SHORT COMMUNICATION

Rediscovery of *Macroplea japana* (Coleoptera: Chrysomelidae: Donaciinae), an aquatic leaf beetle once thought to be extinct in Japan

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Abstract

Macroplea japana (Jacoby, 1885) has not been collected in Japan since the 1960s and was thought to be locally extinct. Recently, we collected this species from submerged aquatic plants growing in the nearshore zone of Lake Biwa, Shiga Prefecture, where it had previously been recorded from the stomach contents of pochards in the 1950s. We conducted a molecular phylogenetic analysis to identify the phylogenetic position of the Japanese *M. japana* within the tribe Haemonini of the Holarctic region, which consists of *Macroplea* in Eurasia and *Neohaemonia* in North America. We found that *M. japana* specimens from Japan and China were genetically close to each other and distantly related to all other known *Macroplea* species from Asia and Europe, indicating the species identity of the Japanese and Chinese populations and the distinct species status of *M. japana*.

Key words: endangered species, molecular phylogeny, Haemonini, *Hydrilla verticillate*

Macroplea japana (Jacoby, 1885) is an aquatic leaf beetle of the subfamily Donaciinae (Coleoptera: Chrysomelidae) that has been recorded in clear freshwater habitats in Japan, China, and Russian Primorsky (Hayashi & Shiyake 2001; Lou *et al.* 2011). This species lives underwater during its entire juvenile stage and most of its adult stage (Zhang *et al.* 2010). Only a dozen specimens have been recorded in Japan, from Chiba, Kanagawa, Shiga (Lake Biwa), Hyogo, Fukuoka, and Okinawa (Hayashi 2006), and no specimens have been collected since the 1960s. Therefore, *M. japana* was designated as extinct by the Ministry of the Environment, Government of Japan.

The first author (MK) recently collected *M. japana* during fieldwork at Lake Biwa, Shiga Prefecture (Fig. 1A, B). The first beetle (Fig. 1C-a) was found in February 2022 in a plastic bag containing samples of the submerged aquatic plant *Hydrilla verticillate*, which were collected on October 30, 2021, and brought to the laboratory to rear chironomids associated with aquatic plants. The beetle was thought to be attached to the *H. verticillate* plant. The habitat was the nearshore zone of Lake Biwa, where submerged aquatic plants including *H. verticillata* (Hydrocharitaceae), *Potamogeton maackianus*, *P. perfoliatus*, and *P. anguillanus* (Potamogetonaceae) grow on the gravelly bottom (Fig. 1A). The second and third beetles (Fig. 1C-b, c) were collected from the remains of *P. maakianus* collected at the same site on February 27, 2022 (Fig. 1B). These beetles were thought to have been attached to the aquatic plants. The presence of *M. japana* in these samples was not noticed upon their initial collection in the field. Due to conservation concerns, we refrain from describing the detailed locality of *M. japana* in this report.

The specimens collected from Lake Biwa (body length: male, 4.0–4.1 mm; female, 4.2 mm) were smaller than *M. japana* collected from Guizhou, China (male, 4.2 mm; female, 5.1–5.3 mm), although the sample sizes are insufficient for comparison (Fig. 1C).

We studied the phylogenetic position of *M. japana* within the tribe Haemonini, which consists of two genera (*Macroplea* and *Neohaemonia*) in the Holarctic. We obtained partial sequence data of mitochondrial *cytochrome c oxidase subunit I* (*COI*) and nuclear *28S rRNA* (*28S*) genes from two *M. japana* specimens from Shiga and three *Macroplea* species from China (Table S1). Total genomic DNA was extracted from thoracic muscles using the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany). Partial sequences of *COI* and *28S* genes were amplified through polymerase chain reaction (PCR) using the primers C1-J-2195 and TL2-N-3014 (Simon *et al.* 1994) for *COI* and 28S-01 and 28S-R01 (Kim *et al.* 2000) for *28S*. The purified PCR products were sequenced following dye terminator cycle-sequencing reactions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher, Waltham, MA, USA). Sequence data were deposited in the DNA Data Bank of Japan (DDBJ)/GenBank (accession nos. LC705464–LC705473). In the phylogenetic analysis, we included sequence data for seven species of Haemonini and nine species of other Donaciinae genera (*Plateumaris*, *Donacia*, and *Sominella*) retrieved from GenBank (data from Kölsch *et al.* 2006; Sota *et al.* 2008; Hayashi & Sota 2014; Table S1). We performed a maximum-likelihood phylogenetic analysis for the partitioned *COI* and *28S* data using the IQ-TREE v2.1.3 software (Minh *et al.* 2020), with optimal data partitioning and using the substitution model selection (option: -m MFP+MERGE).

