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Pollination by Fungus Gnats in *Mitella formosana* (Saxifragaceae)

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Abstract The pollination system of *Mitella formosana*, the last *Asimitellaria* species (genus *Mitella* sect. *Asimitellaria*, Saxifragaceae) in which the information regarding its flower visitors remained unreported, was studied in two study sites in Taiwan in April, 2011. Although the frequency of pollinator visits observed were very low (0.04 visits per hour per inflorescence on average in daytime), the principal pollinators of *M. formosana* were clarified as the fungus gnats of the genus *Boletina* and *Coelosia*, just similar as in the other *Asimitellaria* species in Japan. The apparent similarity of pollination systems of *Asimitellaria* species between Taiwan and Japan implies the long-term maintenance and the evolutionary stability of the *Mitella*–fungus gnats pollination mutualisms across Japan archipelago and Taiwan.

Key words: *Asimitellaria*, *Boletina*, *Coelosia*, Japan, pollination mutualism, Taiwan.

Introduction

A tremendous diversity of flowering plants today is reflected by the diversity of plant-pollinator interactions, and how the diversity of pollinator-associated floral traits has been shaped and how it further facilitated the plant speciation has been one of the major interests in plant evolutionary biology (Proctor *et al.*, 1996; Fenster *et al.*, 2004; Willmer, 2011). To address this issue, it is very useful to have a model plant group in which variation in pollination systems and associated floral traits, as well as the phylogenetic relationships therein are analyzed simultaneously.

Asimitellaria, a section of the genus *Mitella* (Saxifragaceae), is one of such the few plant lineages in which not only the phylogeny but also its relationships with pollinators are comprehensively surveyed (Okuyama *et al.*, 2004, 2005, 2008, 2012). In *Asimitellaria*, almost all species except *M. doiana*, which is presumably an obligate selfer, are known to be pollinated exclusively by fungus gnats (Mycetophilidae) (Okuyama *et al.*, 2004, 2008). Moreover, pollination by fungus gnats in *Asimitellaria* shows a notable variation, where some species are pollinated only by a long tongued fungus gnat species *Gnoriste mikado*, while the other species are pollinated by short-tongued fungus gnats of genus *Boletina* and *Coelosia* exclusively, or together with *Gnoriste mikado* as a co-pollinator. Interestingly, in some populations, two to three *Asimitellaria* species co-occur, and the pollinator isolation mediated by the divergent pollinator fungus gnats support their sympatric nature. Therefore, to analyze the selection force behind the pollinator shifts in *Asimitellaria* should be useful to understand the mechanisms on pollinator-mediated isolation and speciation of flowering plants.

The last *Asimitellaria* species without any information of its relationships with pollinators is *M. formosana*, the only *Asimitellaria* species that distributes outside Japan. *Mitella formosana* also has a notable phylogenetic position in *Asimitellaria*, i.e., forming a well-supported clade with

