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plant *Mitrastemon yamamotoi* (Mitrastemonaceae)  
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1 **Social wasps, crickets, and cockroaches contribute to the pollination of the**  
2 **holoparasitic plant *Mitrastemon yamamotoi* (Mitrastemonaceae) in southern Japan**

3

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9 Running title: Social wasps, crickets, and cockroaches pollination

10

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1 ABSTRACT

2 • *Mitrastemon yamamotoi* is completely embedded within the tissues of its hosts, except  
3 during the reproductive stage, when aboveground parts emerge from the host tissues. Its  
4 highly modified appearance has attracted the attention of many botanists, but very little  
5 is known about *M. yamamotoi* its reproductive system.

6 • Floral visitors of *M. yamamotoi* were observed in southern Japan. Pollination  
7 experiments were conducted to determine the plant's self-compatibility and pollen  
8 limitation, as well as the contribution of diurnal and nocturnal visitors to fruit set and  
9 outcrossing.

10 • *Mitrastemon yamamotoi* was mainly pollinated by social wasps, but previously  
11 unnoticed pollinators (i.e., crickets and cockroaches) are also important, based on  
12 visitation frequency and pollen loads. The results of the pollination experiments  
13 suggested that nocturnal visitors, such as crickets and cockroaches, contribute to  
14 geitonogamous pollination, whereas diurnal visitors, such as social wasps, facilitate  
15 outcrossing.

16 • The unexpected pollinator assemblage of *M. yamamotoi* might be influenced by  
17 multiple factors, including the highly modified flowers that are produced close to the  
18 ground in dark understory environments, the species' winter-flowering habit, and the  
19 location of the study site (i.e., near the northern limit of the species' range). Considering  
20 that *M. yamamotoi* occurs widely in subtropical and tropical forests in Asia, additional  
21 studies are needed to assess the pollinator assemblages of *M. yamamotoi* at other  
22 locations.

23

24 Keywords: Achlorophyllous plant; breeding system; dung beetle; parasitic plant;

1 heterotrophic plant; reproductive biology

2

3 Non-photosynthetic plants have long attracted interest because of their peculiar  
4 morphological features (Kuijt 1969). The genus *Mitrastemon*, the sole member of  
5 Mitrastemonaceae, includes two to six holoparasitic species and has diversification  
6 centers in Southeast Asia and Central America. It was formerly classified in  
7 Rafflesiaceae together with the members of Apodanthaceae and Cytinaceae, whose  
8 vegetative parts are completely embedded within the host tissues except during the  
9 reproductive period, due to their unusual lifestyles (de Vega *et al.* 2007). However,  
10 based on recent phylogenetic studies, the former Rafflesiaceae was split into four  
11 families belonging to four different orders (Barkman *et al.* 2004; Nickrent *et al.* 2004;  
12 Barkman *et al.* 2007; Filipowicz & Renner 2010). Barkman *et al.* (2004) revealed that  
13 the genus *Mitrastemon* belongs to the order Ericales, while Rafflesiaceae itself belongs  
14 to the order Malpighiales, based on mitochondrial markers. *Mitrastemon yamamotoi*  
15 occurs naturally in the subtropical or tropical forests of Borneo, Sumatra, Indochina,  
16 and Japan, while the debate on *Mitrastemon* species delimitation remains unsettled  
17 (Meijer & Velkamp 1993).

18 The extraordinary appearance of *Mitrastemon* species has attracted the interest of  
19 many botanists, but very little is known about its their reproductive systems. For  
20 example, some birds (e.g., *Zosterops*, *Melidectes*, and *Oedistoma* species) have been  
21 reported to forage for nectar among *M. yamamotoi* flowers (Matuda 1947; Beehler  
22 1994). However, no studies have confirmed the effectiveness of birds as pollinators.  
23 Beehler (1994) also hypothesized that small nocturnal mammals could pollinate *M.*  
24 *yamamotoi* flowers. However, nocturnal visitors have never been formally observed.

