

First Record of *Mimobdella japonica* (Hirudinida: Arhynchobdellida: Salifidae) from Okinawajima Island, Ryukyu Islands, Japan, with a Description of the Specimens from the Ryukyu Islands

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Two specimens of the large, predaceous salifid leech *Mimobdella japonica* Blanchard, 1897 were collected from Okinawajima island, Ryukyu Islands, Japan, representing the first record of this species from Okinawajima. A description is provided of the external and internal morphology of these specimens and two additional specimens of *M. japonica* from Amami-oshima island, also in the Ryukyu Islands. COI sequences showed no difference between the two specimens of *M. japonica* from Okinawajima and the two specimens from Amami-oshima (K2P distance=0%). This suggests that *M. japonica* is an introduced species in one or both of these islands.

Key Words: Hirudinida, Salifidae, *Mimobdella japonica*, first record, Okinawajima, Amami-oshima, Ryukyu islands, COI.

Introduction

Mimobdella Blanchard, 1897 is a genus of large, predaceous leeches. The taxonomic position of *Mimobdella* has been ambiguous. In the original description, Blanchard (1897) placed this genus in the family Erpobdellidae (cited by him as Herpobdellidae, which is now regarded as a junior synonym of Erpobdellidae; Moore 1927a). Soós (1966) also concluded that *Mimobdella* belongs among the erpobdellids. However, Sawyer (1986) placed this genus in the Gastrostomobdellidae (cited as the subfamily Gastrostomobdellinae in his work) in the suborder Hirudiniformes along with two other genera of terrestrial macrophagous leeches, *Gastrostomobdella* Moore, 1929 and *Orobdella* Oka, 1895. Recently, Nakano (2011) re-examined the holotype of *Mimobdella japonica* Blanchard, 1897, the type species of *Mimobdella*, and showed that this species possesses diagnostic characters of the Salifidae (e.g., pharyngeal stylets). Accordingly he re-assigned *Mimobdella* to the family Salifidae in the suborder Erpobdelliformes (Nakano 2011; Nakano *et al.* 2012).

Two species were initially included in *Mimobdella*, *M. japonica* from Japan and *M. buttkoferi* Blanchard, 1897 from Borneo (Blanchard 1897). Blanchard (1897) did not designate a type species for *Mimobdella*, but Soós (1966) designated *M. japonica* as the type and placed two additional species in the genus, *M. africana* Moore, 1939 from Lake Tanganyika and *M. thienemanni* Augener, 1931 from Sumatra. Sawyer (1986) concluded that only three species truly belonged to *Mimobdella*, *M. japonica*, *M. buttkoferi*, and *M. thienemanni*. To determine the correct generic assign-

ment of erpobdelliform species, a description of the internal morphology of each species is crucial. Although Nakano (2011) provided information about the internal anatomy of the type species *M. japonica*, the internal morphology of the remaining species of *Mimobdella* has not been described. Thus, *M. japonica* is the only well-established species in this genus (Nakano 2011).

The holotype of *M. japonica* was collected by Philipp Franz von Siebold and is now deposited in the Naturalis Biodiversity Center in Leiden, The Netherlands (Blanchard 1897; Nakano 2011). The locality of collection of this specimen is described as “Japan” on its label (Nakano 2011). After its original description, *M. japonica* has been reported from various places in Japan and China (Oka 1910a, b, 1917, 1923; Yang 1996), but my examination of the holotype (Nakano 2011) led me to conclude that those records were based on misidentified specimens. Thus, the precise distribution of *M. japonica* is not well known. Two studies (Nakano 2011; Nakano *et al.* 2012) reporting *Mimobdella japonica* on the island of Amami-oshima in the Ryukyu Islands of southwest Japan provide the only confirmed locality for this species. Recently, newly collected specimens have come to hand of large salifid leeches from Okinawajima, farther south in the Ryukyu Islands. These specimens agree with those from Amami-oshima and are thus identified as *M. japonica*. As was noted above, information about the internal anatomy of *Mimobdella* species is severely limited. Based on an external and internal morphological examination of specimens from the Ryukyu Islands, an amended description of *M. japonica* is presented here. In addition, the significance of the invariant COI sequences obtained from

