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ORIGINAL RESEARCH PAPER

New information on host plants and distribution ranges of an invasive gall midge, *Contarinia maculipennis* (Diptera: Cecidomyiidae), and its congeners in Japan

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Abstract Gall midges of the genus *Contarinia* (Diptera: Cecidomyiidae) that infest the flower buds of various plant species have been newly found in Japan in recent years. Those infesting the flower buds of *Pseuderanthemum laxiflorum* (A. Gray) Hubbard ex Baillon (Amaranthaceae) and *Jasminum sambac* (Linnaeus) Aiton (Oleaceae) in Okinawa Prefecture, and *Dendrobium* spp. (Orchidaceae) in Mie Prefecture were identified, on the basis of morphological features and molecular information, as an invasive gall midge, *C. maculipennis* Felt. *C. maculipennis* was recorded in Mie Prefecture for the first time, and *P. laxiflorum* is newly regarded as one of the host plants of *C. maculipennis*. Three other *Contarinia* gall midges that we found infesting the flower buds of *Lycopersicon esculentum* Miller, *Capsicum annuum* Linnaeus (Solanaceae), and *Oxalis corniculata* Linnaeus (Oxalidaceae) were not identical with *C. maculipennis*. Among these, the first two, which infested solanaceous plants, were identical. However, the species other than *C. maculipennis* could not be identified to the species level because morphological differences were obscure and DNA sequencing data of allied congeners have not yet been registered on GenBank.

Keywords COI region · *Dendrobium* orchid · New host plant record · Sweet pepper · The blossom midge

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Introduction

The blossom midge, *Contarinia maculipennis* Felt (Diptera: Cecidomyiidae), is an invasive gall midge infesting the flower buds of *Dendrobium* orchid (Orchidaceae), mainly in greenhouses. This gall midge is polyphagous, with a wide host plant range across 15 species belonging to eight plant families in Hawaii (Gagné 1995; Mohan and Manjunath 2005; Uechi et al. 2007), whereas many other gall-inducing cecidomyiids are monophagous or oligophagous, utilizing one or several plant genera within a single plant family (see lists in Skuhravá 1986; Gagné 1989, 1994, 2010; Yukawa and Masuda 1996).

Contarinia maculipennis seems to have originated in Southeast Asia and has extended worldwide through the international trade of *Dendrobium* cut flowers (Gagné 1995). In Japan, this pest has been known to occur since 1989 in greenhouses of *Dendrobium* spp. in Okinawa, Miyazaki, and Fukuoka Prefectures (Tokuda et al. 2002; Uechi et al. 2007). Because the commercial value of orchids with infested flower buds is reduced to nothing, the frequency of insecticide applications has increased to twice as much as previously (Tokuda et al. 2002). *C. maculipennis* also infests the flower buds of the bitter gourd, *Momordica charantia* Linnaeus (Cucurbitaceae), on Okinawa Island

Fig. 1 Flowers and flower buds of *Pseuderanthemum laxiflorum*, a new host plant of *Contarinia maculipennis*. These flower buds were infested by gall midge larvae, although infested flower buds were hardly distinguishable from normal ones

(Uechi et al. 2007), although reduction of fruit yield has not yet been reported.

In 2007 and 2008, larvae of *Contarinia* species were collected from the flower buds of *Pseuderanthemum laxiflorum* (A. Gray) Hubbard ex Baillon "White rabbit" (Acanthaceae) (Fig. 1), jasmine, *Jasminum sambac* (Linnaeus) Aiton (Oleaceae), yellow sorrel, *Oxalis corniculata* Linnaeus (Oxalidaceae), tomato, *Lycopersicon esculentum* Miller (Solanaceae), and *M. charantia* in Okinawa Prefecture. Among these plants, *P. laxiflorum* is an invader of orchid greenhouses, and *J. sambac*, *L. esculentum*, and *M. charantia* are cultivated plants. *Oxalis corniculata* is a wild plant distributed widely in open fields in Okinawa. In 2008, larvae of a *Contarinia* species infesting the flower buds of cultivated *Dendrobium* spp. (Orchidaceae) were found in Mie Prefecture and forwarded to us for species identification. In 2010, other *Contarinia* larvae infesting the flower buds of the sweet pepper, *Capsicum annuum* Linnaeus var. "*grossum*" (Fig. 2) were found in Okinawa Prefecture.