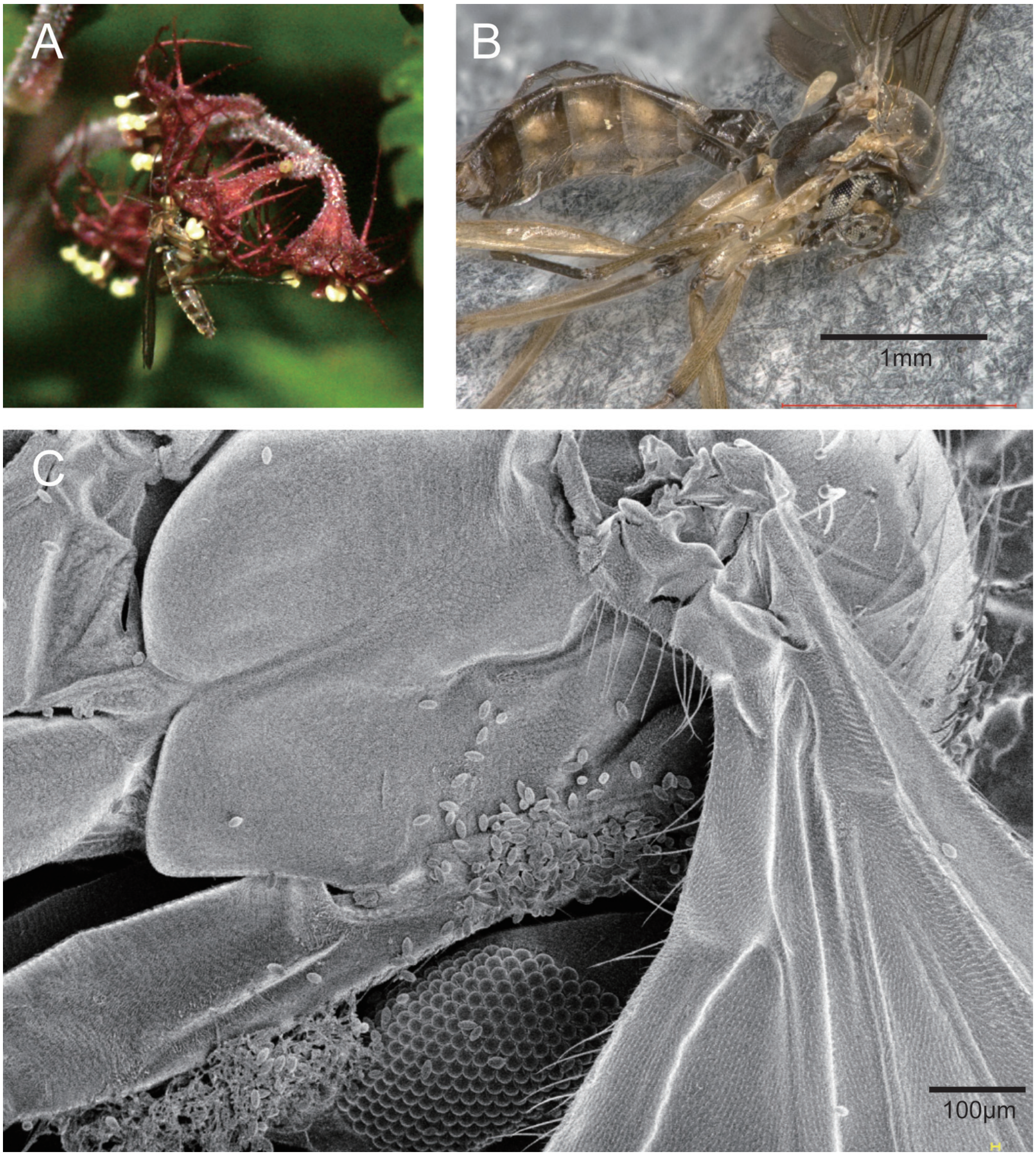

Fig. 1. *Coelosia* sp. (Mycetophilidae) that visited on the *M. formosana* flowers. A, *Coelosia* sp. nectaring on the *M. formosana* flower. B, A microscopic image of *Coelosia* sp. collected on a *M. formosana* flower. Note numerous pollen grains are attached on the anterior side of the body. C, An electron microscopic image of *Coelosia* sp. collected on a *M. formosana* flower. The rugby-ball like morphology of pollen grains typical of the pollen grains of *Mitella* is visible on the insect's surface.