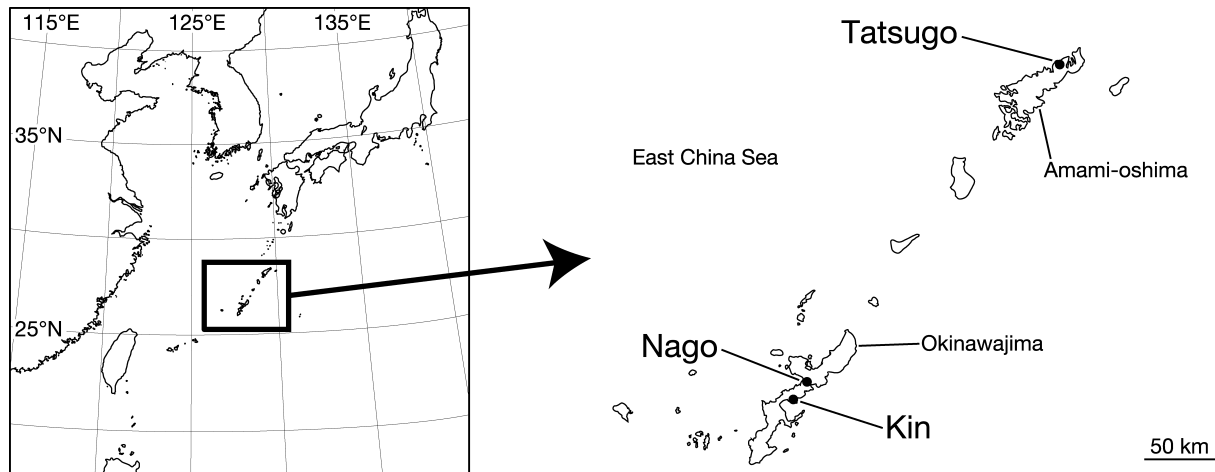


Fig. 1. Map showing the collection localities of the specimens examined in this study: Nago and Kin on Okinawajima island, and Tatsugo on Amami-oshima island.

Table 1. Voucher, locality, and GenBank accession numbers for COI sequences of *Mimobdella japonica* Blanchard, 1897 used in this study.

Voucher	Locality	COI	Source
KUZ Z179	Tatsugo, Amami-oshima	AB679658	Nakano (2012)
KUZ Z227	Nago, Okinawajima	AB761393	Present study
KUZ Z228	Tatsugo, Amami-oshima	AB761394	Present study
KUZ Z229	Kin, Okinawajima	AB761395	Present study

these specimens is also discussed.

Materials and Methods

Leeches were newly collected from the islands of Okinawajima and Amami-oshima, in the Ryukyu Islands, Japan (Fig. 1). When possible, altitudes above sea level and geographical coordinates for localities were obtained using a Garmin eTrex® GPS unit.

The specimens were relaxed by the gradual addition of 95% ethanol to fresh water. For DNA extraction, botryoidal tissue was taken from the posterior part of the body around the caudal sucker of every specimen, and the rest of the bodies were fixed in 10% formalin and preserved in 70% ethanol. Two measurements were taken: body length (BL) from the anterior margin of the oral sucker to the posterior margin of the caudal sucker, and maximum body width (BW). Examination, dissection, and drawing of the specimens were performed under a stereoscopic microscope equipped 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 is based on Moore (1927b): body somites are denoted by Roman numerals, and the annuli in each somite are given an alphanumeric designation.

Extraction of genomic DNA and sequencing of the mitochondrial COI gene were done according to Nakano (2012). DNA sequences of three of the present specimens of *M. japonica* from the Ryukyu Islands were obtained and deposited in GenBank. In addition, the COI sequence of one addi-

tional specimen of *M. japonica* from Amami-oshima (KUZ Z179) was obtained from GenBank (Table 1). COI sequences were aligned by eye because no indels existed. The length of the aligned COI sequences was 1267 bp. Based on the aligned COI sequences, pairwise comparisons of Kimura 2-parameter (K2P) distance (Kimura 1980) of the sequences were calculated using MEGA5 (Tamura *et al.* 2011).

Genus *Mimobdella* Blanchard, 1897
urn:lsid:zoobank.org:act:77DDC749-ABA0-4A38-9B09-89A3D856759D
Mimobdella japonica Blanchard, 1897
urn:lsid:zoobank.org:act:E9ED3E63-39DC-479F-BAB2-F30B1DA0CB6C
(Figs 2–4)

Mimobdella japonica Blanchard, 1897: 94–95, pl. 6, figs 16, 17; Nakano 2011a: 3–7, figs 1–4.