The genus *Contarinia* contains at least 311 species worldwide (Gagné 2010), and eight species and at least seven unidentified congeners in Japan (Yukawa 1971; Yukawa and Masuda 1996; Tokuda et al. 2002; Tokuda and Yukawa 2004; Uechi et al. 2007). They are relatively small, and morphologically similar to each other. In addition, we can easily collect larval specimens from galls on the host plants but it is not easy to rear adults that emerge from pupae on the ground. Therefore, DNA analysis using larval specimens is essential for identification of species and determination of host range. We investigated both morphological features and DNA sequencing data of the aforementioned *Contarinia* species to determine whether they consist of more than one species and if some of them are identical with

Fig. 2 Flower buds of *Capsicum annuum* infested by a *Contarinia* species

C. maculipennis. In this paper, the results of identification are provided, with additional information on the new host plant and distribution range of *C. maculipennis*.

At the same time, we urgently warn those who are responsible for orchid growing against escape of *C. maculipennis* from greenhouses to other crops and the danger of its possible overwintering in open fields, particularly under global warming conditions.

Materials and methods

Collection and preservation of *Contarinia*-like gall midges

Larvae of *Contarinia*-like gall midges were collected in 2007, 2008, and 2010 from the flower buds of various plant species growing inside or outside orchid greenhouses in different localities in Okinawa Prefecture (Table 1). Flower buds collected from various plant species in different localities were kept separately in plastic bags (200 mm long, 170 mm wide) to obtain mature larvae escaping from the flower buds for pupation. In plants other than *C. annuum*, infested flower buds could not be distinguished from normal ones in appearance (e.g., Fig. 1). Therefore, at least 100 flower buds were collected randomly and placed in plastic bags to obtain larvae.

Most of the larvae that escaped from infested buds were placed in 70–75% ethanol for morphological observation or in 99.5% acetone for DNA analysis. To rear adult gall midges, the remaining larvae were maintained with moistened fine vermiculite in plastic containers (100 mm in diameter, 100 mm high) at room temperature (approx. 25°C) and natural photoperiod (from 10.5L–13.5D to 14L–10D). Approximately 2 weeks later, adults emerged and were collected and preserved in 70–75% ethanol.

Table 1 Collection data of *Contarinia* gall midges examined in this study

Host plant	Collection site	Collection date	Stage (sex)	Accession no.
Pseuderanthemum laxiflorum	Motobu Town, Okinawa Prefecture (a greenhouse where *C. maculipennis* occurred)	25 Sep. 2007	5 males, 4 females, 4 larvae	AB597012-15[a]
Jasminum sambac	Nanjo City, Okinawa Prefecture	13 Sep. 2007	3 larvae	AB597016-18[a]
Lycopersicon esculentum	Aguni Village, Okinawa Prefecture	26 Aug. 2008	3 larvae	AB597019-21[a]
Capsicum annuum	Yaese Town, Okinawa Prefecture	24 Jan. 2011	3 males, 2 females	AB618264-66[a]
		26 Jan. 2011	3 females	
		24 Dec. 2010	3 larvae	
Oxalis corniculata	Motobu Town, Okinawa Prefecture. (An open field near a field of bitter gourd. *C. maculipennis* had been collected there previously)	25 Sep. 2007	1 male, 7 females, 4 larvae	AB597022-25[a]
Momordica charantia	Aguni Village, Okinawa Prefecture	26 Aug. 2008	3 larvae	AB597026-28[a]
Dendrobium spp.	Ise City, Mie Prefecture	14 Aug. 2008	3 larvae	AB597029-31[a]

[a] Homological 439 bp was used in the analysis

In August 2008, gall midge larvae were found infesting orchid flower buds in a greenhouse in Ise City, Mie Prefecture. These larvae and the adults reared were sent to us by Mr N. Ohkubo and Ms S. Shimo (Mie Prefecture) for species identification.

