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Colletes jankowskyi (Hymenoptera: Colletidae) newly recorded from Japan, with some biological notes and DNA barcodes

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Abstract. *Colletes jankowskyi* Radoszkowski is recorded from Japan for the first time. It is similar to *C. babai* Hirashima & Tadauchi and *C. floralis* Eversmann of the Japanese congeners, but is separated from these allied species by the combination of following characters: malar space short, less than one-half of basal width of mandible; mesoscutum with whitish hairs mixed with darker ones; first metasomal tergum with dense punctures; and hair zone on disc of seventh metasomal sternum narrow in central area. DNA barcodes are also useful for discrimination among these three species. The habitat of Japanese *C. jankowskyi* may be limited to semi-natural grassland. Adults appeared from mid-August to mid-September. The number of individuals is richest from late August to early September, although the species had been collected earlier in the season on the Asian mainland. Most specimens were collected on flowers of *Lespedeza cyrtobotrya* Miq. (Fabaceae). In Japan, *C. jankowskyi* is suggested as a relict species judging from the distribution and habitat information.

INTRODUCTION

The bee genus *Colletes* Latreille, 1802 (Colletidae: Colletinae) includes 484 species (Ascher & Pickering, 2015) distributed on all continents except for Antarctica, Australia, and Madagascar (Michener, 2007; Kuhlmann, 2014). According to Michener (1989, 2007), the genus is supported as a monophyletic group by two character states: 1) the outwardly arcuate posterior part of the second recurrent vein (2m-cu), and 2) base of propodeum with short subhorizontal to vertical basal zone, usually delimited posteriorly by a carina or sharp change in slope or sculpture and divided by a longitudinal carina.

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The Japanese species of *Colletes* were revised by Ikudome (1989). Subsequent to that work, detailed information such as distribution and biology of each species has been accumulated by various researchers, through the study of the local fauna and their biology (*e.g*., Ikudome, 1991; Maeta & Miyanaga, 1998; Matsuno *et al*., 2009; Miyanaga *et al*., 2015; Shimamoto *et al*., 2006; Yamada & Ikudome, 1992). At present, seven species of *Colletes* are reported from Japan (Ikudome, 2014). In August 2010, R.M. found an unknown species of *Colletes* mainly visiting flowers of *Lespedeza cyrtobotrya* Miq. (Fabaceae) in Oita Prefecture, Kyushu, western Japan. Through our examination, this unknown species was identified as *C. jankowskyi* Radoszkowski, 1891, a species newly recorded for Japan. *Colletes jankowskyi* belongs to the *clypearis* group and is widely distributed from central to eastern Asia (Kuhlmann & Proshchalykin, 2011; Niu *et al*., 2013). Detailed biological information for this species is still unknown.

In the present paper, we report new locality data for *C. jankowskyi*, including a diagnosis to separate the species from allied Japanese species, DNA barcodes, and some biological notes such as phenology, flower preferences, and habitat information in Japan. Finally, based on the presently available data we discuss both the biological notes observed in Kyushu and hypothesize a process by which *C. jankowskyi* colonized Japan.

MATERIAL AND METHODS

COLLECTIONS & TERMINOLOGY: The specimens used in the present study are housed in the collection of the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka, Japan (ELKU), and the private collection of the first author in Fukuoka, Japan (cMur). Morphological terminology used in the present work follows that of Ikudome (1989). Abbreviations used in the text are as follow: d = diameter; IS = interspace between punctures (*e.g*., IS of 0.5d, means 1/2 of the diameter of a puncture).

FIELD SURVEY FOR PHENOLOGY & FLORAL RECORDS: Sampling in the field was conducted at an elevation 1050 m at Chojyabaru, Kokonoe-machi, Kusu-gun, Oita Prefecture, Kyushu, N33°7′6.773′′ E131°13′49.331′′, in May 2011; August 2012; July and August 2013; July, September, and October 2014; and August and September 2015, by R.M. He also conducted periodical sampling at the locality during August and September 2015. During sampling bouts, R.M. initiated collecting on all flowers visited by *C. jankowskyi* from approximately 11:30 am to 1:30 pm.

