

Pollination of *Oberonia japonica* (Orchidaceae) by gall midges (Cecidomyiidae)

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Orchidaceae is the most species-rich plant family, with renowned floral diversity. Floral diversity among orchids is attributed to the diversity of pollination systems, which is a product of specialization within orchid species to different animal pollinators (Micheneau et al., 2009; Schiestl & Johnson, 2013). However, pollination biology remains unknown in over 90% of orchid species (Ackerman et al., 2023), limiting our understanding of the full repertoire of floral adaptation in Orchidaceae. Dipteran insects are important orchid pollinators, often attracted to flowers by unique floral adaptations that exploit the biology of specific fly species (Jiang et al., 2020; Policha et al., 2016; van der Niet et al., 2011). However, considering the large taxonomic and ecological diversity of flies, increased study of orchid–fly interaction will likely enrich our understanding of orchid floral diversity. This is especially true for several of the most species-rich orchid subtribes, such as Bulbophyllinae (>2100 spp.), Malaxidinae (>1200 spp.), and Pleurothallidinae (>5500 spp.), in which dipteran pollination is expected to be prevalent but is yet to be confirmed (Ackerman et al., 2023).

Oberonia Lindl. is a genus of 150–300 species of epiphytic or lithophytic orchids in the subtribe Malaxidinae, distributed throughout the Paleotropics from Africa to Polynesia. *Oberonia* flowers, typically about 2 mm in diameter, are among the smallest of all orchids. Given their

size, they may be pollinated by small insects not previously known as orchid pollinators (Geiger, 2019). Here, we assessed the pollination of *Oberonia japonica* (Maxim.) Makino. The species is native to Japan, Korea, and Taiwan, representing the northern extreme of *Oberonia* distribution (Tsutsumi et al., 2021). The species is epiphytic and often found on moss- and lichen-covered trunks of mature trees. The plant is caulescent with equitant alternating leaves. A single inflorescence bears 50–100 flowers. Flowers are approximately 2 mm in diameter and arranged in whorls of five or six. Flowers are nonresupinate, and because the inflorescence is pendulous, the lips are positively geotropic. Flowers are typically orange, and the lips are slightly darker in color (Figure 1a,b).

Flower visitors were observed on 11–12 and 14 May 2022, at Shinshiro, Aichi, Japan, for a total of 26.5 h. More than 1000 inflorescences were successively checked for flower visitors. For nocturnal observations, a handheld flashlight covered with a thin red plastic film was used because most insects are insensitive to red light (van der Kooi et al., 2021).

The first observation began at 10:30 AM on 11 May and continued until 08:30 AM the next day. At the start of the observation, a deceased gall midge (Cecidomyiidae) with two yellow pollinaria stuck on its head was found on a flower. One of the pollinaria was attached to the stigma, and upon touching the gall midge, the insect was detached