Morphological observation and molecular analysis

Some of the ethanol-preserved specimens were mounted on slides in Canada balsam for microscopic study based on the techniques outlined both in Yukawa (1971) and in Gagné (1989). Relative length of the first to second flagellomeres, the circumfilar loops of male flagellomeres, wing length and pattern, male genitalia, larval sternal spatula and its associated papillae, and larval eighth abdominal segments were compared with those of *C. maculipennis* that had been identified in Tokuda et al. (2002) and with its congeners redescribed by Gagné (1995). Because adults were not reared from larvae infesting the flower buds of *J. sambac*, *L. esculentum*, and *M. charantia*, morphological features of the adults could not be observed.

For every larva, total DNA was extracted from the whole body with the DNeasy Blood and Tissue Kit (Qiagen, Japan), following the manufacturer's instructions. A region of the cytochrome oxidase subunit I (COI) gene of mitochondrial DNA was amplified, purified, sequenced, and electrophoresed following the methods described by Yukawa et al. (2003). The primers used in the analysis were: forward; COIS 5′-GGA TCA CCT GAT ATA GCA TTC CC-3′ or LCO1490 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′ (Folmer et al. 1994) and reverse; COIA 5′-CCC GGT AAA ATT AAA ATA TAA ACT TC-3′ (Funk et al. 1995). COIS has been used to detect intra and interspecific variations in Cecidomyiidae (Uechi et al. 2003; Yukawa et al. 2003; Tokuda et al. 2004, 2008) in combination with COIA (Funk et al. 1995). Another forward primer, LCO1490, has been adopted for the DNA barcoding project of animals (Hebert et al. 2003). The estimated length of the region amplified by use of LCO1490 and COIA was 676 bp that covered a partial COI region used for the DNA barcoding project (658 bp from the 5′-end) (Hebert et al. 2003) and that has been used for molecular phylogenetic analysis of Cecidomyiidae (439 bp from the 3′-end, amplified using COIS and COIA) (Uechi et al. 2003). In this work, homologous 439 bp were used in the analysis to compare DNA sequences with those of *C. maculipennis* and congeners that have been registered on DNA databases. In addition, the sequence data of *C. maculipennis* collected from Okinawa (accession nos. AB105494-AB105493, AB280769-AB280779, and AB201055), Fukuoka (accession nos. AB280767 and AB280768), Miyazaki (accession nos. AB280780-AB280784), Thailand (accession nos. AB105486-AB105490), Hawaii (accession nos. AB105494, AB105499, AB105504, AB105507, AB105510, AB105515, AB105519), and sequence data of congeners, *Contarinia nasturutii* (Kieffer) (accession nos. EU812560 and AY485370), *Contarinia okadai* (Miyoshi) (accession no. AB105485), *Contarinia viburnonum* Kieffer (accession no AB280766), *Contarinia tritici* (Kirby) (accession no. AY485369), *Contarinia pisi* Winnertz (accession no. AY485382), and *Resseliella yagoi* Yukawa and Sato (Diptera: Cecidomyiidae) (accession no. AB506002; as an outgroup taxon) were included in the analysis (Chen et al. 2009; Frey et al. 2004; Uechi et al. 2003, 2007; Yukawa et al. 2009). A neighbor-joining (NJ) tree based on these sequence data was constructed and bootstrap analysis was conducted with 1,000 pseudoreplications using ClustalX ver. 1.83 (Thompson et al. 1997). Evolutionary distances were computed by use of Kimura's two-parameter distances (Kimura 1980).

Results

The number, stage, and sex of gall midge specimens examined in this study are listed in Table 1. On the basis of morphological affinities, we concluded that gall midges collected from the flower buds of *Dendrobium* in Mie Prefecture and those from *P. laxiflorum* in Okinawa Prefecture were identical with *C. maculipennis*. Morphological features of the aforementioned gall midges, for example the circumfilar loops of male flagellomeres, wing length and pattern, male genitalia, larval sternal spatula, and larval eighth abdominal segments, coincided well with those of *C. maculipennis* (data for male and female flagellomeres and larval eighth abdominal segments are given by Tokuda et al. 2002). In particular, the basal node of the female first flagellomere was 1.8–1.9 times as long as that of the second flagellomere, which is one of the important characteristics of *C. maculipennis*. DNA sequencing data supported this identification (Fig. 3). The larvae collected from *J. sambac* and *M. charantia* also proved to be identical with *C. maculipennis* by DNA sequencing data. Monophyly of the clade including *C. maculipennis* was supported with a 100% bootstrap value. *C. maculipennis* was recorded from Mie Prefecture for the first time and *P. laxiflorum* was newly discovered as one of the host plants of *C. maculipennis.*