1           The pollinator assemblage of *M. yamamotoi* is likely influenced by the location of  
2 its flowers. These are produced close to the ground in dark understory environments,  
3 which are generally associated with pollinator species different from that found at open  
4 sites (Kato 1996; Herrera 1997; Moore 1997; Rincon *et al.* 1999). Achlorophyllous  
5 plants, including *M. yamamotoi*, can occupy low-light niches where there is little  
6 competition from autotrophic plants. However, such conditions can inhibit reproduction  
7 if pollinator foraging is negatively influenced by low light intensity. Most bees, for  
8 example, tend to restrict their foraging activities to areas of high light intensity.  
9 Consequently, it is possible that plants in shaded understory habitats experience less  
10 pollination by bees (Kato 1996; Suetsugu 2015; but also see Hentrich *et al.* 2010). In  
11 fact, most mycoheterotrophs studied to date appear to have abandoned bee pollinators in  
12 favor of self-pollination or alternative understory pollinators, such as fruit flies  
13 (Suetsugu 2013; Martos *et al.* 2015; Suetsugu 2015, 2018a). In addition, unexpected  
14 seed dispersal systems, such as endozoochory by camel crickets (Rhaphidophoridae),  
15 have been reported in non-photosynthetic plants, possibly due to the colonization of  
16 dark understory habitats, where wind is an ineffective seed dispersal agent (Suetsugu  
17 2018b,c). Judging from the complex and intriguing reproductive systems of  
18 non-photosynthetic plants growing in dark understory environments, the pollination  
19 biology of *Mitrastemon* species is likely unusual.

20           Notably, *M. yamamotoi* blooms from late autumn to early winter in Japan.  
21 Matuda (1947) reported that *Mitrastemon* species only grow under cool, dry conditions,  
22 when its hosts are relatively inactive. Outside the tropics, winter-flowering plants may  
23 experience pollinator limitation, as insect activity is largely limited by temperature  
24 (Fang *et al.* 2012). Therefore, several studies have suggested that winter-flowering

1 phenologies favor bird pollination in temperate regions (Kunitake *et al.* 2004; Fang *et al.*  
2 2012).

3 Here, I conducted intensive field observations and pollination experiments to  
4 examine the pollination biology and reproductive system of *M. yamamotoi* in a  
5 warm-temperate region, i.e., Yakushima Island, southern Japan. I hypothesized that  
6 avian visitors are the primary pollinators of *M. yamamotoi* in this population because  
7 previous studies reported birds foraging for nectar in *M. yamamotoi* (Matuda 1947;  
8 Beehler 1994; Hansen 1972). However, the results obtained here indicated that  
9 relatively unnoticed floral visitors, including social wasps, crickets, and cockroaches,  
10 contribute significantly to fruit set.

11

## 12 MATERIALS AND METHODS

13

### 14 Study species and study site

15

16 *Mitrastemon yamamotoi* is a holoparasitic plant that is completely embedded within the  
17 tissues of its hosts, except during the reproductive stage, when its flowers emerge from  
18 the host tissues. *Mitrastemon* species produce bisexual, protandrous flowers with  
19 collar-shaped perianth tubes (Fig. 1). The stamens of the flowers are connate, forming a  
20 mitra-shaped androecial tube that is crowned by a fertile zone of pollen-bearing locules  
21 (Nickrent *et al.* 2004), and the staminal tube, which has a small hole at the top,  
22 circumscissily separates from the flower as it is pushed up by the growing gynoecium.  
23 Furthermore, the apical portion of the staminal tube is sterile, whereas the basal portion  
24 possesses a series of vertical rings of numerous, minute pollen sacs. The gynoecia of the

1 flowers are hypogynous and single-locular, with a thick, conical stigma (Nickrent *et al.*  
2 2004), and the large amount of dilute nectar stored in upper scale leaves has been  
3 considered a pollinator reward (Matuda 1947; Beehler 1994).

4 The floral biology of *M. yamamotoi* was investigated in the southern part of  
5 Yakushima Island, Kyushu district, Japan, where *M. yamamotoi* parasitizes the roots of  
6 *Castanopsis sieboldii* (Fagaceae).

