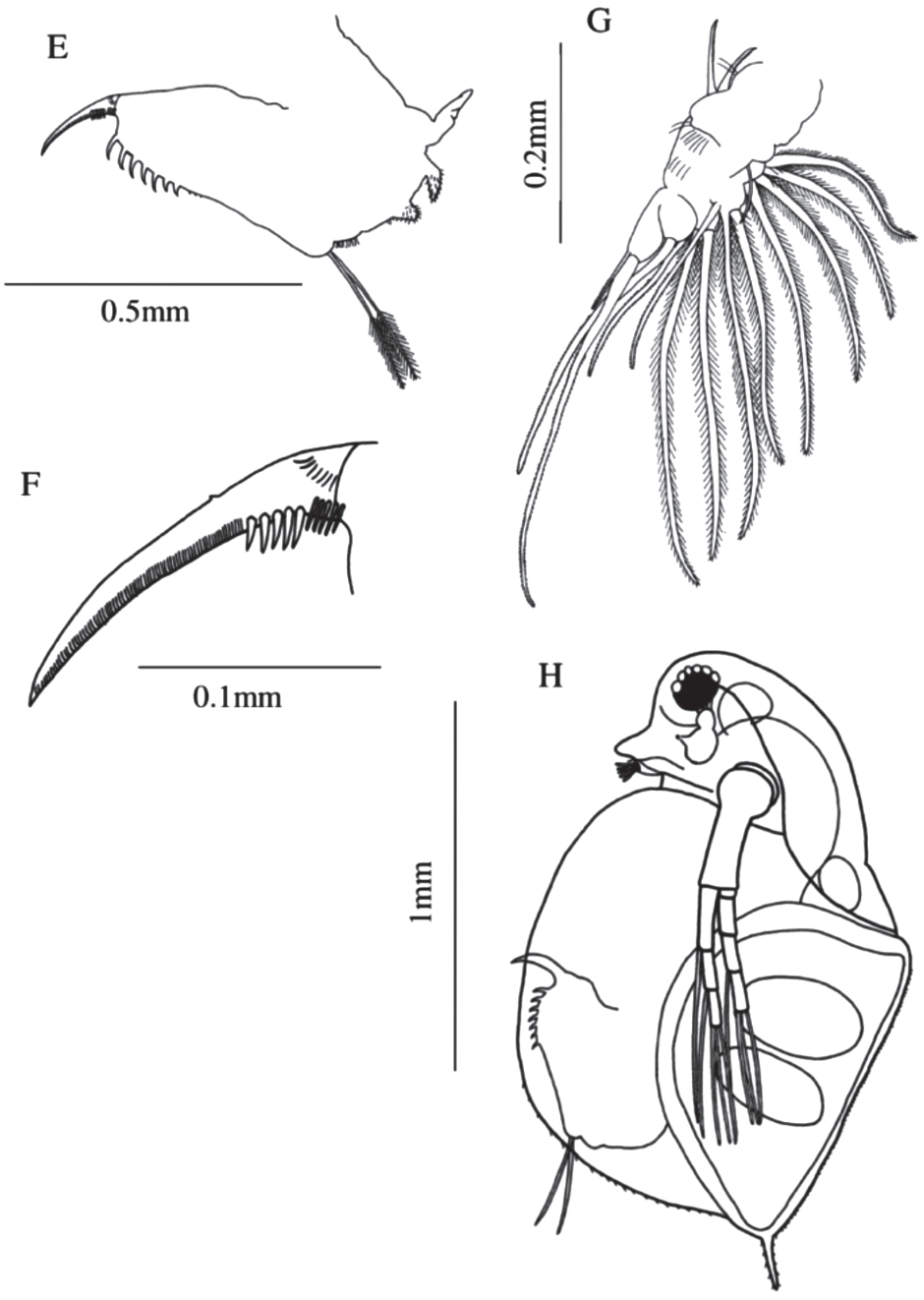

Fig. 2. *Daphnia mitsukuri* Ishikawa, 1896, parthenogenetic female originally collected in Lake Inbanuma and cultured in a laboratory, and ephippial female grown in a laboratory. **(A)** lateral view of parthenogenetic adult female, **(B)** a row of long plumose setae in ventral margin of the carapace, **(C)** lateral view of head, **(D)** second antenna, **(E)** postabdomen, **(F)** postabdominal claw, **(G)** limb I, and **(H)** lateral view of ephippial female.