Diagnosis (amended from Nakano 2011). In life, dorsal surface ochre, ventral surface whitish ochre, paler than dorsal surface. Mid-body somites novem-annulate, generally c1=c2<b2<a2>c9=c10=d21=d22<c12. Clitellum in X c9 to XIII a2. Anus with 2–4 post-anal annuli. Post-crop caeca in pairs in XX c12 to XXII c1. Male gonopore in XI/XII, female gonopore in XII/XIII, gonopores separated by 9 annuli (1 full somite). Sperm ducts reaching to XVI c1. Ovi-sacs reaching to XXIII c1, and then turning anteriorly.

Material examined. Two specimens collected from Okinawajima island, Okinawa Prefecture, Japan, by Masashi Sugimoto: KUZ Z227, dissected, from Nago in

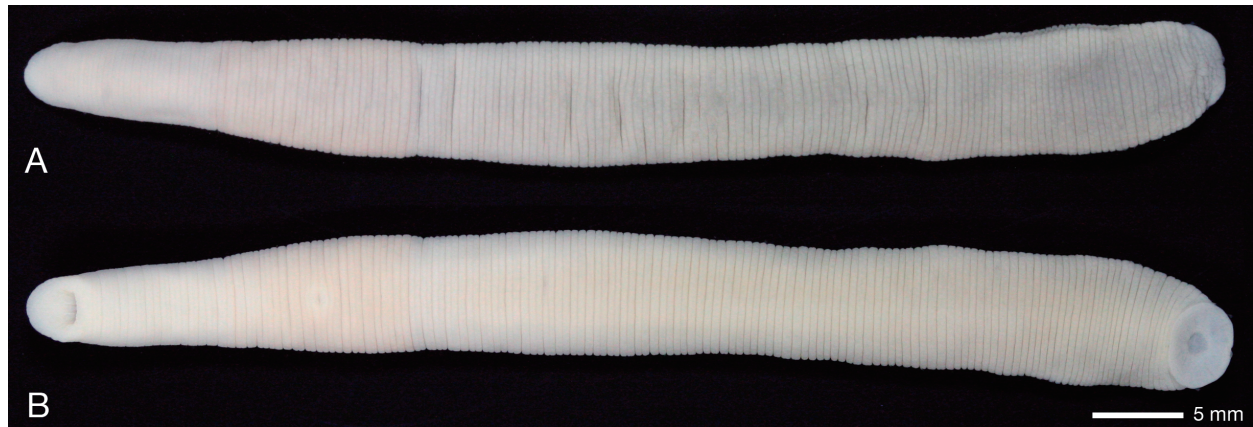


Fig. 2. *Mimobdella japonica* Blanchard, 1897 from Okinawajima, KUZ Z229. A, dorsal view; B, ventral view.



Fig. 3. *Mimobdella japonica* Blanchard, 1897 from Okinawajima, KUZ Z229, photograph of live animal, dorsal view.

spring of 2008, and KUZ Z229, dissected, from Kin on 22 May 2011. Two specimens collected from the underside of stones in a canal in a paddy field in Akina, Tatsugo, Amami-oshima island, Kagoshima Prefecture, Japan, by Takafumi Nakano on 28 April 2010: KUZ Z179 (alt. 0 m, 28°26'35"N, 129°33'36"E), dissected, and KUZ Z228 (alt. 0 m, 28°26'42"N, 129°33'38"E), dissected.

Description of specimens from the Ryukyu Islands. Body firm, muscular, elongated, with constant width in caudal direction, dorsoventrally depressed, maximum BL 106.88 (KUZ Z179), maximum BW 7.13 (KUZ Z227) (Fig. 2A, B). Caudal sucker situated ventrally, oval, its greater diameter smaller than BW (Figs 2A, B, 4C, D). In life, dorsal surface ochre, clitellum yellow ochre, ventral surface paler than dorsal surface, whitish ochre. Colour faded in preservative (Fig. 3).