All samples were pinned, labeled, and deposited in ELKU. The scientific names of flowering plants are cited from the database of "BG Plants Japanese-scientific Names Index (YList)" (Yonekura & Kajita, 2003).

DNA ANALYSIS: 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. The DNA barcode region of the mtDNA cytochrome oxidase subunit I (COI) gene fragment 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 through the DNA Database of Japan (DDBJ) and are also available in the project file "DNA barcode database of Asian bees" of the Barcode of Life Data Systems. Comparative sequences of *C. floralis* Eversmann and *C. jankowskyi* were obtained from GenBank and those of *C. babai* Hirashima & Tadauchi were analyzed by R.M. Table 1 lists the GenBank accession numbers and

BOLD process IDs used in the present paper. Pairwise sequence divergences within and between *C. jankowskyi* and its close congeners were calculated using the Kimura 2-parameter model (K2P) (Kimura, 1980). The analyses were conducted using MEGA5 (Tamura *et al*., 2011).

RESULTS

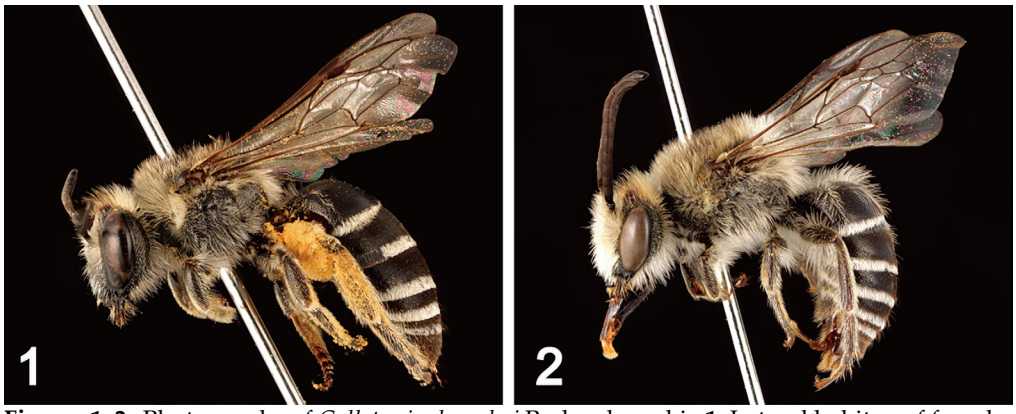
Genus *Colletes* Latreille, 1802
The *clypearis* species group

Diagnosis: This group is characterized by the following character states: 1) 1st metasomal tergum with sparse and long, erect hairs but lacking short, appressed hairs; 2) apical hair bands on metasomal terga well developed, usually complete on female 1st metasomal tergum; 3) malar space generally long, at least approximately half as long as basal width of mandible; 4) disc of male 7th metasomal tergum elongate, apically more or less broadly truncate; and 5) male 7th metasomal sternum elongate and broadened at its base (Noskiewicz, 1936; Niu *et al*., 2013).

Species in Japan: *Colletes babai* Hirashima & Tadauchi, 1979; *C. floralis* Eversmann, 1852; and *C. jankowskyi* Radoszkowski, 1891.

Table 1. List of species of *Colletes* Latreille used in DNA analysis for the present study.

Species	Genbank Accession No.	BOLD Process ID	Length (bp)	Collection Site	References
C. babai	LC102397	ABBOL184-15	609	Mt. Hiko-san, Fukuoka Pref., Kyushu, Japan	Present study
C. babai	LC102398	ABBOL185-15	609	Mt. Hiko-san, Fukuoka Pref., Kyushu, Japan	Present study
C. floralis	DQ872691	GBAH4201-09	663	Nuuriin Khooloi Lake, Mongolia	Almeida & Danforth (2009)
C. floralis	HQ948115	FBAPB978-09	624	Aosta Valley, Italy	Schmidt *et al*. (2015)
C. floralis	HQ948116	FBAPB979-09	622	Aosta Valley, Italy	Schmidt *et al*. (2015)
C. floralis	HQ948117	FBAPB980-09	615	Vallemaggia, Ticino, Switzerland	Schmidt *et al*. (2015)
C. jankowskyi	LC102399	ABBOL187-15	658	Chojyabaru, Oita Pref., Kyushu, Japan	Present study
C. jankowskyi	LC102400	ABBOL188-15	658	Chojyabaru, Oita Pref., Kyushu, Japan	Present study
C. jankowskyi	EF028514		676	nr. Vladivostok, Russia	Kuhlmann *et al*. (2009)



Figures 1–2. Photographs of *Colletes jankowskyi* Radoszkowski. **1.** Lateral habitus of female. **2.** Lateral habitus of male.