RESULTS

Genetic analysis

The genetic analysis revealed that the animals collected in Lake Inbanuma were neither any species of *D. pulex* complex nor *D. obtusa* sensu stricto (Fig. 1). Instead, a search among public DNA sequence databases showed that the DNA sequences of COI and 12S in these animals were identical with those of *Daphnia* JPN sp. 2 collected in Japan and reported by Kotov and Taylor (2010) (Table 1). Phylogenetic tree constructed from ML analysis indicated that our specimen belongs to a group composed of *Daphnia* JPN sp.2 and some *Daphnia* specimens (deposited as *D. pulex* in GenBank) reported from China by Xu et al. (2014) and Geng et al. (2016) with high support values (Fig. 1). Results of a species delimitation analysis using the PTP model supported the conspecificity of all of these specimens. The same result was obtained in a Bayesian phylogenetic tree (Supplementary Figure S1).

Morphological description

Daphnia mitsukuri Ishikawa, 1896

Adult parthenogenetic female. Size 1.5–2.0 mm. Carapace broadly oval in lateral view with tail spine less than one-sixth to one-ninth of carapace length (Fig. 2A); spinules present on posterior half of dorsal and ventral margins of carapace and tail spine; a row of long plumose setae sprouting from the inner lip of middle part of ventral margin of carapace (Fig. 2B). Head relatively short and broadly rounded (Fig. 2C); head length one-fourth to one-fifth of carapace length; ventral side of head almost vertical and curving to rostrum; rostrum bluntly pointed and relatively short; tip of aesthetes of antennule projecting beyond rostrum. Second antennae with some clumps of long setae on root of 2nd segment of exopod and on tip of distal segment of endopod (Fig. 2D). Limb I (Fig. 2G): outer distal lobe with a long seta bilaterally armed distally with short setules and a thin, short seta with setules; endite 4 (inner distal lobe) with a single long anterior seta bilaterally armed distally with short setules; endite 3 with a short anterior seta armed with minute setules and two long posterior setae; endite 2 with a short and thin anterior seta and two long posterior setae; endite 1 with a small anterior seta and four posterior setae; two ejector hooks of unequal length. Postabdomen (Fig. 2E) having four abdominal processes; first abdominal process largest, curving to dorsal side, second abdominal process curving to opposite side from first process, third process moderate, fourth process rudimentary; no notable hair present on first abdominal process, other three abdominal processes hairy; anal teeth