A published short *COI* sequence of *M. japana* from Heilongjiang, China (209 bp; Kölsch *et al.* 2006) was close to the *COI* sequences of Japanese specimens (identity, 96%). In the maximum-likelihood tree (Fig. 1D), the *M. japana* sequences showed a distinct position among Eurasian *Macroplea*, which was sister to North American *Neohaemonia* species. These results confirmed the unique species status of *M. japana*

from Japan and China among *Macroplea* species that was previously observed morphologically (Hayashi & Shiyake 2001). Two other Haemonini species have been described from Eurasia, *Macroplea skomorokhovi* Medvedev, 2006 from Primorsky and *Neohaemonia voronovae* Medvedev, 1977 from Mongolia. Of these, *M. skomorokhvi* should be a junior synonym of *M. japana* (Lou *et al.* 2011). *Neohaemonia voronovae* is morphologically similar to *M. ranina* Lou and Yu, 2011 and may be a member of *Macroplea* (Lou *et al.* 2011); because *M. ranina* is distantly related to *M. japana* according to the molecular phylogeny, it is unlikely that *N. voronovae* is closely related to *M. japana*.

The ecology of *M. japana* was studied in China in 2006–2008, as this species was a potential biological control agent against the invasive aquatic plant *H. verticillata* in North America (Zhang *et al.* 2010). In China, larvae and/or adults of *M. japana* utilized nine plant species including *H. verticillate*; *M. japana* had one generation per year and overwintered as pupae or adults attaching to the base of plants (Zhang *et al.* 2010). Our collection of *M. japana* in Lake Biwa was made possible by this overwintering ecology. In Lake Biwa, an *M. japana* adult was discovered in the stomach contents of the pochard *Aythya ferina*, a winter visitor in Japan, collected from November to February in 1950s (Ikeda 1956). Other stomach contents of the examined pochards included the submerged aquatic plant *Ceratophyllum demersum* and thecorbiculid clam *Corbicula leana* (Ikeda 1956), indicating that the *M. japana* adult was consumed by a pochard along with aquatic plants and that the habitat of *M. japana* in the 1950s was also the nearshore zone, where soft bottoms harbored both submerged aquatic plants and corbiculid clams.

The rediscovery of *M. japana* in Lake Biwa invokes conservation concerns, as *M. japana* has an entirely aquatic life cycle, except for temporary out-of-water activity

during its adult stage, and they are vulnerable to water pollution. The reduction of suitable habitats for the host plants such as *H. verticillate* is also a major threat to the persistence of the *M. japana* population. Predation by invasive fish such as bluegill *Lepomis macrochirus* may be another critical factor, although predation pressure on *M. japana* is unknown, except that its remains have been found in the stomach contents of a frog and the abovementioned water birds (Hayashi 2006). Therefore, it is urgent that the range and size of the extant *M. japana* population in Lake Biwa be clarified to elucidate the urgent threats to this population.

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REFERENCES

Hayashi M (2006) Distribution records of Donaciinae (Coleoptera, Chrysomelidae) in Japan. Part 2. Genera *Donacia* Fabricius and *Macroplea* Samouelle. *Bulletin of the Hoshizaki Green Foundation* **9**, 151–192.