The gall midge collected from *C. annuum* was different from *C. maculipennis* in the following morphological features: wing length is approximately 1.2 mm, slightly shorter than the 1.3–1.5 mm of *C. maculipennis*, and the aedeagus does not extend beyond the hypoproct. DNA sequencing data also supported the results based on morphological comparison. This gall midge was not identified to the species level because we could not obtain a sufficient number of good adult specimens for morphological comparison. DNA sequencing data indicated that the gall midge infesting the flower buds of *C. annuum* was identical with that from *L. esculentum* (Fig. 3).

The gall midge collected from *O. corniculata* was considered to be a *Contarinia* species, because it shared morphological characteristics given in the definition of *Contarinia* by Harris (1966) as follows: palpus 4-segmented; antenna with 2 + 12 segments; node of male flagellomeres subequal in length and diameter, internode and neck relatively long; male genitalia with gonostylus slightly narrowed distally, hypoproct deeply divided; each female flagellomere with a cylindrical node and a comparatively short neck, circumfila not looped; ovipositor retractile, tapering to a pair of narrow terminal lobes. However, this gall midge was different from *C. maculipennis* in the following morphological features: basal node of female first flagellomere approximately 1.5 times as long as that of the second; wing length 1.0–1.5 mm, which is slightly shorter than that of *C. maculipennis*; scales on wing membrane not denser and darker than those of *C. maculipennis*. This gall midge could not be identified to the species level because only one damaged male was available for comparison.

We could not find any registered sequencing data of congeners that matched with those of gall midges from *C. annuum*, *L. esculentum*, and *O. corniculata*.

Discussion

Host range of *C. maculipennis*

Pseuderanthemum laxiflorum is a newly recorded host plant for *C. maculipennis*. This plant is a horticultural variety, native to the Fiji Islands (T. Takaesu 2007, personal communication), which was accidentally brought into a greenhouse in Okinawa. Later, *C. maculipennis* escaped from the greenhouse and invaded another greenhouse where orchids were cultivated. This invasion probably provided an opportunity for *C. maculipennis* to infest the flower buds of *P. laxiflorum* secondarily. Now 16 species belonging to eight plant families are regarded as host plants of *C. maculipennis* (Table 2). *Jasminum sambac* has been recorded as one of the host plants in Hawaii (Gagné 1995) and this is the first record for that host plant in Japan. *Momordica charantia* has been recorded as a host plant of *C. maculipennis* on Okinawa Island (Uechi et al. 2007) and it is now recognized as a host plant also on Aguni Island (N26°34′56″, E127°13′36″), west of Okinawa Island.

Distribution range of *C. maculipennis* in Japan

In addition to Fukuoka, Miyazaki, and Okinawa Prefectures (Tokuda et al. 2002; Uechi et al. 2007), *C. maculipennis* was confirmed to exist in Mie Prefecture, which is the first distribution record of this gall midge in Honshu, Japan. Such a scattered distribution pattern indicates that *C. maculipennis* did not extend its distribution range from prefecture to prefecture but was directly introduced accidentally from somewhere in Southeast Asia. Different haplotypes between localities (Fig. 3) and frequent interception of *C. maculipennis* under plant quarantine inspection at Japanese seaports and airports (Iwaizumi et al. 2007) also support this possibility.