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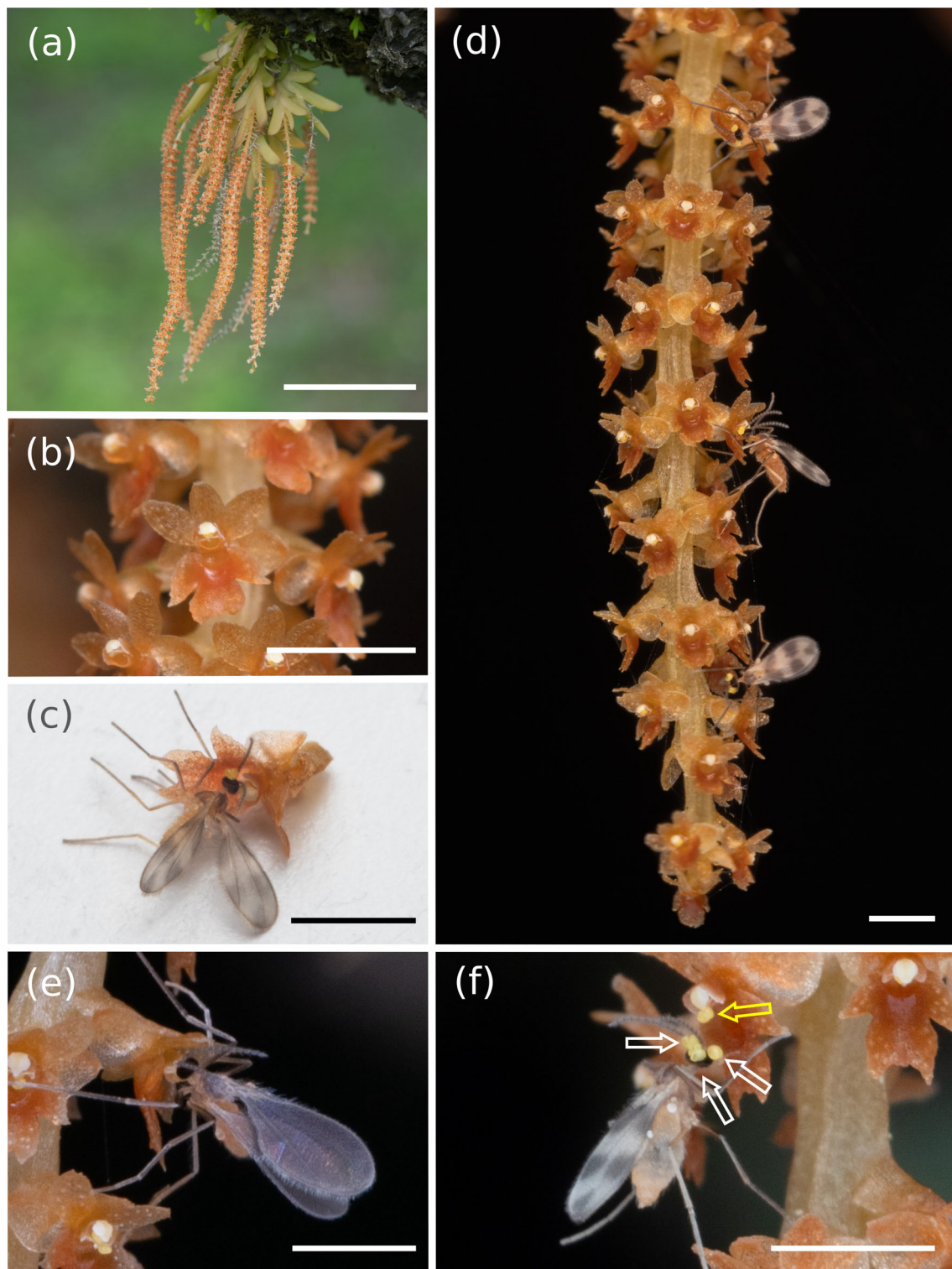


FIGURE 1 *Oberonia japonica* and its flower visitors. (a) Flowering habit of *O. japonica*. (b) Flower size of *O. japonica*. (c) Gall midge trapped in an *O. japonica* flower. (d) Gall midges visiting an *O. japonica* inflorescence. (e) Gall midge sticking its mouthparts on a flower of *O. japonica*. (f) Gall midge carrying three pollinaria (white arrows), and a pollinium deposited on the stigma (yellow arrow). Scale bars: (a) = 4 cm; (b–f) = 2 mm. Photo credits: Yuta Sunakawa.

from the flower, leaving the pollinium on the stigma (Figure 1c). No other visitors were observed during the daytime. After dark, at roughly 08:00 PM, many midges,

presumably of the same species, were found on the flowers (Figure 1d–f). Midges were present throughout the night until around 06:00 AM when dawn arrived. Flower visitors

were collected by an aspirator and preserved in 99.5% ethanol for later identification. An additional observation was made at the same site from 07:30 PM to midnight on 14 May. In total, 135 visitors were collected. The collection contained a small proportion of insect species from other dipteran families (Chloropidae, Chironomidae, Sciaridae, and Psychodidae), while the majority (94.8%) were gall midges (Cecidomyiidae). Of the 128 gall midges collected, all were female, and 102 belonged to a single morphospecies that was clearly distinguished from other gall midges by a characteristic pattern on the wings (Figure 1c,d,f). Six specimens of this morphospecies with a distinct wing pattern were deposited as vouchers at the University Museum, University of Tokyo (UMUT-YS-Dip-001-006).