Hayashi M, Shiyake S (2001) The identity and distribution of Macroplea japana (Jacoby) (Coleoptera, Chrysomelidae, Donaciinae). *Bulletin of the Osaka Museum Natural History* **55**,15–22.

129 Hayashi M, Sota T (2014) Quaternary donaciine beetles (Coleoptera, Chrysomelidae) in
130 Japan: Colonization and divergence patterns inferred from fossil and molecular data.
131 *Quaternary International* **341**, 255–266.
132 Ikeda S (1956) *[Food Habit of Japanese Birds]*. *Chojuchosahokoku* **15**, 1–95. (In
133 Japanese.)
134 Kim CG, Zhou HZ, Imura Y, Tominaga O, Su ZH, Osawa S (2000) Patterns of
135 morphological diversification in the *Leptocarabus* ground beetles (Coleoptera:
136 Carabidae) as deduced from mitochondrial ND5 gene and nuclear 28S rDNA
137 sequences. *Molecular Biology and Evolution* **17**, 137-145.
138 Kölsch G, Biström O, Pedersen BV (2006) Species delimitation in the leaf beetle genus
139 *Macroplea* (Coleoptera, Chrysomelidae) based on mitochondrial DNA, and
140 phylogeographic considerations. *Insect Systematics & Evolution* **37**, 467–479.
141 Kölsch G, Pedersen BV (2008) Molecular phylogeny of reed beetles (Col.,
142 Chrysomelidae, Donaciinae): The signature of ecological specialization and
143 geographical isolation. *Molecular Phylogenetics and Evolution* **48**, 936–952.
144 Lou Q, Yu P, Liang H (2011) Two new species of *Macroplea* Samouelle (Coleoptera:
145 Chrysomelidae: Donaciinae) from China, with a key to all known species. *Zootaxa*
146 **3003**, 1–21.
147 Minh BQ, Schmidt HA, Chernomor O, et al (2020) IQ-TREE 2: New models and
148 efficient methods for phylogenetic inference in the genomic era. *Molecular Biology*
149 *and Evolution* **37**, 1530–1534.
150 Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting,
151 and phylogenetic utility of mitochondrial gene sequences and a compilation of
152 conserved polymerase chain reaction primers. *Annals of the Entomological Society*

of America **87**, 651–701.

Sota T, Bocak L, Hayashi M (2008) Molecular phylogeny and historical biogeography of the Holarctic wetland leaf beetle of the genus *Plateumaris*. *Molecular Phylogenetics and Evolution* **46**, 183–192.

Zhang J, Wheeler GS, Purcell M, Ding J (2010) Biology, distribution, and field host plants of *Macroplea japana* in China: An unsuitable candidate for biological control of *Hydrilla verticillata*. *Florida Entomologist* **93**, 116–119.