#### 7 8 Pollinator observation

9  
10 Pollination observations were conducted from late October to late November from 2008  
11 to 2011. Direct observations were made for ca. 100 h in total, in 4- to 6-h bouts, which  
12 were scheduled to cover all hours within a day. The behavior of potential visitors was  
13 observed by walking around the study site, sitting next to flower patches, or hiding in  
14 the vegetation near (1–2 m) flower clusters. Nocturnal observations involved the use of  
15 red lamps, which minimized the effect of light on potential floral visitors. The  
16 frequency, duration, and visitation pattern (single or sequential) were recorded for each  
17 floral visitor, i.e., for individuals landing on, passing the floral patches, or foraging for  
18 floral nectar or pollen grains.

19 A subset of the observed floral visitors (at least one species from each order)  
20 was captured using a sweep net or aspirator. Pollen grains carried on the bodies of  
21 visitors were counted under a dissecting microscope. Those forming clumps were first  
22 removed from visitor bodies using basic fuchsin jelly (Kearns & Inouye 1993), and  
23 then spread on glass slides.

24 From November 7 to 21 in 2011, a remote camera with built-in infrared motion

1 sensors (Sensor Camera Fieldnote; Marif Co., Ltd., Japan) was also used at all hours of  
2 the day and night to record any additional potential floral visitors that might have been  
3 deterred by our direct observation (e.g., birds and mammals). This camera was set ca. 2  
4 m from the target flowering plants. The observation by the remote camera was used  
5 only for detecting visitations by mammals and birds. In contrast, I used the data based  
6 on direct observations for visitations by invertebrates.

7

## 8 Breeding system

9

10 Pollination experiments were conducted in early November 2008 to determine the  
11 breeding system (i.e., the capacity for self-fertilization) of *M. yamamotoi*. Flower buds  
12 were bagged using nylon netting and subject to one of four treatments: (1) bagged only  
13 to exclude floral visitors (40 flowers; autonomous selfing); (2) self-pollinated by hand  
14 as soon as each flower's androecial tube became circumscissile and dehiscent (40  
15 flowers; artificial self-pollination treatment); (3) cross-pollinated by hand as soon as  
16 each flower's androecial tube became circumscissile and dehiscent. To avoid crossing  
17 genetically identical plants, all plants used for the cross-pollination experiments were  
18 located at least 5 m from their nearest neighbor (40 flowers; artificial cross-pollination  
19 treatment); or (4) the nylon netting had small openings around the stems to allow  
20 visitation by ants (40 flowers; ants-only treatment). In addition, 100 flowers were  
21 marked to analyze fruit set under natural conditions (open treatment).

22 When all stigmas became blackish and unreceptive, the plants in each treatment  
23 group were bagged using nylon-mesh cages to prevent seed consumption by potential  
24 seed dispersers and facilitate a precise determination of fruit set. Fruit number was



1 counted at ca. 2–3 months after the flowering season, and the effect of treatment was  
2 determined using Fisher’s exact test. In addition, 100 randomly selected seeds from  
3 each fruit capsule were examined under a dissecting microscope, to assess the  
4 proportion of seeds with embryos, and the effect of treatment was determined using  
5 analysis of variance (ANOVA).

6

### 7 Effectiveness of diurnal and nocturnal visitors

8

9 The effectiveness of diurnal and nocturnal visitors was evaluated in November 2011.  
10 Based on the observations from 2008 to 2010, most nocturnal visitors were flightless.  
11 The visitors likely contribute to pollination within the same patches. Furthermore,  
12 because the endophytic system of *M. yamamotoi* grows intercellularly within host roots,  
13 it is highly possible that *M. yamamotoi* flowers on the same host plant are genetically  
14 identical. Therefore, the effectiveness of outcrossing pollinators in both diurnal and  
15 nocturnal visitors was examined by emasculating all *M. yamamotoi* flowers that  
16 parasitized a single tree, as these were considered genetically identical.

17 Patches with more than 20 individuals parasitizing the same tree with unopened  
18 flower buds were selected before anthesis, and, within each patch, flowers were  
19 assigned to one of four treatment groups. Five patches were used per treatment: (1)  
20 nocturnal visitors were excluded from sunset to sunrise using a nylon-mesh cage (110  
21 flowers; diurnal natural pollination); (2) diurnal visitors were excluded from sunrise to  
22 sunset using a nylon-mesh cage (110 flowers; nocturnal natural pollination); (3)  
23 nocturnal pollinators were excluded from emasculated flowers from sunset to sunrise  
24 using a nylon-mesh cage (112 flowers; diurnal cross-pollination); or (4) diurnal

1 pollinators were excluded from emasculated flowers from sunrise to sunset using a  
2 nylon-mesh cage (112 flowers; nocturnal cross-pollination).

