

Journal of Melittology

Bee Biology, Ecology, Evolution, & Systematics

The latest buzz in bee biology

No. 84, pp. 1–8

27 March 2019

Halictus hedini hedini (Hymenoptera: Halictidae) newly recorded from Japan, revealed by DNA barcoding and morphology

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Abstract. *Halictus hedini hedini* Blüthgen is newly recorded from Japan. The species had been previously mistaken for the Holarctic species *H. rubicundus* (Christ), which is removed from the Japanese fauna. *Halictus hedini hedini* was previously known from Eastern and Central Asia. *Halictus hedini hedini* is similar to *H. rubicundus*, but is separated by the first metasomal tergum with coarse and deep punctures in both sexes and the male flagellum ventrally with a fringe of short hairs. A key to Japanese species of *Halictus* is provided. DNA barcodes, phenology, and floral records from Japan are reported.

INTRODUCTION

The bee genus *Halictus* Latreille (Halictidae: Halictinae) includes 206 species worldwide (Ascher & Pickering, 2019), distributed across the Holarctic, Oriental, and Neotropical Regions (Michener, 2007). The greatest diversity is recorded from the Palearctic Region (Pesenko, 2005). *Halictus* is notable for its remarkable diversity of social behaviors (Michener, 1974), including social polyethism (Soucy, 2002; Field *et al.*, 2010), making it an important taxon for studies of social behavior and evolution (Schwarz *et al.*, 2007). *Halictus* is mainly distinguished from other halictid bees by the following character states: distal crossveins (1rs-m and 2rs-m) of female forewing as strong as first submarginal crossvein (2nd abscissa of Rs); metasomal terga with dense plumose bands of hairs apically; and ventral gonostylus of male genitalia absent or directed apically, not retrorse (Michener, 2007). In Michener's (2007) classification, *Halictus* is divided into 15 subgenera: *Argalictus* Pesenko, *Halictus* Latreille s.str., *Hexataenites* Pesenko, *Lampralictus* Pesenko, *Monilapis* Cockerell, *Nealictus* Pesenko,

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doi: <http://dx.doi.org/10.17161/jom.v0i84.7565>

Odontalictus Robertson, *Pachyceble* Moure, *Paraseladonia* Pauly, *Platyhalictus* Pesenko, *Protohalictus* Pesenko, *Ramalictus* Pesenko, *Seladonia* Robertson, *Tytthalictus* Pesenko, and *Vestitohalictus* Blüthgen. A morphological phylogeny failed to resolve *Seladonia* and *Pachyceble* (Pesenko, 2004) and molecular phylogenetic analyses reveal that *Pachyceble* is rendered paraphyletic by both *Vestitohalictus* and *Seladonia* s.str. (Danforth *et al.*, 1999; Gibbs *et al.*, 2012). Furthermore, the subgenus *Paraseladonia* is likely to be derived from *Seladonia* s.l. (Pesenko, 2004). As such, justification for the subgenera of *Seladonia* as currently recognized is poor.

The East Asian fauna of *Halictus* has been relatively well studied by various researchers (Dawut & Tadauchi, 2000, 2001, 2002, 2003; Ebmer, 1978a, 1978b, 1982, 2005, 2006; Pesenko, 2005, 2006; Murao *et al.*, 2013; Niu *et al.*, 2004, 2007; Sakagami & Ebmer, 1987). Four species are listed in Japan (Tadauchi & Murao, 2014). One of them, *Halictus rubicundus* (Christ), has been recorded from Hokkaido to Honshu in Japan (Tadauchi & Murao, 2014). This species is Holarctic in distribution (Soucy & Danforth, 2002; Ebmer, 2011). It is a very rare species in Japan (Sakagami, 1992).