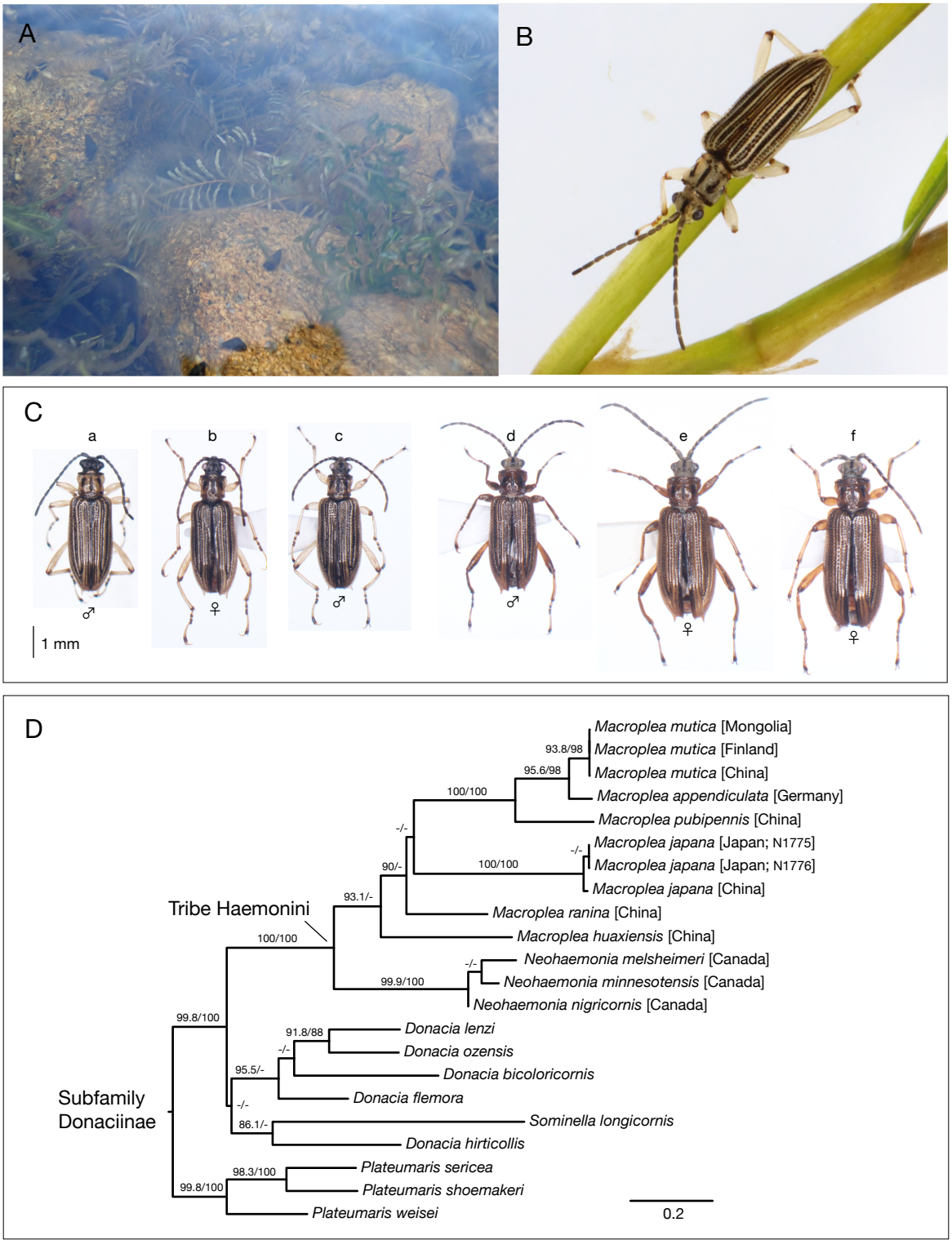
SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Table S1. List of sequence data for Haemonini Species.

Figure legends

Figure 1 (A) Underwater habitat of *Macroplea japana* in Lake Biwa. Photograph by M. Kato. (B) *Macroplea japana* walking on a *Potamogeton maackianus* shoot. Photograph by T. Sota. (C) *Macroplea japana* specimens from Lake Biwa collected in this study (a–c) and Guizhou, China collected by J. Zhang in 2007 (d–f). Photographs by T. Sota. (D) Maximum-likelihood tree of tribe Haemonini species based on partitioned analysis of mitochondrial *COI* and nuclear *28S* gene sequences. *Plateumaris* is shown to be sister to all other groups according to Kölsch and Pedersen (2008). Node support values indicated on branches are the Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT) and ultrafast bootstrap (UF-boot) values (%). Only SH-aLRT values >80% and UF-boot values > 95% are shown. Detailed descriptions of the samples are provided in Table S1.