Annulation of somites I–VII unclear, comprising 14–17 annuli altogether, 1st annulus completely merged with prostomium, 7th–9th annuli forming posterior margin of oral sucker; KUZ Z179 with 15 annuli in this region, 12th and 15th annuli with obvious furrow; KUZ Z227 with 14 annuli, 9th and 11th annuli with slight dorsal furrow, 10th and 14th annuli with slight furrow; KUZ Z228 with 16 annuli, 12th, 13th and 16th annuli with slight furrow; KUZ Z229, comprising 17th annuli, 14th and 17th annuli with slight furrow, 7th and 8th annuli united on venter (Fig. 4A, B). Somite VIII quinquannulate, $b1 > b2 < a2 < b5 > b6$ (KUZ Z227, Z229) (Fig. 4A, B); or sexannulate, $b1$ (with slight furrow: $c1, c2 > b2 < a2 < b5$ ($c9, c10 > c11 > c12$ (KUZ

Z179, Z228). Somite IX quiquannulate, $b1$ ($c1, c2$) = $b2$ ($c3, c4$) $< a2 < b5$ ($c9, c10$) = $b6$ ($c11, c12$) (KUZ Z229); sexannulate, $b1$ ($c1, c2$ dorsally) $> b2 = a2 < b5 > c11 > c12$ (KUZ Z227); or septannulate, $c1 = c2 < b2 < a2 < b5 = c11$ ($c9, c10$) $> c12$ ($d21, d22$) (KUZ Z179, Z228). Somite X sexannulate, $c1 = c2 < b2 < a2 < b5$ ($c9, c10$) $< b6$ ($d21, d22, c12$) (KUZ Z227); octannulate, $c1 = c2 = b2 < a2 < c9$ ($d17, d18$) $> c10$ ($d19, d20$ ventrally) $> c11 = c12$ (KUZ Z229) (Fig. 4E); or novem-annulate, $c1 = c2 < b2 < a2 > c9 = c10 = d21 = d22 < c12$ (KUZ Z179, Z228). Somite XI quiquannulate, $b1$ ($c1, c2$) $> b2 = a2 < b5$ ($c9, c10$) $< b6$ ($d21, d22, c12$) (KUZ Z227); or novem-annulate (KUZ Z179, Z228, Z229) (Fig. 4E). Somite XII sexannulate, $b1$ ($c11, c12$) $> b2 = a2 < b5$ ($c9, c10$) = $c11$ ($d21, d22$) $> c12$ (KUZ Z227); or novem-annulate (KUZ Z179, Z228, Z229) (Fig. 4E). Somite XIII septannulate, $c1 = c2 = b2 > a2 < b5$ ($c9, c10$) = $c11$ ($d21, d22$) $> c12$ (KUZ Z227); or novem-annulate (KUZ Z179, Z228, Z229). X $c9$ and XIII $a2$ respectively being first and last annuli of clitellum (Fig. 4E). Somites XIV–XXIII novem-annulate, or rarely somites XXII and XXIII respectively septannulate, $c1 = c2 < b2 < a2 < b5$ ($c9, c10$) = $c11$ ($d21, d22$) $> c12$, and quiquannulate, $b1$ ($c1, c2$) $> b2 < a2 < b5$ ($c9, c10$) $< b6$ ($d21, d22, c12$) (KUZ Z227). Somite XXIV quiquannulate, $b1$ ($c1, c2$) $> b2 < a2 < b5$ ($c11, c12$) = $b6$ ($c11, c12$) (KUZ Z227); sexannulate, $c1 = c2 = b2 < a2 < b5$ ($c9, c10$) = $b6$ ($c11, c12$) (KUZ Z229) (Fig. 4C, D); septannulate, $c1 = c2 < b2 < a2 < b5$ ($c9, c10$) $> c11$ ($d21, d22$) $> c12$ (KUZ Z228); or octannulate, $c1 = c2 < b2 < a2 > c9 = c10 < c11 > c12$ (KUZ Z179). Annulation of somites XXV–XXVII comprising 7–9 annuli altogether, but hardly decidable; possibly XXV triannulate $a1$ ($b1, b2$) $> a2 < a3$; XXVI triannulate, $a1 = a2 = a3$; and XXVII uni- (KUZ Z227), bi- (KUZ Z228), or triannulate (KUZ Z179, Z229); XXVI $a1$ (KUZ Z227, Z229), or $a3$ (KUZ Z179, Z228) being last complete annulus on venter; anus at XXVI $a2/a3$ with 2–4 post-anal annuli (Fig. 4C, D).

Eyes undetectable. Nephridiopores in 17 pairs in VIII–XXIV, situated ventrally at middle of $b2$ of each somite (Fig. 4B, D, E). Papillae numerous, minute, mainly 1 row on every annulus, and 2 or 3 rows on annuli with slight furrow(s).