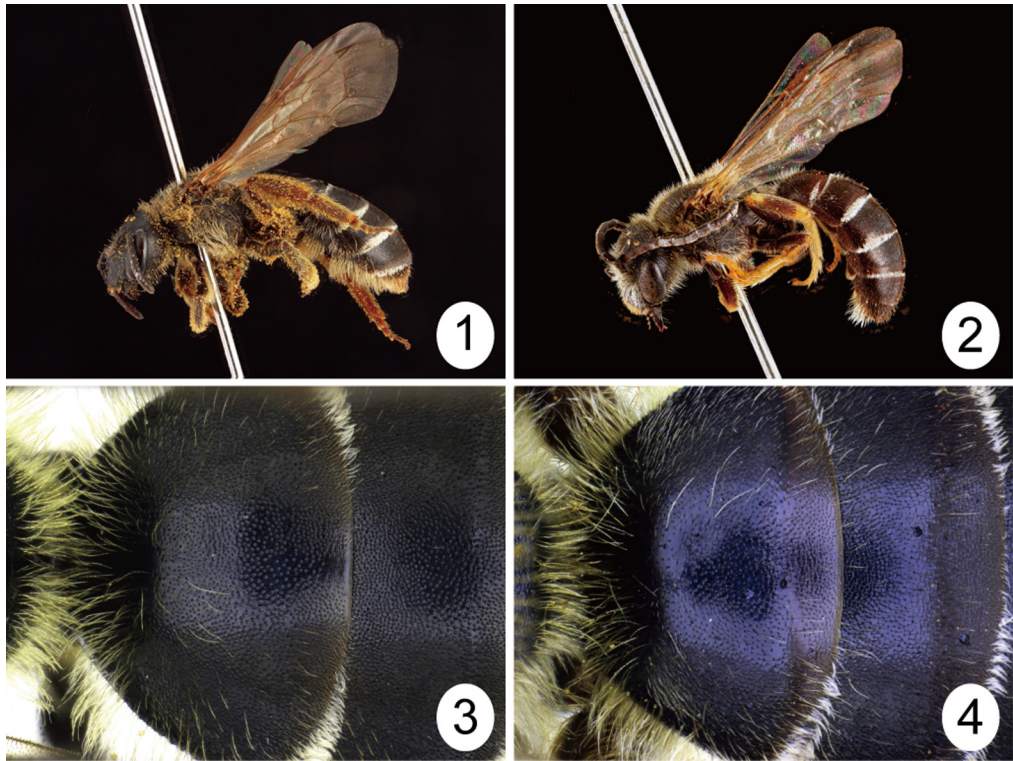
In the course of molecular systematics studies of halictid bees, we noticed that sequence data of Japanese *H. rubicundus* were quite distinct from either those of North America and Europe. Through re-examination of morphological characters, the Japanese specimens were identified as *H. hedini hedini* Blüthgen, a new record for Japan. In the present paper, we report new locality data for *H. hedini hedini* including flight and flower records, DNA barcodes, and a key to Japanese species of *Halictus*.

MATERIAL AND METHODS

COLLECTION: This study is based on the specimens deposited on the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka, Japan (ELKU), J.B. Wallis-R.E. Roughley Museum of Entomology, Manitoba, Canada (WRME), and Yasuo Maeta's collection in the Laboratory of Insect Ecology, Faculty of Life and Environmental Science, Shimane University, Shimane, Japan (SULE).

IDENTIFICATION AND FLORAL RECORDS: Identification is based on specimens of Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia and the diagnostic morphological characters described by Pesenko (2005). Records of flower visited by *H. hedini* are based on specimen label data. The scientific names of flowering plants are cited from the database of "BG Plants Japanese-scientific Names Index (YList)" (Yonekura & Kajita, 2003).

DNA BARCODES: DNA extraction and PCR were conducted at the Kyushu University Museum (Fukuoka, Japan). DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen, Tokyo, Japan) following the manufacturer's instructions. DNA barcode region of the cytochrome oxidase subunit I (COI) gene fragment of mtDNA was amplified (using the primers LCO1490 and HCO2198), purified, and electrophoresed following the methods described by Murao *et al.* (2015). DNA sequencing was outsourced to the FASMAC Co. Ltd. (Kanagawa, Japan). The sequences analyzed in the present study are deposited in GenBank and are available in the project file "DNA barcode database of Asian bees" of the Barcode of Life Data Systems. Table 1 lists the GenBank accession numbers and BOLD process IDs for sequences used in the present paper. DNA extractions and sequencing were also done independently at Cornell University following protocols described in Gibbs *et al.* (2012) for a single specimen. New sequences were compared to public sequence data for *H. rubicundus*.



Figures 1–4. Photographs of *Halictus hedini hedini* Blüthgen and *H. rubicundus* (Christ). 1–2. Lateral habitus of *H. hedini hedini* (1 female, 2 male). 3–4. 1st metasomal tergum of female (3 *H. hedini hedini*, 4 *H. rubicundus*).

SYSTEMATICS

Genus *Halictus* Latreille
Subgenus *Protohalictus* Pesenko

Halictus (Protohalictus) hedini Blüthgen, 1934
(Figs. 1–4)

Halictus hedini Blüthgen, 1934: 5.

DIAGNOSIS: *Halictus hedini hedini* is morphologically similar to its consubgener *H. rubicundus*. It is separated from *H. rubicundus* by the male flagellum ventrally with a fringe of short hairs 0.3–0.5× as long as flagellomere diameter and first metasomal tergum with coarse and deep punctures in both sexes (Fig. 3). In contrast, *H. rubicundus* has the male flagellum ventrally without a distinct fringe and the first metasomal tergum with fine and shallow punctures in both sexes (Fig. 4) (Pesenko, 2005). The dark legs of the female separate *H. hedini hedini* from the subspecies *H. hedini hebeiensis* Pesenko & Wu, 1997, which was described from China.