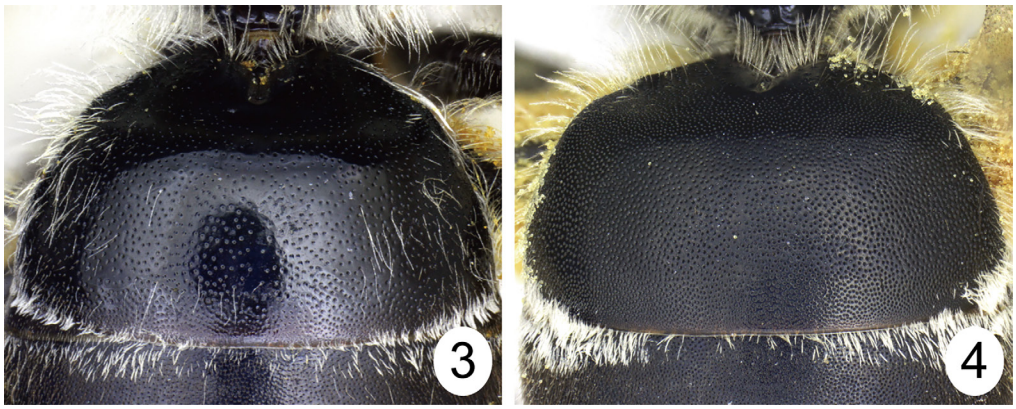
Colletes jankowskyi Radoszkowski, 1891
(Figs. 1, 2, 4, 7, 9)

Colletes jankowskyi Radoszkowski, 1891: 253.
Colletes ventralis Pérez, 1903: 225. Synonymy *vide* Kuhlmann, 2000: 180.
Colletes ventraliformis Cockerell, 1924: 595. Synonymy *vide* Kuhlmann, 2000: 180.

SPECIMENS EXAMINED: [JAPAN] **Honshu:** 1♂, Karuizawa (Kitasaku-dist.), Nagano Pref., 30.viii.1949 [30 August 1949] (R. Ishikawa, ELKU). **Kyushu:** 1♂, Kujû (Bungo), 3.viii.1940 [3 August 1940] (T. Esaki, ELKU; a paratype of *C. babai*); 1♀, 2♂♂, Kokonoe-machi, Kusu-gun, Kuju, Oita Pref., 13.ix.2009 [13 September 2009] (O. Tadauchi, ELKU); 1♀, 1♂, Chojyabaru, alt. 1,050 m, Kokonoe-machi, Kusu-gun, Kuju, Oita Pref., N33°7′6.773′′ E131°13′49.331′′, 5.ix.2010 [5 September 2010] (R. Murao, on *Lespedeza cyrtobotrya* Miq., cMur); 2♀♀, 7♂♂, same locality, 28.viii.2011 [28 August 2011] (R. Murao & Y. Murao, on *L. cyrtobotrya*, ELKU); 6♀♀, same locality, 31.viii.2012 [31 August 2012] (R. Murao, on *L. cyrtobotrya*, ELKU); 4♀♀, same locality, 17.ix.2014 [17 September 2014] (R. Murao, on *L. cyrtobotrya*, ELKU); 2♀♀, 4♂♂, same locality, 15.viii.2015 [15 August 2015] (R. Murao, on *L. cyrtobotrya*, ELKU); 14♀♀, 2♂♂, same locality, 28.viii.2015 [28 August 2015] (R. Murao, on *L. cyrtobotrya*, ELKU); 19♀♀, same locality, 5.ix.2015 [5 September 2015] (R. Murao, on *L. cyrtobotrya*, ELKU); 2♀♀, same locality, 14.ix.2015 [14 September 2015] (R. Murao, on *L. cyrtobotrya*, ELKU).