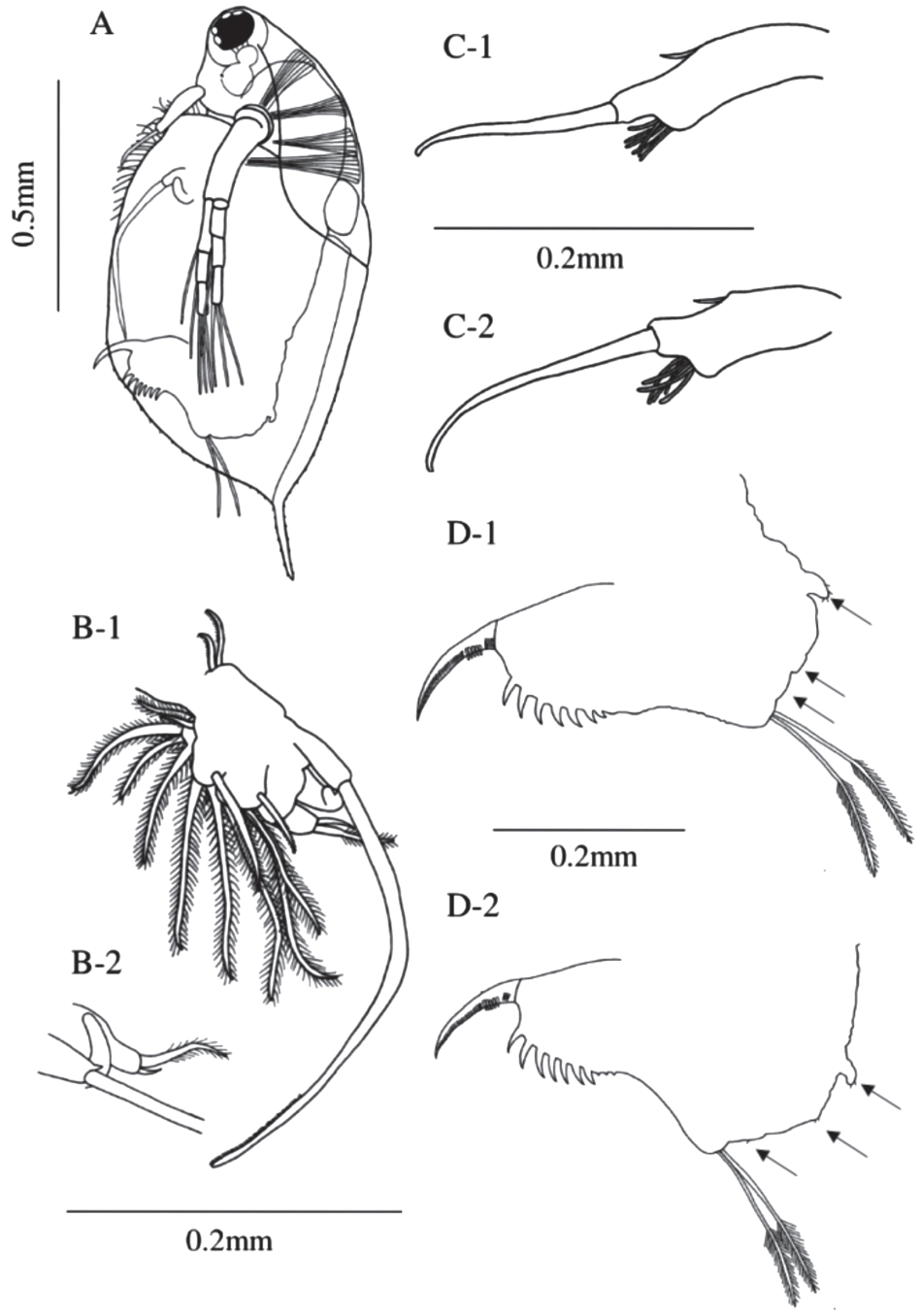

Fig. 3. *Daphnia mitsukuri* Ishikawa, 1896, male produced in a laboratory by a descendant of an individual collected from Lake Inbanuma. **(A)** lateral view, **(B)** limb I, **(C)** first antenna, and **(D)** postabdomen: abdominal processes are denoted by arrows.

generally 7–10 in number, slightly curved, increasing in size toward postabdominal claw. Postabdominal claw (Fig. 2F) regularly bent with pointed tip; three successive pectens on outer side; teeth of proximal pecten slender but robust, teeth of middle pecten thicker than those of proximal pecten, distal pecten composed of a row of small fine teeth.

Ephippial female. Size 1.5–2.0 mm. Carapace and head (Fig. 2H) generally similar to parthenogenetic female except for dorsal side of carapace owing to development of ephippium. Ephippium nearly straight on dorsal side, without spinules; one spine present at end of posterior side; one pair of dark colored resting eggs, with slightly brown margins.

Male. Size 0.7–1.3 mm. Carapace smaller and slenderer than female (Fig. 3A); length of tail spine one-seventh of carapace; anterior margin of ventral side angulated, middle section of ventral margin inflating; spinules present on posterior half of dorsal and ventral margins and tail spine; numerous long pubescences arise from anterior part of ventral side; a row of long and straight setae sprout on ventral margin under pubescences. Head short and wide relative to height; rostrum short; dorsal side curved smoothly to carapace; head length one-fourth to one-fifth of carapace; ocellus under compound eye. Limb I (Fig. 3B): cylindrically large outer distal lobe with a very large seta supplied with unilaterally minute setules distally and rudimentary seta; endite 4 (inner distal lobe) with a bent copulatory hook and two setae of different length; endite 3 with two small anterior seta and two posterior setae; endite 2 with a moderate anterior seta and two posterior setae; endite 1 with a small anterior seta and four posterior setae of different length; two ejector hooks of different length. Antennule (Fig. 3C) arising from antero-ventral part; flagellum as long as base of antennule, curved slightly, its tip not hook-shaped. Postabdomen (Fig. 3D) having three abdominal processes with hairs; first process largest and curved to the ventral side, second process with appearance of a small hill, third process rudimentary; lower part of postabdomen moderately or strongly concaved just behind anus; anal teeth and postabdominal claw as female.