3 After all the stigmas had become blackish and unreceptive, the plants in each  
4 treatment group were bagged using nylon-mesh cages, in order to prevent seed  
5 consumption by potential seed dispersers and facilitate a precise determination of fruit  
6 set. Fruit number was counted at ca. 2–3 months after the flowering season, and the  
7 effect of treatment was determined using Fisher’s exact test. In addition, 10 fruit  
8 capsules were randomly collected from each treatment group. After that, 100 randomly  
9 selected seeds from each of these fruit capsules were examined under a dissecting  
10 microscope, to assess the proportion of seeds with embryos, and the effect of treatment  
11 was determined using ANOVA.

## 12 13 RESULTS

### 14 15 Observations of floral visitors

16  
17 Recordings by the motion-sensor-equipped camera showed that *Zosterops japonicus*  
18 (*Zosteropidae*) rarely landed on *M. yamamotoi* patches and never foraged for nectar. In  
19 contrast, direct observation showed that *M. yamamotoi* flowers were frequently visited  
20 by social wasps, crickets, cockroaches, flies, dung beetles, stag beetles, and ants (Fig. 2;  
21 Table 1).

22 Social wasps (e.g., *Vespa mandarinia*, *Vespa analis*, *Vespa simillima xanthoptera*,  
23 *Vespula flaviceps*, and *Vespula shidai*) were the main diurnal visitors and were observed  
24 to sequentially visit multiple plants during foraging. Multiple wasps visited the same *M.*

1 *yamamotoi* patches simultaneously and they were often observed to aggressively defend  
2 their feeding territories from one another. Furthermore, even though social wasps did  
3 not actively collect pollen grains from the *M. yamamotoi* flowers, these were always  
4 attached to the wasps' legs, heads, abdomens, and mouthparts (Table 2).

5 Other diurnal visitors included flies, such as *Drosophila* species and members of  
6 the Calliphoridae, Sarcophagidae, Muscidae, and Tachinidae, and ants, such as  
7 *Crematogaster* species. Flies sometimes visited multiple plants within the patches, at a  
8 single visitation, while foraging for nectar. However, despite touching the anthers and  
9 stigmas of the flowers with their legs and mouthparts, pollen grains were rarely found  
10 attached to either (Table 2). Ants also visited *M. yamamotoi* flowers to harvest nectar,  
11 but usually only visited single flowers, where they stayed for a long time, and rarely  
12 made successive visits to multiple flowers.

13 Nocturnal visitors included various crane fly, orthopteran, cockroach, beetle,  
14 centipede, and moth species. Crane fly *Limonia* sp. occasionally visited *M. yamamotoi*  
15 flowers to lay eggs but only oviposited on the plants' scale-leaves and rarely touched  
16 either the male or female flowers. However, orthopterans, such as camel crickets  
17 (*Diestrammena yakumontana*) and field crickets (*Duolandrevus ivani*), which were  
18 some of the most frequent nocturnal floral visitors, usually visited multiple plants  
19 sequentially, touched anthers and stigmas during feeding, and, consequently, carried  
20 pollen grains on their legs, heads, and mouthparts (Table 2). Similarly, cockroaches,  
21 such as *Opisthoptatia orientalis* and *Onychostylus pallidiolus*, were also observed to  
22 visit multiple plants sequentially, touch anthers and stigmas, and carry pollen grains  
23 (Table 2).

24 The dung beetle *Onthophagus yakuinsulanus* often visited multiple flowers to

1 forage for nectar and pollen grains, and specimens sometimes exhibited pollen grains  
2 attached to their legs and abdomens. The stag beetle *Aegus laevicollis* visited multiple  
3 plants to forage for nectar, and pollen grains were also found attached to their legs and  
4 abdomens (Table 2). The centipedes *Thereuopoda clunifera* and *Scolopendra*  
5 *subspinipes* were observed to visit *M. yamamotoi* flowers, possibly to feed on nectar or  
6 prey on other animals attracted by the nectar, and pollen grains were sometimes found  
7 attached to their legs. Finally, an unidentified pyralid moth was observed to land on  
8 flowers and to oviposit on the surface of scale-leaves.