M. japonica and *M. yoshinagae* (Fig. 2, Okuyama and Kato, 2009), which presumably reflects the ancient floristic link between Taiwan and Japan. Okuyama *et al.* (2008) classified the floral morphology of *M. formosana* as "saucer-shape", implying this species might have relationship with short-tongued fungus gnat species of the genus such as *Boletina* and *Coelosia*. To test this prediction and to expand the knowledge on pollination systems of *Asimitellaria* into the whole lineage and geographic distribution, I conducted a survey on pollination biology of *M. for-*

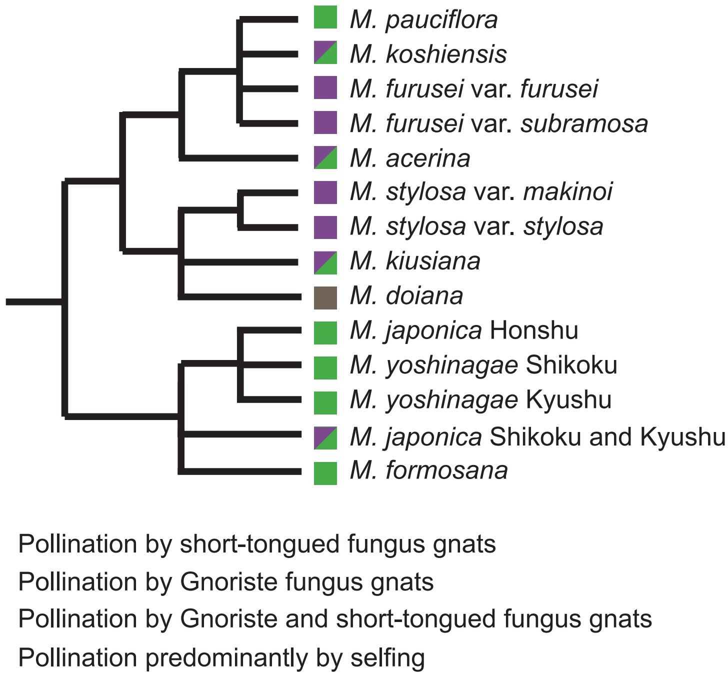

Fig. 2. Phylogenetic distribution of pollination systems in *Asimitellaria*. Phylogenetic tree was redrawn from Okuyama and Kato (2009).

mosana in April, 2011. The present study revealed that *M. formosana*, a geographically isolated and the southernmost species of *Asimitellaria*, maintains a surprisingly similar pollination system to those of species in Japan.

Materials and Methods

Direct observation of pollinator visits to flowers of *M. formosana* were made at Lalashan site (N24°42′17.88″, E121°26′44.53″) during 1200–1700 on April 7 and 1600–1700 on April 10, and Szuyuanyakou site (N24°23′15.62″, E121°21′28.37″) during 0900–1700 on April 9 and 1100–1200 on April 10. All insects flew and landed to the flowers were collected and stored dry separately to inspect their pollen loads under a KEYENCE VHX-2000 digital microscope (Keyence, Tokyo). In addition, pollinator visits were further monitored with time-lapse photography using Optio W90 cameras (Pentax, Tokyo), each set in front of an individual of *M. formosana*, keeping >20 cm distance from the plants and <5 m to each other. The time-intervals between the shots were set to 2 min, as this is the minimum time-interval for camera battery to be sustained overnight (>12h). This setting is reasonable because the pollinator fungus gnats in other *Asimitellaria* species were known to stay on flowers for >2 min, on average (Okuyama *et al.*, 2004), and there is little discrepancy between the flower-visitation data from direct observation and time-lapse photography in *Asimitellaria* (Yudai Okuyama, personal observation; see also the Results section). Five cameras were set each in Lalashan site and Szuyuanyakou site for 13–46 h, focusing on 1–12 inflorescences in full bloom (Table 1). Each insect individual >5 mm in body size photographed at nectaring on a flower was count as a single visit, and the insect on the same flower taken in the subsequent shot was not count.

