First Record of *Mimobdella japonica* (Hirudinida: Arhynchobdellida: Salifidae) from Hachijojima Island, Izu Islands, Japan, with a Comment on the Genetic Diversity of the Species

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Three specimens of the predatory salifid leech *Mimobdella japonica* Blanchard, 1897 were collected from Hachijojima island, Izu Islands, Japan. This species was previously known only from Okinawajima and Amamioshima islands in the Ryukyu Islands, Japan; this is the first record of this species from the Izu Islands. A morphological description based on these specimens is presented here. In addition, we evaluated the genetic diversity of this species based on sequences of cytochrome *c* oxidase subunit I, tRNA<sup>Cys</sup>, tRNA<sup>Met</sup>, 12S rRNA, tRNA<sup>Val</sup>, 16S rRNA, tRNA<sup>Leu</sup>, and NADH dehydrogenase subunit 1 obtained from specimens of *M. japonica* collected recently from all three islands. The only difference noted among them was a unique ND1 sequence detected from two specimens collected on Okinawajima. The possibility of human-mediated introduction of *M. japonica* to one or more of these islands is discussed.

**Key Words:** *Mimobdella japonica*, terrestrial leech, new record, island fauna, genetic diversity.

**Introduction**

The salifid leech genus *Mimobdella* Blanchard, 1897 includes terrestrial and semi-terrestrial predatory species from East and Southeast Asia. According to Sawyer (1986), this genus includes three species: *M. buettikoferi* Blanchard, 1897, *M. japonica* Blanchard, 1897 (the type species), and *M. thienemanni* Augener, 1931. Although the holotype of *M. japonica* was reexamined by Nakano (2011), the taxonomic status of the other two species has not been reconsidered since they were first described.

Although the taxonomic identity of *M. japonica* has been clarified, its distribution and biogeographical history have not yet been elucidated. The type locality of this species was originally only stated as Japan (Blanchard 1897; Nakano 2011), and its confirmed range of distribution has until now been limited to Okinawajima and Amamioshima islands in the Ryukyu Islands, Japan (Nakano 2013). Nakano (2013) thought it likely to be an introduced species on one or both of these islands because the partial sequences of cytochrome *c* oxidase subunit I (COI) obtained from specimens collected on both islands were identical.

Salifid leeches identified as *M. japonica* were recently collected from Hachijojima island, Izu Islands, Japan. This is the first record of the family Salifidae from the Izu Islands. Here, we provide a brief morphological description of these new specimens of *M. japonica* and also present an assessment of the genetic diversity of this species based on sequences of COI, tRNA<sup>Cys</sup>, tRNA<sup>Met</sup>, 12S rRNA, tRNA<sup>Val</sup>, 16S rRNA, tRNA<sup>Leu</sup>, and NADH dehydrogenase subunit 1 obtained from specimens collected from all three of these islands in recent years.

**Materials and Methods**

Three leeches were collected from a locality (33.071886°N, 139.811194°E) on Hachijojima island, Izu Islands, Japan. All of the specimens were relaxed by the gradual addition of absolute ethanol (EtOH) to freshwater. For DNA extraction, botryoidal tissue was removed from the posterior part of the body around the caudal sucker of every specimen, and then preserved in absolute EtOH. The remainder of the body was fixed in 10% formalin and preserved in 70% EtOH. Four measurements were taken: body length from the anterior margin of the oral sucker to the posterior margin of the caudal sucker (BL), maximum body width (BW), caudal sucker length from the anterior to the posterior margin of the sucker (CL), and caudal sucker width from the right to the left margin of the sucker (CW). Examination, dissection, and drawing of the specimens were conducted using a stereoscopic microscope with a drawing tube (Leica M125). Specimens used in this study have been deposited in the Zoological Collection of Kyoto University (KUZ). The numbering convention of leech morphology is based on Moore (1927): body somites are denoted by Roman numerals, and the annulli in each somite are given alphanumeric designations.
The extraction of genomic DNA from botryoidal tissues preserved in absolute EtOH followed Nakano (2012a). Primer sets for the PCR and cycle sequencing (CS) reactions used in this study were as follows: for cytochrome c oxidase subunit I (COI), LCO 1490 and HCO 2198 (PCR and CS) (Folmer et al. 1994), and LCO-in and HCO-out (PCR and CS) (Nakano 2012a); for tRNA\(^{\text{Cys}}\), tRNA\(^{\text{Met}}\), 12S ribosomal RNA, tRNA\(^{\text{Leu}}\), and 16S ribosomal RNA (tRNA\(^{\text{Cys}}\)–16S), 12SA-out and 12SB-in (PCR and CS), and 12SA-in and 12SB-out (PCR and CS) (Nakano 2012a); for tRNA\(^{\text{Leu}}\) and NADH dehydrogenase subunit 1 (tRNA\(^{\text{Leu}}\)–ND1), LND3000 and HND1932 (PCR and CS) (Light and Siddall 1999). The PCR reactions and DNA sequencing were performed using the modified method outlined by Nakano (2012b). The PCR reactions were performed using a GeneAmp PCR System 2700 and a GeneAmp PCR System 9700 (Applied Biosystems, Waltham, USA) as well as a T100 Thermal Cycler (Bio-Rad, Hercules, USA). The PCR mixtures were heated to 94°C for 5 min, followed by 35 cycles at 94°C (10 s each), 48°C for COI, 42°C for tRNA\(^{\text{Cys}}\)–16S, and 44°C for tRNA\(^{\text{Leu}}\)–ND1 (20 s), and 72°C (42 s each), and then a final extension at 72°C for 6 min. The sequencing mixtures were heated at 96°C for 2 min, followed by 40 cycles at 96°C (10 s each), 50°C (5 s each), and 60°C (42 s each). The obtained sequences were edited using DNA BASER (Heraclès Biosoft S.R.L., Pitești, Romania). The DNA sequences listed in Table 1 were newly obtained in this study and were deposited with the DNA Data Bank of Japan (DDBJ).