9

#### 10 Breeding system

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12 Flowers in the pollinator-excluded treatment showed low levels of fruit set (Table 3).  
13 The fruit set observed in bagged flowers was unexpected; the stamen tube was pushed  
14 off by the growth of the pistil after the pollen had been shed and, therefore, its floral  
15 structure should have prevented self-fertilization. Low levels of fruit set in bagged  
16 flowers were possibly due to unintended ant intrusion or apomixis. There were more  
17 developed fruits in the ants-only treatment than in the bagged-only treatment, although  
18 the difference was insignificant ( $P = 0.59$ ). High fruit set and seed viability were  
19 observed in both the artificial self-pollination and cross-pollination groups, whereas the  
20 fruit set and seed viability of the open-pollinated group were significantly lower thereby  
21 indicating some pollinator limitation (Table 3).

22

#### 23 Effectiveness of diurnal and nocturnal visitors

24

1 In both the diurnal and nocturnal treatment groups, approximately half of the flowers  
2 developed fruit capsules, and there were no significant differences between the fruit set  
3 and seed viability of the two groups ( $P = 0.50$ ). This indicated that both diurnal and  
4 nocturnal floral visitors contributed to pollination (Table 4). Although seed viability was  
5 similar for nocturnal and diurnal cross-pollination groups, ( $P = 0.41$ ), fruit set in the  
6 nocturnal cross-pollination group was significantly lower than that in the diurnal  
7 cross-pollination group ( $P < 0.01$ ). This indicated that the relative contribution of  
8 diurnal cross-pollinators to fruit set was higher than that of nocturnal cross-pollinators.

9

## 10 DISCUSSION

11

12 The findings of the present study indicate that unexpected floral visitors, such as social  
13 wasps, crickets, and cockroaches, contribute significantly to *M. yamamotoi* fruit set.  
14 This unexpected pollinator assemblage seems to be influenced by multiple factors,  
15 including the highly modified flowers produced close to the ground in dark understory  
16 environments and the winter-flowering habit of *M. yamamotoi*, as well as the location of  
17 the study site (i.e., near the northern limit of the species' range). In addition, pollination  
18 experiments suggested diurnal and nocturnal pollinators have a differential contribution  
19 to reproductive success. While there were no significant differences in fruit set and seed  
20 viability between the diurnal- and nocturnal-pollinators treatment groups, fruit set in the  
21 nocturnal cross-pollination group was significantly lower than that in the diurnal  
22 cross-pollination group. These results indicate that nocturnal visitors, such as crickets,  
23 cockroaches, and dung beetles, mainly contributed to the geitonogamous pollination.

24 The potential factor contributing to the observed difference is that most nocturnal

1 visitors were flightless arthropods. It is intriguing that endemic flightless insects such as  
2 *Diestrammena yakumontana* and *Onthophagus yakuinsulanus* appear to contribute to  
3 fruit set of *M. yamamotoi* in Yakushima Island. Flightless insects might outcompete  
4 other species on island environments because mainland predators are often absent  
5 (Carlquist 1974). Thus, pollination by flightless insects might represent unique  
6 pollination biology in island ecosystems, since Yakushima Island is known to form a  
7 unique ecosystem harboring many endemic taxa (Yahara *et al.* 1987).

8 In the present study, social wasps were the main diurnal visitors of *M. yamamotoi*,  
9 and both visitor observation and pollination experiments suggested that social wasps are  
10 the most effective pollinators of *M. yamamotoi*, as they transfer pollen grains between  
11 patches. Because *Vespa* species do not harvest pollen to feed their brood (Richter 2000),  
12 wasps are not considered typical floral visitors (Brodmann *et al.* 2008, 2009). However,  
13 social wasps often feed on nectar to fuel their own activity (Richter 2000). In addition,  
14 Vislobokov & Galinskaya (2018) reported that wasps are the main pollinators of the  
15 holoparasitic plant *Balanophora harlandii*, which occupies an ecological niche that is  
16 similar to that of *Mitrastemon* species.