Pharynx strepsilaematous, reaching to XIV $d22/c12$ –XIV/XV, with 3 myognaths separated by triangular parag-naths, each myognath bearing 2 conical stylets arranged in

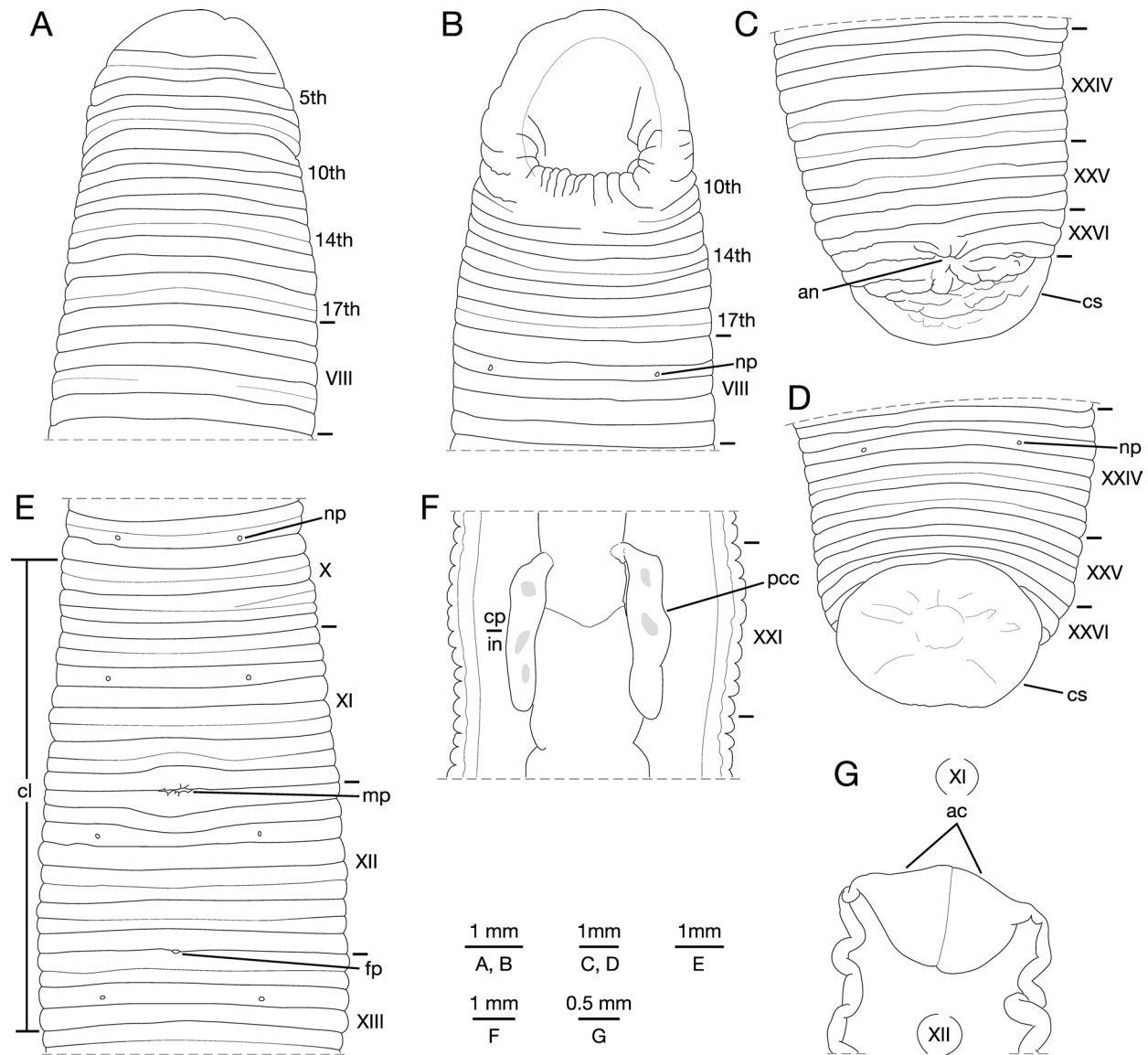


Fig. 4. *Mimobdella japonica* Blanchard, 1897 from Okinawajima, KUZ Z229. A, dorsal view of somites I–VIII; B, ventral view of somites I–VIII; C, dorsal view of somites XXIV–XXVII and caudal sucker; D, ventral view of somites XXIV–XXVII and caudal sucker; E, ventral view of somites X–XIII; F, ventral view of post-crop caeca; G, dorsal view of male atrium showing positions of ganglia XI and XII. Abbreviations: ac, atrial cornu; an, anus; cl, clitellum; cp, crop; cs, caudal sucker; fp, female gonopore; in, intestine; mp, male gonopore; np, nephridiopore; pcc, post-crop caecum.