A
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1 mm
D
Macroplea mutica [Mongolia]
Macroplea mutica [Finland]
Macroplea mutica [China]
Macroplea appendiculata [Germany]
Macroplea pubipennis [China]
Macroplea japana [Japan; N1775]
Macroplea japana [Japan; N1776]
Macroplea japana [China]
Macroplea ranina [China]
Macroplea huaxiensis [China]
Neohaemonia melsheimeri [Canada]
Neohaemonia minnesotensis [Canada]
Neohaemonia nigricornis [Canada]
Donacia lenzi
Donacia ozensis
Donacia bicoloricornis
Donacia flemora
Sominella longicornis
Donacia hirticollis
Plateumaris sericea
Plateumaris shoemakeri
Plateumaris weisei
93.8/98
95.6/98
100/100
-/-
-/-
90/-
100/100
93.1/-
100/100
-/-
99.9/100
99.8/100
91.8/88
-/-
95.5/-
-/-
86.1/-
98.3/100
99.8/100
Tribe Haemonini
Subfamily
Donaciinae
0.2

Table S1. List of sequence data for Haemonini Species.

Species	Sample ID	Locality [year, collector*]	DDBJ/GenBank accession no. COI/28S [ref.]
Tribe Haemonini			
Macroplea japana	jap01	Heilongjiang, China	DQ887744/NA [1]
Macroplea japana	N1775	Shiga, Japan [2022, M. Kato]	LC705467/LC705472 [0]
Macroplea japana	N1776	Shiga, Japan [2022, M. Kato]	LC705468/LC705473 [0]
Macroplea mutica	mut15	Finland	DQ887730/NA [1]
Macroplea mutica	N1769	Hebei, China	AB820481/AB820524 [2]
Macroplea mutica	N1773	Bulgan, Mongolia [2010, H.B. Liang & C.M. Shi]	LC705466/LC705471 [0]
Macroplea ranina	N1596	Sichuan, China [T. Sota & H.B. Liang, 2007]	LC705464/LC705469 [0]
Macroplea huaxiensis	N1637	Guizhou, China [Y. Liu, 2007]	LC705465/LC705470 [0]
Macroplea appendiculata	app05	Schleswig-Holstein, Germany	DQ887736/NA [1]
Macroplea pubipennis	pub05	Heilongjiang, China	DQ887742/NA [1]
Neohaemonia melsheimeri	N1401	Ontario, Canada	AB820470/AB820525 [2]
Neohaemonia minnesotensis	N1403	Ontario, Canada	EF532502/EF532412 [2]
Neohaemonia nigricornis	N1405	Manitoba, Canada	EF532503/EF532413 [2]
Outgroup			
Plateumaris sericea	N1028	Hokkaido, Japan	EF532541/EF532451 [3]
Plateumaris shoemakeri	N1471	Manitoba, Canada	EF532444/EF532489 [3]
Plateumaris weisei	N0667	Hokkaido, Japan	EF532527/EF532437 [3]
Sominella longicornis	N1576	Sichuan, China	AB820482/AB820526 [2]
Donacia hirticollis	N1393	Manitoba, Canada	AB820465/AB820495 [2]
Donacia flemora	N0769	Tottori, Japan	AB820378/AB820493 [2]
Donacia bicoloricornis	N0059	Ibaraki, Japan	AY232497/AB820487 [2]
Donacia ozensis	N0103	Niigata, Japan	AY232525 /AB820506 [2]
Donacia lenzi	N0831	Kagawa, Japan	AB820390 /AB820501 [2]

* For specimens newly reported in this study.

References

[0] This study.

[1] Kölsch G, Biström O, Pedersen BV (2006) Species delimitation in the leaf beetle genus *Macroplea* (Coleoptera, Chrysomelidae) based on mitochondrial DNA, and phylogeographic considerations. *Insect Systematics & Evolution* **37**, 467–479

[2] Hayashi M, Sota T (2014) Quaternary donaciine beetles (Coleoptera, Chrysomelidae) in Japan: Colonization and divergence patterns inferred from fossil and molecular data. *Quaternary International* **341**, 255–266.

[3] Sota T, Bocak L, Hayashi M (2008) Molecular phylogeny and historical biogeography of the Holarctic wetland leaf beetle of the genus *Plateumaris*. *Molecular Phylogenetics and Evolution* **46**, 183–192.