DIAGNOSIS: In Japan, this species is similar to *C. babai* and *C. floralis*. It can be differentiated from these species by the following key:

1. ♀♀ 2
—. ♂♂ 4
2(1). Malar space long, approximately one-half of basal width of mandible; punctures on 1st metasomal tergum sparser, as in figure 3 (IS = 3d at maximum) ... . *C. floralis* Eversmann
—. Malar space short, less than half of basal width of mandible; punctures on 1st metasomal tergum denser, as in figure 4 (IS = 0.5–2d) 3
3(2). Mesoscutum with yellowish-brown hairs, excluding darker setae .... *C. babai* Hirashima & Tadauchi



Figures 3–4. Photographs of first metasomal tergum of females in dorsal view. **3.** *Colletes floralis* Eversmann. **4.** *C. jankowskyi* Radoszkowski.

—. Mesoscutum with whitish hairs excluding dark setae .... *C. jankowskyi* Radoszkowski

4(1). Pilosity of mesoscutum and mesoscutellum without dark hairs; base of 7th metasomal sternum with lateral process (blue arrow in Fig. 5); hair zone on disc of 7th metasomal sternum occupies over half of width of disc in central area (red arrow in Fig. 5) ..... *C. floralis* Eversmann

—. Pilosity of mesoscutum and mesoscutellum mixed with dark hairs; base of 7th metasomal sternum laterally rounded or angulate (blue arrow in Figs. 6–7); hair zone on disc of 7th metasomal sternum occupies approximately half or less than half of width of disc in central area (red arrow in Figs. 6–7) 5

5(4). Base of 7th metasomal sternum laterally rounded (blue arrow in Fig. 6); hair zone on disc of 7th metasomal sternum occupies approximately half of width of disc in central area (red arrow in Fig. 6) ..... *C. babai* Hirashima & Tadauchi

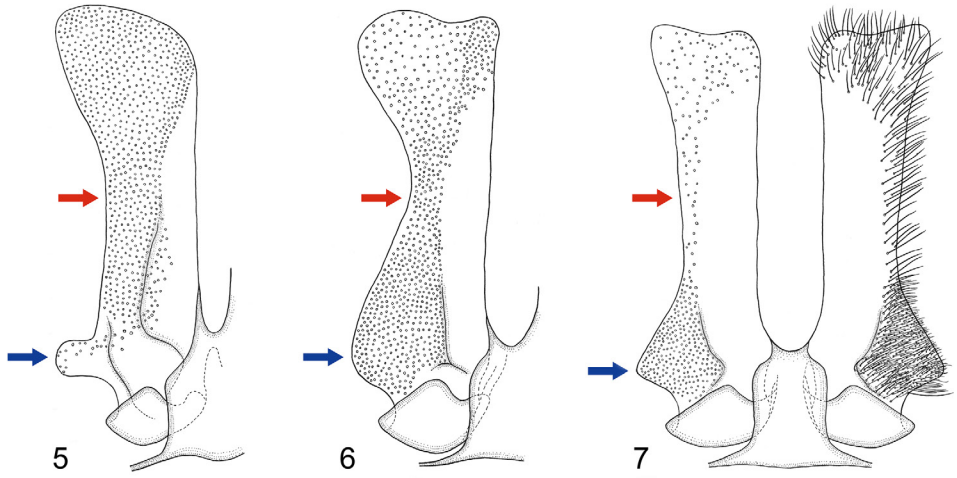
—. Base of 7th metasomal sternum laterally angulate (blue arrow in Fig. 7); hair zone on disc of 7th metasomal sternum narrow, occupies only outer margin of disc in central area (red arrow in Fig. 7) ..... *C. jankowskyi* Radoszkowski

Distribution: Japan (central Honshu and Kyushu); Russia, Mongolia, China, Tajikistan (Proshchalykin & Kuhlmann, 2015).