In Okinawa, *C. maculipennis* has already spread from orchid greenhouses to open fields and is now infesting the flower buds of the bitter gourd, *M. charantia* (Uechi et al. 2007) and *J. sambac* (this study). In contrast, its occurrence is restricted to greenhouses of *Dendrobium* orchids in Mie, Fukuoka, and Miyazaki Prefectures, which are located well to the north of Okinawa Prefecture. There is no sign at the moment that *C. maculipennis* has spread to open fields in

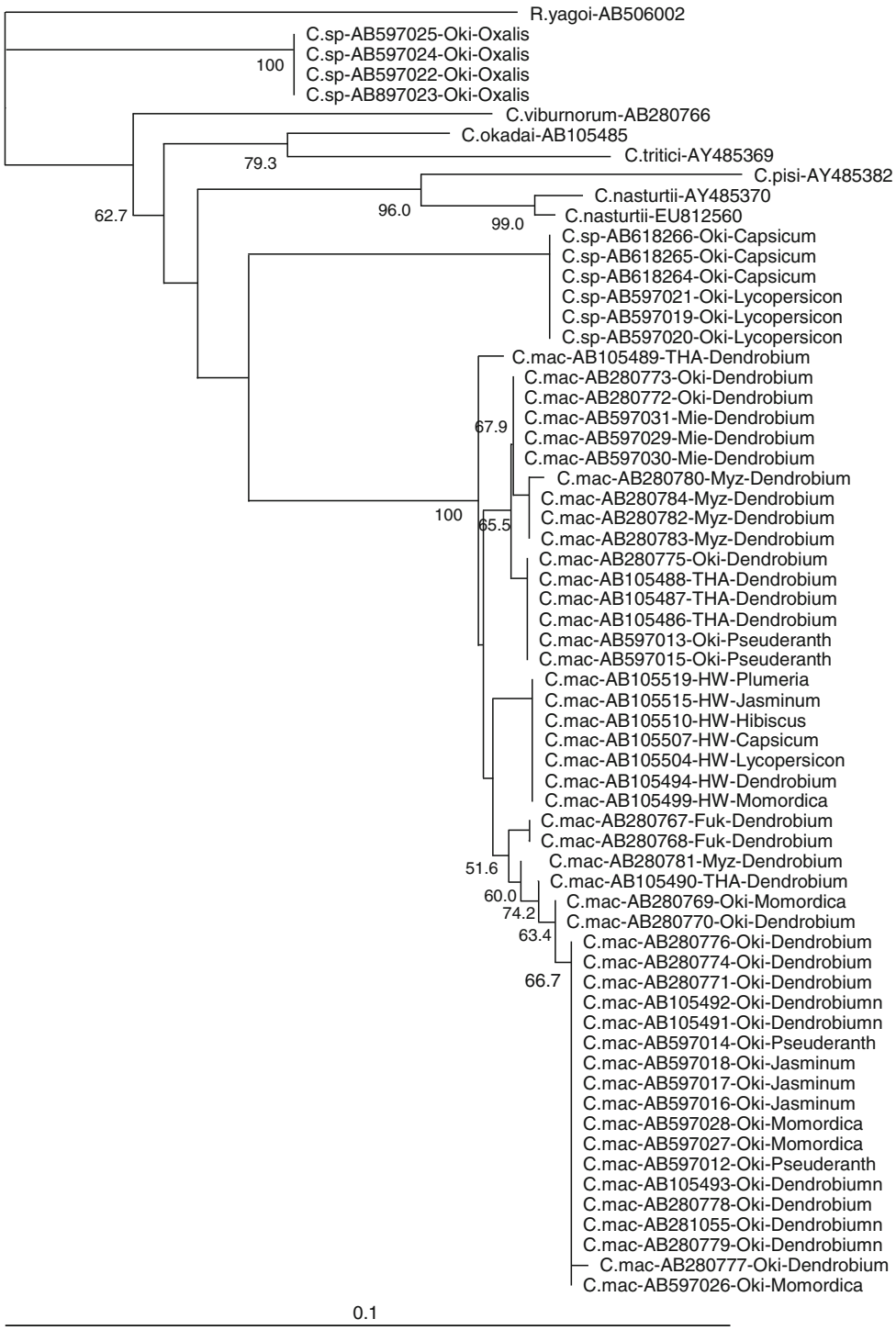

Fig. 3 Neighbor-joining tree for *Contarinia* gall midges based on 439 bp of the mitochondrial cytochrome oxidase subunit I gene. Bootstrap values are indicated for nodes gaining more than 50% support in 1,000 pseudoreplications. *Resseliella yagoi* was used as an outgroup species. Sample names consist of species name, accession number, locality, and host plant. *Fuk* Fukuoka Prefecture, *Oki* Okinawa Prefecture, *Myz* Miyazaki Prefecture, *THA* Thailand, *HW* Hawaii, USA. Among specimens from Thailand, "C. mac-AB105489-THA-Dendrobium" and "C. mac-AB105490-THA-Dendrobium" were those intercepted at Narita Airport when they were imported from Thailand

