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

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Running title: Social wasps, crickets, and cockroaches pollination

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ABSTRACT

- *Mitrastemon yamamotoi* is completely embedded within the tissues of its hosts, except during the reproductive stage, when aboveground parts emerge from the host tissues. Its highly modified appearance has attracted the attention of many botanists, but very little is known about *M. yamamotoi* its reproductive system.
- Floral visitors of *M. yamamotoi* were observed in southern Japan. Pollination experiments were conducted to determine the plant's self-compatibility and pollen limitation, as well as the contribution of diurnal and nocturnal visitors to fruit set and outcrossing.
- *Mitrastemon yamamotoi* was mainly pollinated by social wasps, but previously unnoticed pollinators (i.e., crickets and cockroaches) are also important, based on visitation frequency and pollen loads. The results of the pollination experiments suggested that nocturnal visitors, such as crickets and cockroaches, contribute to geitonogamous pollination, whereas diurnal visitors, such as social wasps, facilitate outcrossing.
- The unexpected pollinator assemblage of *M. yamamotoi* might be influenced by multiple factors, including the highly modified flowers that are produced close to the ground in dark understory environments, the species' winter-flowering habit, and the location of the study site (i.e., near the northern limit of the species' range). Considering that *M. yamamotoi* occurs widely in subtropical and tropical forests in Asia, additional studies are needed to assess the pollinator assemblages of *M. yamamotoi* at other locations.

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

heterotrophic plant; reproductive biology

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

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

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

Notably, *M. yamamoto*i blooms from late autumn to early winter in Japan. Matuda (1947) reported that *Mitrastemon* species only grow under cool, dry conditions, when its hosts are relatively inactive. Outside the tropics, winter-flowering plants may experience pollinator limitation, as insect activity is largely limited by temperature (Fang *et al.* 2012). Therefore, several studies have suggested that winter-flowering phenologies favor bird pollination in temperate regions (Kunitake *et al.* 2004; Fang e*t al.* 2012).

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

MATERIALS AND METHODS

Study species and study site

Mitrastemon yamamotoi is a holoparasitic plant that is completely embedded within the tissues of its hosts, except during the reproductive stage, when its flowers emerge from the host tissues. *Mitrastemon* species produce bisexual, protandrous flowers with collar-shaped perianth tubes (Fig. 1). The stamens of the flowers are connate, forming a mitra-shaped androecial tube that is crowned by a fertile zone of pollen-bearing locules (Nickrent *et al.* 2004), and the staminal tube, which has a small hole at the top, circumscissily separates from the flower as it is pushed up by the growing gynoecium. Furthermore, the apical portion of the staminal tube is sterile, whereas the basal portion possesses a series of vertical rings of numerous, minute pollen sacs. The gynoecia of the flowers are hypogynous and single-locular, with a thick, conical stigma (Nickrent *et al.* 2004), and the large amount of dilute nectar stored in upper scale leaves has been considered a pollinator reward (Matuda 1947; Beehler 1994).

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

Pollinator observation

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

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

From November 7 to 21 in 2011, a remote camera with built-in infrared motion sensors (Sensor Camera Fieldnote; Marif Co., Ltd., Japan) was also used at all hours of the day and night to record any additional potential floral visitors that might have been deterred by our direct observation (e.g., birds and mammals). This camera was set ca. 2 m from the target flowering plants. The observation by the remote camera was used only for detecting visitations by mammals and birds. In contrast, I used the data based on direct observations for visitations by invertebrates.

Breeding system

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

When all stigmas became blackish and unreceptive, the plants in each treatment group were bagged using nylon-mesh cages to prevent seed consumption by potential seed dispersers and facilitate a precise determination of fruit set. Fruit number was counted at ca. 2–3 months after the flowering season, and the effect of treatment was determined using Fisher’s exact test. In addition, 100 randomly selected seeds from each fruit capsule were examined under a dissecting microscope, to assess the proportion of seeds with embryos, and the effect of treatment was determined using analysis of variance (ANOVA).

Effectiveness of diurnal and nocturnal visitors

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

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

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

RESULTS

Observations of floral visitors

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

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

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

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

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

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

Breeding system

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

Effectiveness of diurnal and nocturnal visitors

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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Table 1. Floral visitors of *Mitrastemon yamamotoi*.

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

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	*Opisthoplatia orientalis*	78.8 ± 32.9	n = 5
	Onychostylus pallidiolus	19.4 ± 8.6	n = 5
Coleoptera	*Onthophagus yakuinsulanus*	12.8 ± 7.7	n = 5
Diptera	*Drosophila* spp.	2.6 ± 1.1	n = 20
	Calliphoridae spp.	3.6 ± 1.5	n = 20
	Limonia sp.	0	n = 5
Hymenoptera	*Vespa analis*	118.2 ± 27.2	n = 5
	Vespa mandarinia	156.4 ± 49.2	n = 5
	Vespa simillima xanthoptera	71.0 ± 17.0	n = 5
	Paratrechina flavipes	3.0 ± 1.2	n =20
Lepidoptera	Pyralidae sp.	0	n = 2
Orthoptera	*Diestrammena yakumontana*	66.6 ± 31.0	n = 5
	Duolandrevus ivani	78.8 ± 26.8	n = 5
Scutigeromorpha	*Thereuopoda clunifera*	9.7 ± 5.2	n = 3
Chilopoda	*Scolopendra subspinipes*	6.3 ± 6.3	n = 3

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

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^{a}	67.5^{b}	65.0^{b}	25.0^{a}	43.0^{ab}
Seeds with embryo	18.0 ± 12.0^{a}	60.5 ± 20.8^{b}	67.2 ± 21.5^{b}	27.7 ± 15.7^{a}	48.3 ± 21.3^{c}

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

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^{a}	48.2^{a}	42.0^{a}	26.8^{b}
Seeds with embryo	48.2 ± 20.6^{a}	42.7 ± 17.8^{a}	41.7 ± 20.3^{a}	34.3 ± 20.0^{a}

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

Figures

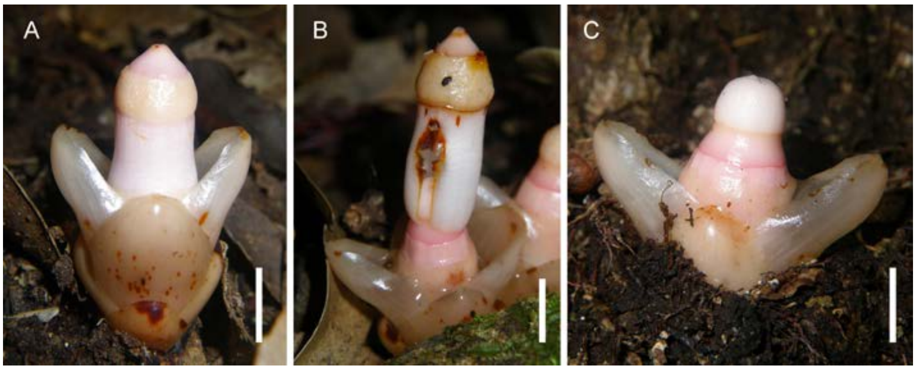

Fig. 1. Flowering of *Mitrastemon yamamotoi*. (A) Male stage. (B) Transitional stage (the stamen tube begun to fall off). (C) Female stage.

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