DISCUSSION

This study demonstrates that the DNA sequences of specimens collected in Lake Inbanuma, Japan, do not match with those of *D. obtusa* and *D. pulex*. Rather, these were genetically identical to that of *Daphnia* JPN sp. 2 collected in Japan and reported by Kotov and Taylor (2010) in a study analyzing the phylogenetic relationship among *D. obtusa*-like species from several locations around the world. To identify this species, we examined the morphological characteristics

of female and male specimens collected in Lake Inbanuma.

Since *D. obtusa*-like species and *D. pulex* were reported in Japan (Uéno, 1926; Tanaka and Shigaki, 1987; So et al., 2015), we first compared the morphological characteristics of our specimens with those of *D. obtusa* and *D. pulex*. According to Scourfield (1942), a row of plumose setae on the inner lips of middle part of ventral margin of the carapace is a key morphological characteristic distinguishing *D. obtusa* from *D. pulex*. In our morphological observations, we noted these setae in our specimens. Thus, the examined specimens in this study were morphologically similar to *D. obtusa* rather than *D. pulex*. However, the specimens were not morphologically congruent with *D. obtusa* described originally in Europe. According to the description by Kurz (1874) and the re-description by Scourfield (1942), for example, the lower part of the postabdomen of male *D. obtusa* is not concaved. However, in the male specimens examined in this study, the lower part of the postabdomen is moderately concaved as in *D. pulex*. In addition, although our specimens have several clumps of long setae on the tip of distal endopod segment of the second antenna, such morphological features were not described for *D. obtus*a in Europe (Scourfield, 1942).

As mentioned earlier, Ishikawa (1895a, b, 1896) described three new *Daphnia* species, *D. morsei*, *D. whitmani* and *D. mitsukuri*, in Japan. Based on the morphological descriptions of these species, Kotov et al. (2006) suggested that *D. morsei* is the closest congener of *D. sinevi* while *D. whitmani* and *D. mitsukuri* are *curvirostris*-like and *obtusa*-like species, respectively. Ishida et al. (2006) also suggested that *D. whitmani* is an ecological morph of *D. curvirostris*. Unfortunately, Ishikawa appears not to have deposited any of the *Daphnia* specimens he examined. Indeed, we could find neither type specimens nor plankton samples deposited by C. Ishikawa in National Museum of Nature and Science and the University Museum of the Tokyo University where he worked. Therefore, we compared morphological characteristics of our specimens with those described for *D. morsei* (Ishikawa, 1895a), *D. whitmani* (Ishikawa, 1895b) and *D. mitsukuri* (Ishikawa, 1896).

In specimens examined in this study, postabdominal claws consist of three pectens as in the three species described by Ishikawa (1895a, b, 1896). In addition, as described above, plumose setae sprout from the inner lip of the ventral margin of the carapace in our specimens. Ishikawa did not mention on such plumose setae in his description. Since these plumose setae are often hard to observe, the possibility is that Ishikawa overlooked these setae. However, a row of the plumose setae on the middle part of ventral margin of the carapace was depicted in the sketch of *D. mitsukuri* (fig. 1 in Ishikawa, 1896), although these were not recognized in the sketches of *D. morsei* (figs. 1 and 3 in Ishikawa, 1895a) and *D. whitmani* (figs. 1 and 4 in Ishikawa, 1895b).

Female specimens examined in this study also differed morphologically from those of *D. whitmani* and *D. morsei* in several important characteristics. In *D. whitmani*, all of the four abdominal processes are covered by small hairs, while, in the present specimens, only three posterior abdominal processes are covered by hairs as in the case of both *D. morsei* and *D. mitsukuri*. Unlike *D. morsei* and *D. whitmani*, but similar to *D. mitsukuri*, the examined specimens have several clumps of long setae on the tip of distal endopod segment of the second antennae. Thus, female specimens of *Daphnia* we examined are morphologically close to *D. mitsukuri*.