these prefectures, possibly because the gall midge that originated in Southeast Asia (Gagné 1995) cannot overwinter in open fields under lower temperature conditions in these prefectures than in Okinawa. Nevertheless, we must be aware of the danger of its possible overwintering in open fields particularly under global warming conditions. We must pay attention to any sign of flower bud infestation of other crops and wild plants in open fields near greenhouses where *C. maculipennis* occurs. When any infestation is found, flower buds should be removed immediately and destroyed appropriately.

Congeners newly found in Okinawa Prefecture

It seems to be natural that the gall midges from tomato *L. esculentum* and sweet pepper *C. annuum* are identical to

Table 2 Host plants of *Contarinia maculipennis*, based on Felt (1933), Gagné (1995), Jensen (1946), Mohan and Manjunath (2005), Nakahara (1981), Tokuda et al. (2002), Uechi et al. (2003), and this paper recording new hosts

Plant family and species	Source
Amaranthaceae	
Crossandra[a], *Pseuderanthemum laxiflorum* (A.Gray) Hubbard ex Baill.	Mohan and Manjunath (2005); this paper
Apocynaceae	
Plumeria rubra L.	Nakahara (1981)
Brassicaceae	
Brassica chinensis L.[b]	Jensen (1946)
Cucurbitaceae	
Momordica charantia L.	Gagné (1995); Uechi et al. (2003, 2007)
Malvaceae	
Hibiscus sp., *Hibiscus rosa-sinensis* L. (hybrid species)[c]	Felt (1933); Jensen (1946); Uechi et al. (2003)
Oleaceae	
Jasminum sambac (L.) Ait.	Jensen (1946); this paper
Orchidaceae	
Dendrobium phalaenopsis Fitz., *Dendrobium* spp.	Gagné (1995); Tokuda et al. (2002)
Solanaceae	
Capsicum frutescens L., *C. annuum* L., *Lycopersicon chilense* Dun., *L. esculentum* Mill., *L. peruvianum* (L.) Mill., *Solanum melongena* L., *S. tuberosum* L., *S. rantonnetti* Carr.	Jensen (1946); Jensen (1950); Uechi et al. (2003)

Plant families are arranged in alphabetical order

[a] Mohan and Manjunath (2005) treated ecological data of the pests of Crossandra; detailed information on species identification of gall midges was not given

[b] The record of *B. chinensis* was based on a single observation in a glasshouse (Jensen 1946) and has not been confirmed by subsequent observations

[c] Felt (1933) did not mention the specific name of hibiscus, but *C. maculipennis* larvae were collected from *H. rosa-sinensis* by Uechi et al. (2003)

each other, because both host plants belong to Solanaceae. Tomato is the only host plant of *Contarinia lycopersici* Felt (Gagné 2010), which occasionally becomes a pest in the West Indies (Harris 1966). In addition, *C. maculipennis* and four other congeners, *Contarinia lycii* Debski, *Contarinia lyciicola* Fedotova, *Contarinia pravdini* Becknazarova and Mamaeva, and *Contarinia solani* (Rübsaamen), have been known to utilize solanaceous plants as hosts (Gagné 2010). The gall midge infesting the flower buds of tomato and sweet pepper in Okinawa is possibly identical to either *C. lycopersici* or one of its four congeners, but we cannot confirm this at the moment because morphological differences are obscure and DNA sequencing data of *C. lycopersici* and the congeners have not yet been registered on GenBank. The gall midge infesting the flower buds of *O. corniculata* was not identical to *C. maculipennis* either. *Oxalis corniculata* has never been recorded as a host plant of any *Contarinia* species (Gagné 2010).

To confirm whether *L. esculentum*, *C. annuum*, and *O. corniculata* are hosting undescribed species of *Contarinia* or are included in the host plant range of known polyphagous species of *Contarinia*, more extensive field surveys are needed to detect congeners and their host plants in Japan or elsewhere for further morphological and molecular studies.

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