Phenology in Japan: The phenology based on the data of both the field survey and ELKU collection is as follows: females emerged from mid-August to mid-September and males from early August to mid-September. The emergence of both females and males could not be confirmed in other seasons. Thus, *C. jankowskyi* seems to be a univoltine species in Japan. Figure 8 shows the phenology of *C. jankowskyi* by number of individuals sampled in Oita Prefecture, Kyushu, in 2015. The number of female individuals was highest from late August to early September and distinctly decreased thereafter.

Habitat in Japan: This species is found in semi-natural grassland (Fig. 10) at an elevation of approximately 1000 m.

Floral records in Japan: The flower records obtained from the field survey were from three species in three families: *Aralia cordata* Thunb. (Araliaceae) (Fig. 12), *Echinops setifer* Iljin (Asteraceae) (Fig. 13), and *L. cyrtobotrya* (Fabaceae) (Fig. 11). The flowers most frequently visited by *C. jankowskyi* were those of *L. cyrtobotrya* (96♀♀, 15♂♂:



Figures 5–7. Illustrations of 7th metasomal sternum for three species of *Colletes* Latreille (red arrows indicate central areas, blue arrows indicate lateral processes; setae omitted except for right side of *Colletes jankowskyi* Radoszkowski, right sides omitted from other species). **5.** *Colletes floralis* Eversmann. **6.** *C. babai* Hirashima & Tadauchi. **7.** *C. jankowskyi*.

50♀♀, 14♂♂ by sampling, and 46♀♀, 1♂ from observations at flowers), followed by *A. cordata* (5♀♀, 1♂ from observations at flowers), and *E. setifer* (1♂ from observation at flowers). During the periodical sampling conducted from August to September 2015, *C. jankowskyi* visited only *L. cyrtobotrya* (37♀♀, 6♂♂), despite the presence of many other flowers (44 species) in bloom during the active season of *C. jankowskyi*. At least at the study site, the population of *C. jankowskyi* appears to have a floral preference for *L. cyrtobotrya*.

DNA BARCODES: The pair-wise sequence divergence was 7.5–7.8% between *C. jankowskyi* and *C. floralis* and 2.9–3.5% between *C. jankowskyi* and *C. babai*. The intraspecific divergence was 0.3–0.7% (inter- and intraspecific divergence summarized in Table 2). Thus, DNA barcoding data can be used for discrimination of *C. jankowskyi* from both *C. babai* and *C. floralis*.

DISCUSSION

Phenology

In Japan, *C. jankowskyi* seems to be a univoltine species, being active in early August to mid-September. However, it is also collected from the early season in the con-

Table 2. Inter- and intraspecific sequence divergences based on 609 bp of the mtDNA COI gene among *Colletes babai* Hirashima & Tadauchi, *C. floralis* Eversmann, and *C. jankowskyi* Radoszkowski (Kimura's two-parameter pairwise distances).

Species	*C. babai*	*C. floralis*	*C. jankowskyi*
C. babai (n = 2)	0–0.013	—	—
C. floralis (n = 4)	0.078–0.089	0–0.008	—
C. jankowskyi (n = 3)	0.029–0.035	0.075–0.078	0.003–0.007