Six published sequences were obtained from the DDBJ for use in genetic distance analyses (Table 1). The lengths of the COI, tRNA\(^{\text{Cys}}\)–16S, and tRNA\(^{\text{Leu}}\)–ND1 sequences were 1,267, 1,004, and 628 bp, respectively. Within the sequences of COI, tRNA\(^{\text{Cys}}\)–16S, and tRNA\(^{\text{Leu}}\), respectively, no differences were detected. The alignment of ND1 was trivial, as no indels were observed. Pairwise comparisons of uncorrected p-distances for nine ND1 sequences (594 bp) were calculated using MEGA6.06 (Tamura et al. 2013).

Family Salifidae Johansson, 1909
Genus *Mimobdella* Blanchard, 1897
*Mimobdella japonica* Blanchard, 1897
(Figs 1–3)

*Mimobdella japonica* Blanchard, 1897: 94, 95, pl. 6, figs 16; Nakano 2011: 3–7, figs 1–4; Nakano 2013: 100–103, figs 2–4.

**Material examined.** Three specimens collected from Nakanogo, Hachijo, Hachijojima island, Tokyo, Japan (33.071886°N, 139.811194°E, elevation 130 m), by Victor Benno Meyer-Rochow: KUZ Z1653, dissected, collected from inside a rotten wooden log, on 19 April 2015; KUZ Z1657, in soil under a rotten wooden log, on 10 July 2015;

### Table 1. Specimens of *Mimobdella japonica* Blanchard, 1897 used for molecular analysis, with voucher registration numbers, collection localities, and DNA Data Bank of Japan (DDBJ) accession numbers. Sequences marked with an asterisk (*) were obtained for the first time in the present study. Acronym: KUZ, Zoological Collection of Kyoto University.

<table>
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<tr>
<th>Voucher Reg. No.</th>
<th>Island</th>
<th>Sequenced Genes</th>
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<th>tRNA(^{\text{Leu}})–ND1</th>
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Fig. 1. *Mimobdella japonica* Blanchard, 1897, from Hachijojima island, Izu Islands, KUZ Z1653. A, dorsal view; B, ventral view. Scale bar: 10 mm.
Mimobdella japonica from the Izu Islands and KUZ Z1658, dissected, in soil under a rotten wooden log, on 11 July 2015.