Results

Throughout the observation period, insect vis-

Table 1. The detailed information of time-lapse photography in the present study

	No. of inflorescence	Photographing time of day	Total time	No. of shots	No. of insect visits*
Lalashan site					
Camera 1	1	1247 April 7–1041 April 9	46 h	1380	1 (1)
Camera 2	1	1356 April 7–1038 April 8	20.5 h	615	0 (0)
Camera 3	3	1414 April 7–0733 April 9	41 h	1230	9 (7)
Camera 4	3	1236–1734 April 7, 0953–1141 April 8	7 h	210	0 (0)
Camera 5	2	1332 April 7–0202 April 8	13 h	390	1 (1)
Szuyuanyakou site					
Camera 6	3	1849 April 8–1128 April 10	41 h	1230	3 (0)
Camera 7	6	1834 April 8–1349 April 9	19 h	570	0 (0)
Camera 8	12	1628 April 9–1101 April 10	18.5 h	555	0 (0)
Camera 9	4	1851 April 8–1135 April 10	40.5 h	1215	3 (0)
Camera 10	6	1838 April 8–1543 April 9	21 h	630	0 (0)

*The numbers in parentheses indicate the number of mycetophilid fungus gnats

its were rare, although certainly present. Except for mordellid beetles continuously stayed and crawled on *M. formosana* flowers, only I had observed were three visits of *Coelosia* sp. (Mycetophilidae) and two visits of the other dipterans in Lalashan site, and a single visit of *Boletina* sp. (Mycetophilidae) in Szuyuanyakou site. This very rare insect visits were also confirmed by a time-lapse photography that shot only 17 insect visits in a grand total of 118 h daytime (0.04 visits per hour per inflorescence on average), in which 9 of 17 were mycetophilid fungus gnats and the other 8 were other dipterans (Table 1). The all four mycetophilid fungus gnats (3 *Coelosia* sp. and 1 *Boletina* sp.) directly collected on the flowers were confirmed to carry >100 pollen grains on their body (Fig. 1), whereas the other dipterans collected on the flowers of *M. formosana* had <10 pollen grains.

Discussion

The present research partly clarified the pollination system of *M. formosana* that has never been reported before. From the direct observation and time-lapse photography, the major flower visitors of *M. formosana* was confirmed to be the short-tongued fungus gnats of the genus *Boletina* and *Coelosia*, just similar as in *Asimitellaria* in Japan. Because these fungus gnats collected on the *M. formosana* flowers always had numerous pollen grains (>100) on their body enough to fertilize all ovules of the flower, it would be reasonable to conclude these fungus gnats are the principal pollinators of *M. formosana*. The other insects, non-mycetophilid dipterans and mordellid beetles, visited on the flowers were never found to carry pollen loads enough to fertilize a large proportion of ovules of the flower, or they seldom move across the inflorescences (mordellid beetles). Therefore, they are unlikely to contribute largely to the cross pollination of the species, although mordellid beetles might partly contribute to self-pollination.

Although the present research is limited to a short time-period in only one year, the following characteristics of pollination system of *M. formosana* were clarified. First, *M. formosana* has surprisingly similar pollination system to those of *Asimitellaria* species in Japan that involves the same mycetophilid genera such as *Boletina* and *Coelosia*. Second, the fungus gnats of the genus *Gnoriste* are unlikely to be involved in the pollination of *M. formosana*, confirming what is expected from the floral morphology (Okuyama *et al*., 2008) and the fact that there is no record of collection of the genus *Gnoriste* in Taiwan. The apparent similarity of pollination systems of *Asimitellaria* species between Taiwan (*M. formosana*) and Japan (*M. pauciflora, M. japonica* Honshu group, and *M. yoshinagae*) implies the long-term maintenance and the evolutionary stability of the *Mitella*–fungus gnats pollination mutualisms across the Japan archipelago and

Taiwan.

It is also noteworthy that, from the present study, the complete picture of the pattern and variation of pollination systems in *Asimitellaria* became available (Fig. 2). As is evident looking at Fig. 2, the different pollination modes in the *Asimitellaria* are scattered across the phylogeny, indicating the pollinator shifts occurred recurrently in the lineage. This suggests that, using the present information, the common ecological and physiological mechanisms behind the pollinator shifts in *Asimitellaria* could be identified, which is the topic of forthcoming research.

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