SPECIMENS EXAMINED: [JAPAN]: **Hokkaido:** 15♀♀, Shiretoko-goko~Ohashi, Shiretoko, 15.vi.1984 [15 June 1984] (O. Tadauchi, ELKU); Shotoshibetsu, Rikubetsu, 4♀♀, 27.v.1984 [27 May 1984] (O. Tadauchi, ELKU); 24♀♀, 13.vi.1984 [13 June 1984] (O.

Tadauchi, ELKU); 1♂, 9.viii.1984 [9 August 1984] (O. Tadauchi, ELKU); 1♀, Utoro, Shiretoko, 2.vi.1975 [2 June 1975] (O. Tadauchi, ELKU); Akan-ko, Kushiro Prov., 1♀, 21.v.1984 [21 May 1984] (O. Tadauchi, ELKU); 1♀, 30.vi.2013 [30 June 2013] (O. Tadauchi, ELKU); Kussharo-ko, Teshikaga, 10♀♀, 26.v.1984 [26 May 1984] (O. Tadauchi, ELKU); 49♀♀, 14.vi.1984 [14 June 1984] (O. Tadauchi, ELKU); Kyushu University Forests, Ashoro, 6♀♀, 6.vi.1984 [6 June 1984] (O. Tadauchi, ELKU); 3♀♀, 9.vi.1984 [9 June 1984] (O. Tadauchi, ELKU); 4♀♀, 22.vi.1984 [22 June 1984] (O. Tadauchi, ELKU); 1♀, Ashoro, Ashoro-ko, 2.vii.2013 [2 July 2013] (O. Tadauchi, ELKU); 1♀, Ashoro, 2.vi.2010 [2 June 2010] (O. Tadauchi, WRME); 1♀, Nishiashoro (Tokachi), 9.viii.1958 [9 August 1958] (Y. Hirashima, cMur); 1♀, 2♂♂, Oketo, Kitami Prov., 21.viii.1969 [21 August 1969] (T. Kawamichi, ELKU); Tokachimitsumata, Kamiashoro, 1♀, 12.vi.1984 [12 June 1984] (O. Tadauchi, ELKU); 2♀♀, 30.vi.1984 [30 June 1984] (O. Tadauchi, ELKU); 2♀♀, 27.viii.1984 [27 August 1984] (O. Tadauchi, ELKU); 1♂, 11.ix.1984 [11 September 1984] (O. Tadauchi, ELKU); 1♀, Hobetsu, Honbetsu-cho, 1.vii.2013 [1 July 2013] (O. Tadauchi, ELKU); 1♂, Akaigawa, 17.ix.1976 [17 September 1976] (M. Usui, ELKU); 1♀, Pe-pan river, Asahikawa, 7.vi.1987 [7 June 1987] (T. Inaoka, ELKU). **Honshu:** 1♀, Sennin Pass, Iwate Pref., 31.viii.1980 [31 August 1980] (Y. Maeta, SULE).

DNA BARCODE (Table 1): Most publicly available COI sequence data for *H. rubicundus* comes from other primer sets that overlap only partially with the DNA barcode fragment (Soucy & Danforth, 2002). Our results show a 2.9% genetic distance over 429 bp shared with *H. rubicundus* sequences. A single downstream sequence of 863 bp was compared to 39 published *H. rubicundus* COI sequences and was found to have a minimum of 3.0% genetic distance, consistent with the DNA barcode fragment.

DISTRIBUTION: Eastern to Central Asia: Japan (Hokkaido and northern to central Honshu), China, Mongolia, Russian Far East, Siberia, Kazakhstan (Pesenko, 2005).

FLIGHT RECORDS IN JAPAN: Female: May to August. Male: August to September (Tadauchi & Murao, 2014, as *Halictus rubicundus*).

FLORAL RECORDS IN JAPAN: *Halictus hedini hedini* has been recorded from the following 12 plant species in four families in Japan. Asteraceae: *Erigeron annuus* (L.) Pers., *Crepidiastrum denticulatum* (Houtt.) J.H.Pak et Kawano, *Hieracium umbellatum* L., *Leucanthemum vulgare* Lam., *Picris hieracioides* L. subsp. *japonica* (Thunb.) Krylov, *Solidago gigantea* Aiton subsp. *serotina* (Kuntze) McNeill, *Sonchus brachyotus* DC., *Taraxacum officinale* Weber ex F.H.Wigg., *Taraxacum* sp. Brassicaceae: *Barbarea vulgaris* R.Br. Fabaceae: *Trifolium repens* L. Salicaceae: *Salix caprea* L.