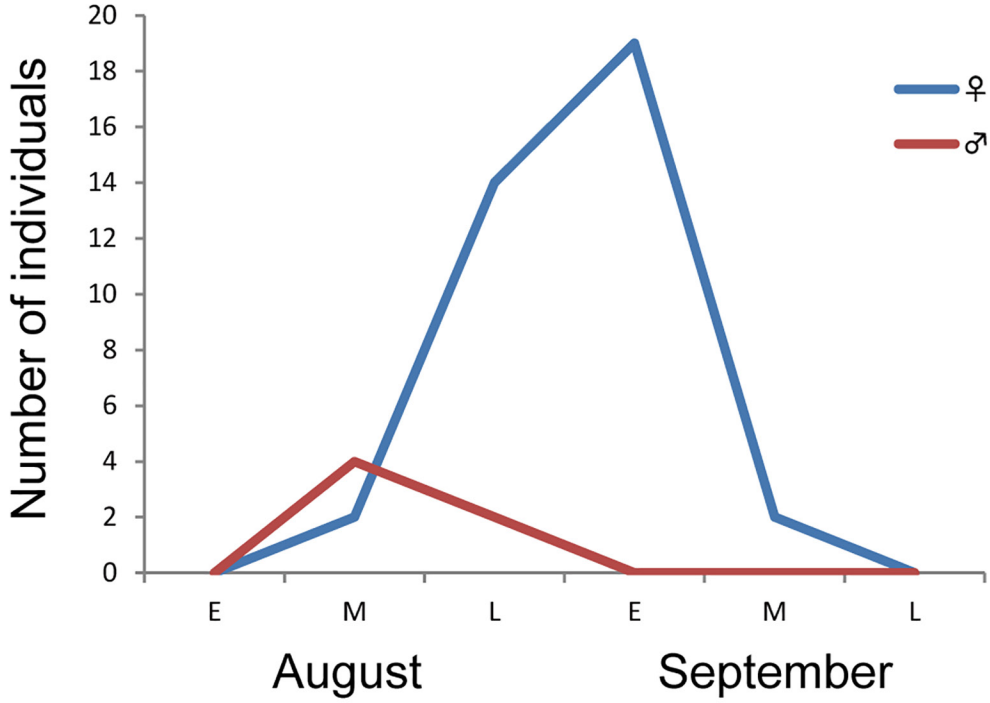
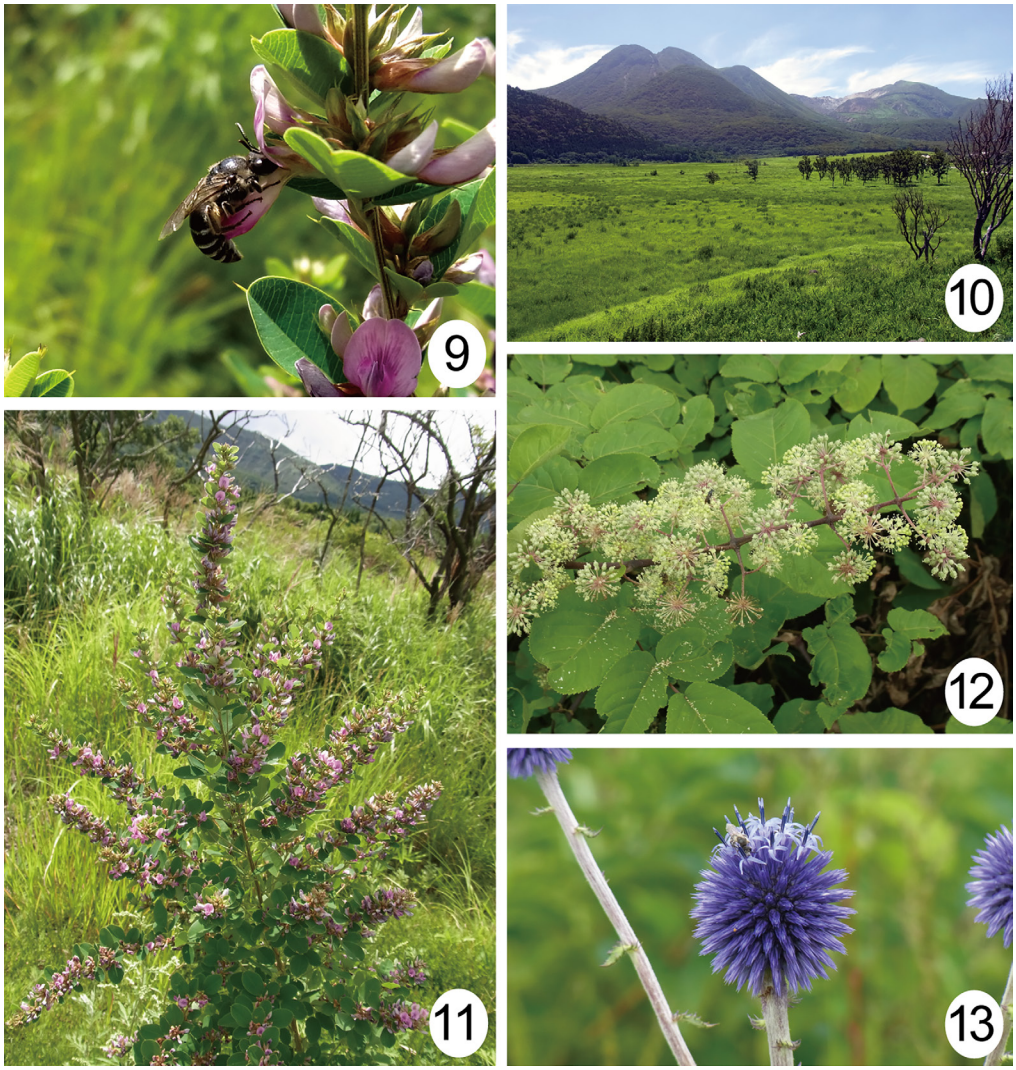