**Description.** BL 68.2–91.1 mm, BW 6.2–8.8 mm (Fig. 1). Caudal sucker ventral, elliptic, CL 3.8–4.2 mm, CW 4.3–5.0 mm (Figs 1B, 2D). Annulation of somites I–VII unclear, comprising 16–17

Fig. 2. *Mimobdella japonica* Blanchard, 1897, from Hachijo island, Izu Islands, KUZ Z1653. A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXIV–XXVII and caudal sucker; D, ventral view of somites XXIV–XXVI and caudal sucker; E, ventral view of somites X–XIII; F, ventral view of junction between crop and intestine with post-crop caeca; G, dorsal view of terminal part of ovisacs with testisacs, including ventral nervous system. Scale bars: 2 mm. Abbreviations: an, anus; cl, clitellum; cp, crop; fg, female gonopore; in, intestine; mg, male gonopore; np, nephridiopore; ov, ovisac; pcc, post-crop caecum; sph, sphincter; ts, testisac.
annuli altogether; according to annuli formation, annulation of somites I–VII tentatively interpreted as follows: somite I merged with prostomium; somite II uniannulate; somite III uni- or biannulate; somite IV bi- or triannulate; somite V triannulate; somite VI 4-annulate; somite VII basically 4-annulate; possibly somite V a3 to somite VI a2 forming posterior margin of oral sucker (Fig. 2A, B). Somite VIII 5-, or 6-annulate. Somite IX 6-, or 7-annulate. Somites X and XI 7-annulate (Fig. 2E). Somites XII and XIII 7-, or 9-annulate, a2 with slight furrow (Fig. 2E). Somites XIV–XXIII 9-annulate; a2 of each somite in somites XVII–XXIII with slight secondary furrow. Somite XXIV 6-annulate (Fig. 2C, D). Annulation of somites XXV–XXVII, comprising 5–8 annuli altogether, hardly discernable; 158th or 159th annulus last complete annulus on venter; according to annuli formation, annulation of somites XXV–XXVII tentatively interpreted as follows: somite XXV tri-, or 4-annulate; somite XXVI bi-annulate; somite XXVII uni- or triannulate. Anus between 159th [XXVI (a1 + a2)] and 160th (XXVI a3) annuli with 2 or 3 post-anal annuli (Fig. 2C, D).

X b5 (c9) and XIII a2, respectively, first and last annuli of clitellum (Fig. 2E).

Male gonopore in XI/XII. Female gonopore in XII/XIII (Fig. 2E). Gonopores separated by one full somite.

Eyes undetectable. Papillae numerous, minute, hardly visible, one row on every annulus, and 2 or 3 rows on annuli with secondary furrow(s). Nephridiopores, in 17 pairs, in somites VIII–XXIV (Fig. 2B, D, E).

Pharynx reaching to XIV/XV (KUZ Z1653) or XV c1 (KUZ Z1658), with 3 myognaths separated by triangular paragnaths, each myognath bearing two conical stylets arranged in tandem. Crop reaching to XXI a2/c9; terminal end of crop forming sphincter between crop and intestine, in XIX c1 (KUZ Z1653) or c1/c2 (KUZ Z1658) to XXI a2/c9, pair of post-crop caeca, right post-crop caecum in XXI c1–d22 (KUZ Z1653) or XX d22–XI d22 (KUZ Z1658), left post-crop caecum in XXI c1–d22 (KUZ Z1653) or XX c12–XXI c21 (KUZ Z1658) (Fig. 2F). Intestine reaching to XXIV c2/b2 (KUZ Z1653) or a2/b5 (c9) (KUZ Z1658). Rectum tubular, thin-walled, reaching straight to anus.

Testisacs multiple (Fig. 2G), ca. 220–260 on each side (KUZ Z1653) or uncountable (KUZ Z1658); on right side, in XVI c2 to 155th (KUZ Z1653) or 156th (KUZ Z1658) annulus (XXV b1), in total ca. 220 testisacs, ca. 20 in XVI, 30 in XVII, 27 in XVIII, 27 in XIX, 26 in XX, 26 in XXI, 23 in XXII, 27 in XXIII, 15 in XXIV, 5 in XXV; on left side, in XVI b2 to 156th (KUZ Z1653) (XXV b2) or 155th (KUZ Z1658) annulus (XXIV b6), in total ca. 260 testisacs, ca. 16 in XVI, 30 in XVII, 32 in XVIII, 26 in XIX, 31 in XX, 35 in XXI, 33 in XXII, 19 in XXIII, 32 in XXIV, 5 in XXV. Sperm duct paired; right sperm duct in XI c12 to XVI c2 (KUZ Z1653) or b2/a2 (KUZ Z1658); left sperm duct in XI c12 to XVI b2 (KUZ Z1653) or a2/c9 (KUZ Z1658). Paired atrial cornua in XI c1 (d21) to XII c1–c2. Male atrium in XI c11 (d21) to XII c1–c2.