17 Although both ants and flies were main diurnal visitors, and occasionally visited  
18 multiple plants within patches during their nectar foraging, the pollen loads on their  
19 bodies were much lower than those found for social wasps, crickets, and cockroaches.  
20 Therefore, their effectiveness as pollinators was lower than that of other frequent  
21 visitors. This result was also supported by the pollination experiment results, as there  
22 were no significant differences on fruit set between the ants-only treatment and the  
23 bagged-only treatment.

24 Some orthopteran species were the most frequent nocturnal visitors. Orthopterans

1 are generally considered herbivores, rather than pollinators (Suetsugu & Tanaka 2014;  
2 Tan *et al.* 2017). In the present study, *D. yakumontana* and *D. ivani* consumed pollen  
3 grains and nectar of *M. yamamotoi*, but did not damage the other floral parts such as  
4 stigmas. In addition, they often visited both male and female flowers sequentially to  
5 forage for nectar. Consequently, they had many pollen grains attached to their bodies,  
6 which clearly suggests that orthopterans can pollinate *Mitrastemon* species, even though  
7 the relationship between *Mitrastemon* and crickets is unspecialized. These results, along  
8 those of previous studies (Micheneau *et al.* 2010; Tan & Tan 2018), suggest that  
9 orthopteran pollination might be more common than previously recognized.

10       Cockroaches were also among the most common nocturnal visitors. Cockroaches  
11 are generally omnivorous scavengers and detritus feeders (Schal *et al.* 1984). However,  
12 cockroach pollination has been reported in at least three plant species, including *Uvaria*  
13 *elmeri* (Annonaceae; Nagamitsu & Inoue 1997), *Balanophora tobiracola*  
14 (Balanophoraceae; Kawakita & Kato 2002), and *Clusia sellowiana* (Clusiaceae;  
15 Vlasáková *et al.* 2008). Here, cockroaches visited multiple flowers and had numerous  
16 pollen grains attached to their bodies, thereby confirming their pollinator status.

17       The dung beetle *Onthophagus yakuinsulanus* often visited multiple flowers in  
18 succession and carried pollen grains (Table 2). Indeed, *Onthophagus* species have been  
19 reported to pollinate other plant species, such as *Orchidantha inouei* (Lowiaceae) in  
20 Sarawak, Malaysia (Sakai & Inoue 1999). Because some dung beetles are excellent  
21 dung searchers and fly long distances to locate specific types of dung, Sakai & Inoue  
22 (1999) considered that they could function as long-distance pollinators. However, the  
23 elytra of *O. yakuinsulanus* are fused, thereby forming a closed carapace and rendering  
24 the species flightless. Nevertheless, based on its visitation behavior and ability to carry

1 pollen, *O. yakuinsulanus* can at least work as a geitonogamous pollinator.

2       It is intriguing that most *M. yamamotoi* visitors included social wasps, crickets,  
3 and cockroaches, which are known to forage for foods such as fermented sap, using  
4 olfactory cues (Yoshimoto et al. 2005; Brodmann et al. 2008; Dormont et al. 2010;  
5 Micheneau et al. 2010; Stökl et al. 2010). Because the scale-leaves of *M. yamamotoi*  
6 store nectar that sometimes smells fermented, the unique assembly of *M. yamamotoi*  
7 pollinators might be attracted by volatiles produced by nectarivorous yeasts. As these  
8 yeasts degrade floral nectar by metabolizing nectar sugar, they are often regarded as  
9 exploitative antagonists of plant-pollinator mutualistic relationships (Herrera et al.  
10 2008). However, nectarivorous yeasts may enhance pollination by altering volatile  
11 profiles emitted from flowers (Rering et al. 2018). Thus, how nectar-dwelling yeasts  
12 affect *M. yamamotoi* pollination success should be addressed in future studies.