Gall midges on inflorescences moved continuously from flower to flower, and some also flew to other inflorescences. Of the 128 gall midges collected, 42 (33%) carried pollinaria on their heads. However, many pollinaria were detached from the gall midges during preservation in ethanol. Based on the 42 preserved specimens retaining pollinaria on the head, up to five pollinaria were found on one individual (mode = 1.6 pollinaria). The overall average number of pollinaria per gall midge based on all pollinaria in the preservation jar (193) was 1.5. Inspection of *Oberonia* flowers in the wild indicated that many had received pollinaria on the stigma, meaning that pollinarium deposition occurred regularly (Figure 1f). Although we have not ruled out the possibility of autogamy or abiotic pollination, we consider that unlikely because *Oberonia* orchids rarely set fruit under cultivation (Geiger, 2019).

Overall, these observations strongly suggest that gall midge is an effective pollinator of *O. japonica*. While there are some reports of gall midges carrying orchid pollinaria (Bogarín et al., 2016; Damon & Roblero, 2007; Karremans & Diaz-Morales, 2017), to our knowledge, this is the most detailed observation of gall midge pollination in Orchidaceae ever reported.

Gall midge pollination is a rare pollination system currently found in 43 species belonging to 10 plant families (Bogner, 2011; Etl et al., 2022; Gan et al., 2022; Kawakita et al., 2022; Matsumoto et al., 2022). Some known examples of gall midge pollination involve brood-site rewards (Kawakita et al., 2022; Luo et al., 2010), but brood-site pollination is unlikely in *Oberonia* because no oviposition behavior was observed, and no larvae emerged from sampled inflorescences. Instead, feeding-like behavior, meaning the action of a midge sticking its mouthparts to the “sac structure” at the center of the lip (Geiger, 2014), was repeatedly observed (Figure 1e). Hence, there is a possibility that gall midges obtain a food reward from *Oberonia* flowers, although we were unable to sample nectar or other secretions from the lip of *O. japonica*.

Another possibility is that gall midges are lured to *Oberonia* flowers by deception. The fact that the sampled midges were all females suggests that a visual and/or olfactory stimulus to which female gall midges respond positively may be involved in their attraction. To the human eye, the color of *O. japonica* flowers resembles the body color of the gall midges (Figure 1c–f). There is therefore some potential that the inflorescence of *O. japonica* mimics lek formation by male gall midges. Floral scent may also be involved, as we were able to detect a weak but distinct smell, and floral scent has been shown to attract gall midges to other plants (Etl et al., 2022). Additional study at different locations to confirm specificity of the gall midge species, as well as analysis of floral scent that might attract female gall midges, is necessary to fully understand the mechanism by which gall midges are attracted to *Oberonia* flowers.

In conclusion, this study demonstrates gall midge pollination in *O. japonica*, adding a new insect family, Cecidomyiidae, to the list of animals known to pollinate orchids. While *Oberonia* is a diverse genus of 150–300 species, there are notable similarities in morphology among them, with minute flowers in monochromatic or sometimes dichromatic colors ranging from light to dark green to yellow, orange, or red. These colors resemble those of other gall midge-pollinated flowers found in different plant families (Arnold et al., 2019; Bogner, 2011; Etl et al., 2022; Gan et al., 2022; Gardner et al., 2018; Kawakita et al., 2022; Luo et al., 2010; Thien et al., 2003; Vislobokov et al., 2014), suggesting that gall midge pollination could be widespread across the genus. Because gall midges are small and are often active at night (Gan et al., 2022), orchid pollination by gall midges may go unnoticed in other genera. For example, small, orange flowers arranged on arching floral stems are features shared by other species such as *Octarrhena angraecoides* (Thelasiinae), *Dendrochilum bicallosum* (Coelogyinae), and *Aerangis hariatiana* (Angraecinae). Considering the large taxonomic and ecological diversity of dipteran insects, the interaction between orchid flowers and Diptera is likely to be more diverse than currently known. Accordingly, study of such interactions is thus a key to our understanding of orchid floral evolution.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Sunakawa, 2023) are available in Figshare at <https://doi.org/10.6084/m9.figshare.23896737>.

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