DISCUSSION

At present, approximately 390 bee species are listed in Japan (Tadauchi & Murao, 2014; Murao *et al.*, 2016). The Japanese bee fauna is among the best characterized in the world due to the efforts of many taxonomists (*e.g.*, Hirashima, 1961, 1962, 1974; Iku-dome, 1989; Mitai & Tadauchi, 2007, 2013; Murao & Tadauchi, 2007; Murao *et al.*, 2009; Nagase, 2006; Sakagami & Tadauchi, 1995; Shiokawa, 2009; Shiokawa & Hirashima, 1982; Tadauchi, 1985; Yasumatsu & Hirashima, 1950, 1956, 1969). However, correct sex association remains problematic for cleptoparasitic taxa such as *Nomada* Scopoli and *Sphecodes* Latreille (Tadauchi *et al.*, 2014). Species delimitation and identification remain a challenge for many halictid bees globally. DNA barcoding has contributed to the taxonomic study of bees, such as sex associations, discovery of new or cryptic species, and synonymies, etc. (Droege *et al.*, 2010; Gibbs, 2009a, 2009b; Monckton, 2016; Packer & Ruz, 2017; Sheffield *et al.*, 2009). DNA barcode data for the Japanese

Table 1. *Halictus hedini hedini* Blüthgen included in this study with Genbank accession numbers and BOLD process ID.

Species	Genbank Accession No.	BOLD Process ID	Length (bp)	Collection Site	Collection Data
<i>Halictus hedini hedini</i>	LC375190	ABBOL 189-18	572	Hobetsu, Honbetsu-cho, Hokkaido	1. vii. 2013
<i>Halictus hedini hedini</i>	LC375191	ABBOL 190-18	486	Ashoro, Ashoro-cho, Hokkaido	2. vii. 2013
<i>Halictus hedini hedini</i>	LC375192	ABBOL 191-18	603	Lake Akan, Hokkaido	30. vi. 2013

bee fauna are still limited, but as this study attests, accumulation of DNA barcodes for Japanese bees may result in additional taxonomic discoveries.

Key to Japanese Species of *Halictus*

1. Body entirely black in both sexes 2
- Body gold or green-metallic luster in both sexes 3
2. Propodeum punctate on lateral and posterior surfaces in female; male genital area deeply concave; male second flagellomere approximately 2.4× first flagellomere *Halictus (Monilapis) tsingtouensis* Strand
- Propodeum wrinkled on lateral surface in female; male genital area nearly flat; male second flagellomere approximately 1.2× first flagellomere *Halictus (Protohalictus) hedini hedini* Blüthgen
3. Distance between lateral ocellus and vertex marginally long, approximately over 3× diameter of ocelli in female; both sexes with pronotum with transverse ridges in both sexes; male six metasomal sternum flat *Halictus (Seladonia) aerarius* Smith
- Distance between lateral ocellus and vertex marginally short, approximately 1.6–2.4× diameter of ocelli in female; both sexes pronotum nearly smooth in both sexes; male six metasomal sternum deeply concave *Halictus (Seladonia) tumulorum ferripennis* (Cockerell)

ACKNOWLEDGEMENTS

We are grateful to Osamu Tadauchi and Bryan Danforth (Cornell University) for support with molecular studies, and to Yulia Astafurova (Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia), Ryoichi Miyanaga (Shimane University, Matsue, Japan), and Toshiharu Mita (Kyushu University, Fukuoka, Japan) for their help in examining specimens. This research was supported in part by the Environmental Research and Technology Development Fund [S-9-2(8)] of the Ministry of the Environment, Japan (grant to Osamu Tadauchi).

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The *Journal of Melittology* is an international, open access journal that seeks to rapidly disseminate the results of research conducted on bees (Apoidea: Anthophila) in their broadest sense. Our mission is to promote the understanding and conservation of wild and managed bees and to facilitate communication and collaboration among researchers and the public worldwide. The *Journal* covers all aspects of bee research including but not limited to: anatomy, behavioral ecology, biodiversity, biogeography, chemical ecology, comparative morphology, conservation, cultural aspects, cytogenetics, ecology, ethnobiology, history, identification (keys), invasion ecology, management, melittopalynology, molecular ecology, neurobiology, occurrence data, paleontology, parasitism, phenology, phylogeny, physiology, pollination biology, sociobiology, systematics, and taxonomy.

The *Journal of Melittology* was established at the University of Kansas through the efforts of Michael S. Engel, Victor H. Gonzalez, Ismael A. Hinojosa-Díaz, and Charles D. Michener in 2013 and each article is published as its own number, with issues appearing online as soon as they are ready. Papers are composed using Microsoft Word® and Adobe InDesign® in Lawrence, Kansas, USA.

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ISSN 2325-4467