tandem, parallel to body axis. Crop tubular, reaching to XXI b2–XXI d21; pair of post crop-caeca thin-walled in XX c12–XXI c2 to XXI c10–XXII c1 (Fig. 4F). Intestine tubular and acaecate, reaching to XXIII b1–XXIII c10/d21. Rectum tubular, thin-walled.

Male gonopore in XI/XII (Fig. 4E). Female gonopore in XII/XIII (Fig. 4E). Gonopores separated by 9 annuli (1 full somite). Testisacs multiple, in XV c12–XVI c2 to XXV a2–XXV a3, several testisacs on each side in each annulus. Sperm ducts in XI c12 to XV c10–XVI c1/c2, coiled, narrowing at junction with atrial cornu, then turning gradually inward toward atrial cornua without pre-atrial loop. Pair of atrial cornua curved laterad in XI c12 and XII c1 (Fig. 4G). Atrium short, muscular, globular, in XI c12 and XII c1. One pair of ovisacs long, thin-walled, slightly folded, tubular in XIII c1 to XXI c2/b2–XXIII c1/c2; right ovisac turned anteriorly in

XXI c2/b2–XXIII c1/c2, then reaching to XX a2–XXII b2; left ovisac also turned anteriorly in XXI c2/b2–XXII c10, then reaching to XX a2–XXI d21, both ovisacs converging in XIII c1, directly descending to female gonopore.

Distribution and habitat. Known from Tasugo, Amami-oshima island, Japan, and lowland regions of Okinawajima island, Japan. Semi-aquatic species.

Genetic variation. The K2P distance (COI) between the two specimens of *M. japonica* from Okinawajima (AB761393, AB761395) and those from Amami-oshima (AB679658, AB761394) was 0%. No difference was detected among the COI sequences obtained from these four specimens.

Remarks. Four specimens from the Ryukyu Islands were unambiguously identified as *M. japonica* based on their possession of the following characteristics: mid-body somites novem-annulate; clitellum in X c9 to XIII a2; male

gonopore in XI/XII, female gonopore in XII/XIII, 9 annuli between gonopores; pair of post-crop caeca mainly in XXI; and paired sperm ducts reaching to XVI c1. Although the holotype of *M. japonica* possesses two post-anal annuli (Nakano 2011), the Ryukyu specimens have two to four post-anal annuli. In addition, the ovisacs of the present specimens are longer than those of the holotype of *M. japonica*. Therefore, the diagnosis of *M. japonica* is amended herein above to take these differences into account.

The COI sequence divergence among these four specimens is 0%. This low genetic diversity may indicate that the leeches have dispersed to these islands in a recent rapid range expansion, as have, e.g., species of *Hirudo* Linnaeus, 1758 in Europe (Trontelj and Utevsky 2012), or that they have been introduced by human activities. The K2P distance among the specimens of *M. japonica* is abnormally low relative to that between *Orobdella shimadae* Nakano, 2011 and *O. dolichopharynx* Nakano, 2011 (8.5–11%, mean=9.7%, Nakano, unpub. data), which are also predaceous, but fully terrestrial leeches that are distributed in Okinawajima and Amami-oshima, respectively. *Mimobdella japonica* from Okinawajima has been found mainly in cultivated areas (Sugimoto, pers. comm.). Additionally, specimens of *M. japonica* from Amami-oshima have been collected only from paddy fields. Thus, *M. japonica* may have been introduced to either Okinawajima or Amami-oshima, or to both islands, via human activity. However, the origin of *M. japonica* in the Ryukyu Islands and the distributional range of this species elsewhere are still unclear. For example, large salifid leeches from Kyushu, Japan, are identified as *Odontobdella blanchardi* (Oka, 1910), not *M. japonica* (Nakano, pers. obs.). Despite the fact that *M. japonica* can be regarded as a likely introduced species in the Ryukyu Islands according to its COI sequence divergence, its only confirmed localities are in these islands. Further faunal surveys are needed to confirm whether *M. japonica* is truly a rapidly dispersing alien species, and to fully reveal its range of distribution and biogeographical history.

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