One pair of ovisacs; right ovisac descending directly to XXII c9 (KUZ Z1653) or descending to XIX c10/d21, turning anteriorly to reach XIX c1, then either turning posteriorly to XIX c2 (KUZ Z1658) (Fig. 2G); left ovisac descending directly to XXII a2 (KUZ Z1653) or descending to XXI b2/a2, turning anteriorly to reach XX c10, then either turning posteriorly to XX d21 (KUZ Z1658) (Fig. 2G); both ovisacs converging in XIII c1; then each ovisac descending directly to female gonopore.

**Colouration.** In life, dorsal surface ochre; ventral surface pinkish gray (Fig. 3). Colour faded in preserved specimens; clitellum obvious, slightly deeper than surrounding body parts (Fig. 1).

**Distribution.** This species has been recorded previously from Amamioshima and Okinawajima islands in the Ryukyu Islands, Japan (Nakano 2013). Herein, we report its first record from Hachijojoima island, Izu Islands, Japan (Fig. 4).

**Natural history.** *Mimobdella japonica* has been reported to be semi-aquatic (Nakano 2013), but all three individuals from Hachijojoima were collected from terrestrial habitats. All had an obvious clitellum. Based on their dates of collection, we deduce that the reproductive season of this species on Hachijojoima begins in April and continues until at least early July. It seems to have established a breeding population on this island despite the island’s being ~500–700 km further north and having a colder climate than its other known localities in the Ryukyu Islands.
Genetic distances. Three specimens from Hachijojima, two from Amamioshima, and four from Okinawajima had completely identical sequences of COI (1,267 bp), tRNA\(^{Gln}\)–16S (1,004 bp), and tRNA\(^{Aua}\) (34 bp). The pairwise ND1 uncorrected \(p\)-distance among these nine specimens was 0.0–0.2% (Table 2). The 570th bp position (counted from the first position, thus in the third position of the codon) of the ND1 sequences of the two specimens from Okinawajima (KUZ Z721 and Z722) was T (it was C in the other seven sequences; Fig. 4).

Remarks. The specimens collected from Hachijojima were clearly identified as *Mimobdella japonica* based on the diagnostic characteristics defined by Nakano (2013): mid-body somites 9-annulate; anus with 2 or 3 post-anal annuli; male gonopore in XI/XII, female gonopore in XII/XIII, gonopores separated by one full somite; post-crop caeca in pairs in XX and XXI; sperm duct reaching to level anterior of middle part of XVI; and ovisacs long, reaching to XIX–XXII. In addition, their COI, tRNA\(^{Gln}\)–16S, and tRNA\(^{Aua}\)–ND1 sequences were identical to those of specimens collected from the Ryukyu Islands. These genetic data confirm the implications of the morphological data that these specimens are indeed *M. japonica*.

Considering that all of the examined specimens including individuals from Hachijojima have essentially identical sequences of mitochondrial genes, it is conceivable that *M. japonica* has arrived in its known localities via recent dispersal events, or that it has been introduced via human activities. Hirano *et al.* (2014) considered the low genetic diversity of the bradybaenid land snail *Bradybuena phaeogramma* (Ancey, 1888), which is distributed in the Izu Islands as well as the Ryukyu Islands, and concluded that its distribution is the result of long-distance dispersal via an oceanic current. Despite this, some other terrestrial invertebrates are considered to have been introduced into Hachijojima via human activities. Karasawa *et al.* (2015) and Umezawa (1973) reported the whip scorpion *Typopeltis stimpsonii* (Wood, 1862), originally from Amamioshima and adjacent islands, in Hachijojima. Umezawa (1973) suggested that this whip scorpion was introduced to the island attaching with cycads in 1968. Fujiyama *et al.* (2012) and Meyer-Rochow (2015) reported mass outbreaks on Hachijojima of the polydesmid diplopod *Chamberlinius hualienensis* Wang, 1956, originally known from Hualien County, Taiwan (Wang 1956). Fujiyama *et al.* (2012) stated that this Taiwanese diplopod was first found on Hachijojima in 2002.

The distributional history of this species remains unveiled because the number of examined specimens of *M. japonica* is still severely limited, and the distribution of this leech species is not fully revealed. Although two of the four specimens from Okinawajima island had ND1 sequence that were identical to specimens from the other two islands, the other two specimens from Okinawajima had unique ND1 sequences (Fig. 4). However, it is possible that this unique ND1 sequence will be detected from leeches in other localities in the future, which could further elucidate the distributional history of this species. Further faunal, distributional, and genetic studies are thus essential to determine the distributional history of *M. japonica*.

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Anzeiger 35: 1–5.