13       It is puzzling that no birds visited *M. yamamotoi* flowers for nectar, especially  
14 because *Zosterops*, *Melidectes*, and *Oedistoma* species have been previously reported to  
15 forage for nectar in *M. yamamotoi* patches (Matuda 1947, Beehler 1994) and because *M.*  
16 *yamamotoi* produces large amounts of dilute nectar that is well suited for avian  
17 pollination (Cronk & Ojeda 2008). However, preliminary observations suggested that *Z.*  
18 *japonicus*, *Turdus pallidus*, and *Erithacus akahige* only visit *M. yamamotoi* during the  
19 fruiting season (Suetsugu, unpublished data). Therefore, records of bird visitation might  
20 have been incorrectly attributed to floral visitation, rather than visitation during the  
21 fruiting season, at least in Japanese populations. Yet, Beehler (1994) reported that  
22 *Melidectes* and *Oedistoma* species aggressively and frequently forage for nectar among  
23 *M. yamamotoi* flowers in New Guinea.

24       It should be noted that the investigated population is near the northern limit of *M.*



1 *yamamotoi* distribution. Because pollinator assemblages can vary among locations (e.g.,  
2 Aguiar *et al.* 2012), other *M. yamamotoi* populations might be ecologically distinct and  
3 utilize different pollinators, including birds. Therefore, further research is needed to  
4 elucidate the pollinator assemblage of *M. yamamotoi* in other areas. In addition, the  
5 discovery of pollination by camel crickets and cockroaches suggests that pollination  
6 systems involving unusual and unexpected taxa might be more widespread than  
7 previously thought, especially in non-photosynthetic plants with highly modified floral  
8 morphology.

9

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19

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Table 1. Floral visitors of *Mitrastemon yamamotoi*.

Order	Species	Number of individuals on flowers
Blattodea	<i>Opisthoplatia orientalis</i>	67
	<i>Onychostylus pallidiolus</i>	35
Coleoptera	<i>Onthophagus yakuinsulanus</i>	21
	<i>Allecula fuliginosa</i>	2
	<i>Aegus laevicollis</i>	1
Diptera	<i>Drosophila</i> spp.	>200
	Calliphoridae spp.	>200
	Sarcophagidae spp.	>100
	Muscidae spp.	>100
	Tachinidae spp.	>200
	<i>Limonia</i> sp.	13
	Mycetophilidae spp.	9
Hymenoptera	<i>Vespa analis</i>	183
	<i>Vespa mandarinia</i>	56
	<i>Vespa simillima xanthoptera</i>	38
	<i>Vespula flaviceps</i>	24
	<i>Vespula shidai</i>	11
	<i>Paratrechina flavipes</i>	>100
	<i>Crematogaster</i> spp.	>100
	<i>Camponotus</i> sp.	15
	<i>Odontomachus monticola</i>	3
Lepidoptera	Pyralidae sp.	4
Orthoptera	<i>Diestrammena yakumontana</i>	89
	<i>Duolandrevus ivani</i>	41
	<i>Aphonoides rufescens</i>	8
	<i>Ornebius kanetataki</i>	5
Scutigeromorpha	<i>Thereuopoda clunifera</i>	10
Chilopoda	<i>Scolopendra subspinipes</i>	14

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Table 2. Number of *Mitrastemon yamamotoi* pollen grains on the bodies of floral visitors

Order	Species	Number of pollen grains on visitor bodies	Number of specimens examined
Blattodea	<i>Opisthoplatia orientalis</i>	78.8 ± 32.9	n = 5
	<i>Onychostylus pallidiolus</i>	19.4 ± 8.6	n = 5
Coleoptera	<i>Onthophagus yakuinsulanus</i>	12.8 ± 7.7	n = 5
Diptera	<i>Drosophila</i> spp.	2.6 ± 1.1	n = 20
	Calliphoridae spp.	3.6 ± 1.5	n = 20
	<i>Limonia</i> sp.	0	n = 5
Hymenoptera	<i>Vespa analis</i>	118.2 ± 27.2	n = 5
	<i>Vespa mandarinia</i>	156.4 ± 49.2	n = 5
	<i>Vespa simillima xanthoptera</i>	71.0 ± 17.0	n = 5
	<i>Paratrechina flavipes</i>	3.0 ± 1.2	n = 20
Lepidoptera	Pyralidae sp.	0	n = 2
Orthoptera	<i>Diestrammena yakumontana</i>	66.6 ± 31.0	n = 5
	<i>Duolandrevus ivani</i>	78.8 ± 26.8	n = 5
Scutigeromorpha	<i>Thereuopoda clunifera</i>	9.7 ± 5.2	n = 3
Chilopoda	<i>Scolopendra subspinipes</i>	6.3 ± 6.3	n = 3

The number of pollen grains on the bodies is indicated as mean ± standard error.