Male specimens examined in this study also differed in some key characteristics from those of *D. whitmani* and *D. morsei*. In the male of *D. morsei*, the anterior part of the dorsal margin is slightly elevated, appearing as a small hump (Ishikawa, 1895a). However, such a characteristic was not observed in our male specimens. In addition, our male specimens displayed a row of long, straight setae on the ventral margin of carapace. However, such setae were not described in the male of *D. morsei*, although these are shown in the descriptions of *D. whitmani* and *D. mitsukuri*. Differing from the other two species, the antennule of *D. whitmani* is very specific in configuration: the flagellum is apparently longer than the base of the antennule and ends in a hook shape. In addition, unlike our male specimens, the lower part of the postabdomen is not concave behind the anus in males of *D. whitmani*. However, according to the sketch by Ishikawa (1896), the lower part of the postabdomen is moderately concave around the anus in males of *D. mitsukuri* as in our male specimens. In addition, in accordance with our male specimen, the flagellum of the antennule in males of *D. mitsukuri* is similar to the base of the antennule in length and bent slightly but never ends in a hook shape. Moreover, the number of abdominal processes in male individuals is three in *D. mitsukuri* as in our male specimens, but two and four in *D. morse*i and *D. whitmani*, respectively. Thus, even in male individuals, morphological characteristics of our specimens are congruent with that of *D. mitsukuri*. Therefore, it is reasonable to identify *Daphnia* specimens collected originally in Lake Inbanuma as *D. mitsukuri*.

According to Ishikawa (1896), *D. mitsukuri* individuals reached up to 2.6 to 2.7 mm in body length. If this length included the tail spine, body size of our specimens was comparable to those in Ishikawa. If the tail spine is not included in the length, the body sizes of our specimens were somewhat smaller. However, it is not usual for *Daphnia* species, such as *Daphnia obtusa*, to survive for several months and grow to a large size when not subjected to predators such as fish (Urabe and Sterner, 2001). Thus, the body size itself is not necessarily a key characteristic in distinguishing *Daphnia* species.

Our search among public DNA sequence databases showed that *Daphnia* with the same COI and 12S rRNA sequences to *D. mitsukuri* occurs in China (Geng et al., 2014, 2016; Xu et al., 2014; Jiang et al., 2017). However, these studies have not taxonomically examined the *Daphnia* specimens and have treated them instead as *Daphnia pulex* without any required morphological examinations. Nonetheless, the genotype matching in this study suggests that *D. mitsukuri* is distributed across East Asia.

Ishikawa (1896) found *D. mitsukuri* several times in a small pool in the village of Omori, within the present-day metropolitan area of Tokyo. Uéno (1926) stated that *D. obtusa*-like species were common in the city of Kyoto, although he never found individuals having characteristics similar to *D. mitsukuri*. However, although we found *D. pulex* in 36 out of the total 301 ponds and small lakes across

Japan, we never collected *D. mitsukuri* or *D. obtusa*-like species in these ponds and lakes (So et al., 2015). Tanaka and Shigaki (1987) also collected *D. obtusa*-like species from only one pond during a plankton survey of a number of irrigation ponds in Toyama Prefecture. We do not know whether *D. obtusa*-like species reported by Uéno (1926) and Tanaka and Shigaki (1987) were *D. mitsukuri* or not. Nonetheless, the circumstantial evidence suggests that *D. mitsukuri* is a rare species in Japan at present.

In ponds and small lakes in Japanese lowland areas, *D. pulex*, an invasive species from North America, were often found (So et al., 2015). Thus, the habitats of *D. pulex* seems to overlap with that of *D. mitsukuri*. Competitive interactions are known to be strong among *Daphnia* species (Iwabuchi and Urabe, 2012). Thus, due to competition with *D. pulex*, distribution of *D. mitsukuri* may have been limited in Japan. Fortunately, *D. mitsukuri* has an ability to produce resting eggs that are stored in the sediments for a long period. The appearance of *D. mitsukuri* in Lake Inbanuma in recent years might therefore have been due to accidentally induced hatching of resting eggs.

Other than species identified as *D. mitsukuri* in this study, Kotov and Taylor (2010) recorded genetically distinct *Daphnia* species (*Daphnia* JPN sp. 1 in Fig. 1) from a small pond in Japan. If both male as well as female individuals of that species were collected, it would be interesting to examine whether that species is also one of the *Daphnia* species described by Ishikawa (1895a, b, 1896). This chance would progress our understanding of the biogeography and evolution of *Daphnia* species in East Asia.

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

JU and NM designed the study. NM and JU performed morphological analysis. HO and WM performed genetic analysis. JU and NM wrote the first draft and all authors contributed the final manuscript.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: http://www.bioone.org/doi/suppl/10.2108/zs170081).

Supplementary Table S1. List of species names, collection areas, accession numbers of sequence data and their reference literatures used for phylogenetic analyses.

Supplementary Figure S1. Phylogenetic tree of Daphnia species constructed by a Bayesian analysis (Proportional_CodonProportional model) based on the same data used for ML tree (Fig. 1).

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