Figure 8. Phenology of *Colletes jankowskyi* Radoszkowski at Chojyabaru, Kokonoe-machi, Kusugun, Oita Prefecture, Kyushu, Japan, in 2015.

tinent (Kuhlmann & Proshchalykin, 2011; Niu *et al*., 2013; Proshchalykin & Kuhlmann, 2015). According to these works, the earliest records were in mid-May in both China and Russia. Localities in Russia are at a higher latitude compared to Japan, most specimens of both females and males were mainly collected in July (21♀♀, 476♂♂ and 43♀♀, 523♂♂). The difference in phenology among local populations is thought to be influenced by the thermal and climatic differences in local areas. Our phenological data for *C. jankowskyi* in Japan are mostly based on specimens collected from a single site (Oita Prefecture, Kyushu, western Japan). If *C. jankowskyi* is discovered from northern Japan in a future study, the phenology of northern populations in Japan may be different from those of our study.

Floral Preferences

Through the field survey of the first author, *C. jankowskyi* at the studied locality in Oita Prefecture is suggested to have a floral preference for *L. cyrtobotrya*, a species that is distributed across eastern China, the Russian Far East, Korean Peninsula, and Japan (except for Hokkaido) (Ohba *et al*., 2007). As stated above, *C. jankowskyi* is widely distributed from central to eastern Asia. The distribution between the bees and this host plant do not coincide completely. According to Niu *et al*. (2013), *C. jankowskyi* was collected from the flowers of 10 species in five families in China. Considering these facts, we think *C. jankowskyi* is a generalist forager and despite its heavy use of *L. cyrtobotrya* at the study site.



Figures 9–13. Photographs of *Colletes jankowskyi* Radoszkowski, its habitat, and floral hosts in Japan. **9.** Female on flowers of *Lespedeza cyrtobotrya* Miq. **10.** Habitat in Japan (Chojyabaru, Kokonoe-machi, Kusu-gun, Oita Prefecture, Kyushu). **11.** *L. cyrtobotrya*. **12.** *Aralia cordata* Thunb. **13.** *Echinops setifer* Iljin.

Hypothesis for Geographical History in Japan

Colletes jankowskyi was discovered from only two localities (Karuizawa, Nagano Prefecture, Honshu, and Kuju Plateau, Oita Prefecture, Kyushu) in Japan. During the process of our examination of specimens of Japanese *Colletes*, we studied those unidentified specimens collected from northern Japan (Hokkaido to northern Honshu) and re-examined previously identified material of both *C. babai* and *C. floralis* from northern Japan. We did not detect *C. jankowskyi* from northern Japan. In Japan, *C. jankowskyi* is only known from two semi-natural grassland sites which were managed by human activities, such as pasture, meadow, and "Noyaki" (meaning to burn the dry grasses of the preceding year, because of the disturbance of vegetation succession). Some insect

and plant species in Japan, such as *Fabriciana nerippe* (C. & R. Felder) (Nymphalidae), *Saussurea pulchella* (Fisch. ex Hornem) Fisch. (Asteraceae), and *Shijimiaeoides divinus* (Fixsen) (Lycaenidae), depend on semi-natural grassland (Suka, 2008). It has also been suggested that these are relict species which have survived in such grasslands after colonization from the continent to Japan during glacial periods (Suka, 2008). Japanese *C. jankowskyi* , too, may be a relict species, judging from both its distribution and habitat information.

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