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Table 3. Effect of pollination treatments on the proportions (%) of fruit set and seeds with embryo in *Mitrastemon yamamotoi*.

	Pollinator-excluded	Manual autogamy	Manual allogamy	Ants-only	Open
Fruit set	17.5 <sup>a</sup>	67.5 <sup>b</sup>	65.0 <sup>b</sup>	25.0 <sup>a</sup>	43.0 <sup>ab</sup>
Seeds with embryo	18.0 ± 12.0 <sup>a</sup>	60.5 ± 20.8 <sup>b</sup>	67.2 ± 21.5 <sup>b</sup>	27.7 ± 15.7 <sup>a</sup>	48.3 ± 21.3 <sup>c</sup>

Pollination treatments producing significant differences ( $P < 0.05$ ) are indicated by different superscript letters.

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Table 4. Effect of diurnal and nocturnal visitors on the proportions (%) of fruit set and seeds with embryo in *Mitrastemon yamamotoi*.

	Diurnal natural	Nocturnal natural	Diurnal cross	Nocturnal cross
Fruit set	49.1 <sup>a</sup>	48.2 <sup>a</sup>	42.0 <sup>a</sup>	26.8 <sup>b</sup>
Seeds with embryo	48.2 ± 20.6 <sup>a</sup>	42.7 ± 17.8 <sup>a</sup>	41.7 ± 20.3 <sup>a</sup>	34.3 ± 20.0 <sup>a</sup>

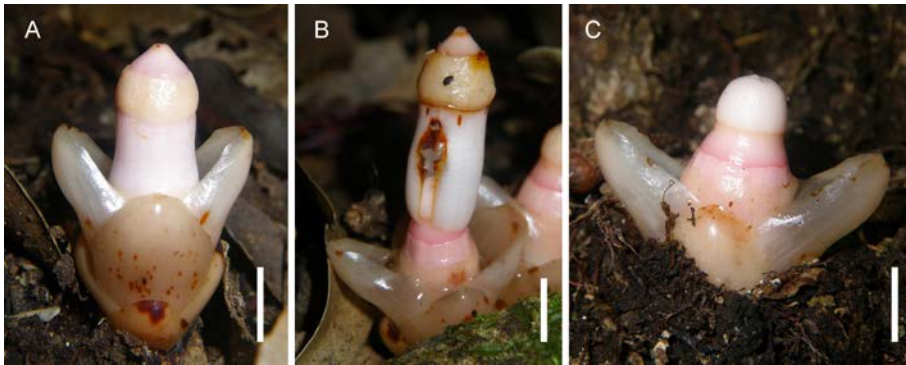
Pollination treatments producing significant differences ( $P < 0.05$ ) are indicated by different superscript letters.

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1 **Figures**



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3 **Fig. 1.** Flowering of *Mitrastemon yamamotoi*. (A) Male stage. (B) Transitional stage  
4 (the stamen tube begun to fall off). (C) Female stage.

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2 **Fig. 2.** Floral visitors of *Mitrastemon yamamotoi*. (A) *Vespa simillima xanthoptera*. (B)  
 3 *Vespa analis*. (C) *Vespula flaviceps*. (D) *Vespa mandarinia*. (E) *Duolandrevus ivani*. (F)  
 4 *Diestrammena yakumontana*. (G) *Ornebius kanetataki*. (H) *Aphonoides rufescens*. (I)  
 5 *Aegus laevicollis*. (J) *Onthophagus yakuinsulanus*. (K) *Opisthoplatia orientalis*. (L)  
 6 *Onychostylus pallidiolus*. (M) Tachinidae sp. (N) Sarcophagidae sp. (O) *Limonia* sp. (P)  
 7 *Drosophila* sp. (Q) *Paratrechina flavipes*. (R) *Thereuopoda clunifera*.

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