Article

DIVISION OF LABOUR BETWEEN DIMORPHIC STAMENS IN *MELASTOMA CANDIDUM* (MELASTOMATACEAE): ROLE OF STAMEN STRENGTH IN THE BIOMECHANICS OF POLLINATION

Shogo Hachiman[1], Masashi Uejo[1], Tetsuo Denda[2]*

[1]*Graduate School of Engineering and Science, University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 903-0213, Japan*
[2]*Laboratory of Ecology and Systematics, Faculty of Science, University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 903-0213, Japan*

Journal of Pollination Ecology, 37(18), 2024, pp 284-302
DOI: 10.26786/1920-7603(2024)810

Received 19 June 2024, accepted 30 September 2024

*Corresponding author: denda@sci.u-ryukyu.ac.jp

Abstract—The division of labour hypothesis suggests that plants exhibiting heteranthery utilise different stamen types for distinct purposes; feeding stamens provide pollen as a reward for pollinators, whereas pollinating stamens ensure successful reproduction. However, the biomechanical factors that influence pollinator behaviour in relation to different stamen types remain underexplored. In this study, we investigated *Melastoma candidum*, a species characterised by dimorphic stamens and poricidal anthers, to elucidate how flower morphology, pollen fertility, and biomechanics of pollinator interactions affect pollination efficiency. We observed the behaviour and stamen preference of multiple bee species visiting the flowers, with a particular focus on the frequency of flower visitation and stigma contact. Among the pollinators studied, *Xylocopa flavifrons* emerged as the primary pollinator on Okinawajima Island, Japan. Our analysis revealed that while both stamen types exhibit comparable pollen fertility, *X. flavifrons* preferentially buzzed feeding stamens with the mechanical advantage of pollen collection due to their structural integrity. This preference was further corroborated by biomechanical interactions, wherein the weaker pollinating stamens could not bear the weight of the bee, thereby relegating their function in pollen release. These findings elucidate the significant influence of biomechanical factors on pollinator behaviour and stamen function, providing novel insights into the mechanisms underlying division of labour in plant-pollinator interactions.

Keywords—Buzz pollination, division of labour, heteranthery, pollinator behaviour, poricidal anthers, stamen selectivity

INTRODUCTION

Pollination is a critical process for the sexual reproduction of most angiosperms and involves the transfer of pollen (male gametes) to the stigma on the pistil. Pollen contained within anthers is typically released through various dehiscence mechanisms, including longitudinal, transverse, poricidal, and valvate (Islam et al. 2008; Åstrand et al. 2021). Poricidal dehiscence is characterised by pollen discharge through pores or slits at the anther tips and is observed in 15,000–20,000 angiosperm species, spanning 544 genera and 72 families (Buchmann 1983), including Leguminosae (Marazzi et al. 2007), Solanaceae (Falcão et al. 2016), and Melastomataceae (Renner 1989).

Poricidal anthers generally attract pollen-collecting insects as they do not produce nectar (Buchmann & Hurley 1978). Many bee species utilise buzz pollination, a specialised technique to extract pollen by vibrating their indirect flight muscles to release pollen (Buchmann 1983; King et al. 1996; Thorp 2000; King & Buchmann 2003). Buzz pollination has evolved independently approximately 45 times across various unrelated taxonomic groups such as Colletidae, Oxaeidae, Halictidae, Anthophoridae, and Apidae (Thorp 2000; Vallejo-Marín et al. 2010; Cardinal et al. 2018).

Plants that rely on buzz-pollinating bees often offer only pollen as a reward, posing the 'pollen

dilemma' - a challenge where plants must balance the reward for pollinators with their need for successful pollination (Buchmann & Buchmann 1981; Westerkamp 1996; 1997; Vallejo-Marín et al. 2009; Lunau et al. 2015). Several strategies have been developed to address this 'pollen dilemma', such as offering non-pollen rewards, producing specialised pollen forms, and using 'ungroomed' spots on insect bodies for pollen attachment, and heteranthery (Luo et al. 2008; Praz et al. 2008; Lunau et al. 2015; Brito et al. 2016; 2017; Koch et al. 2017; Pacini & Franchi 2020; Barrett 2021; Trevizan et al. 2023).

Heteranthery is a strategy in which different stamens serve distinct functions, some as a resource for pollen to reward pollinating bees and others for pollen necessary for the transport of male gametes for fertilisation (Barrett 2021). According to Trevizan et al. (2023), these are termed feeding stamens and pollinating stamens, respectively. This strategy helps resolve the pollen dilemma by distinguishing the roles of pollen: reward and fertilisation. This concept, initially mentioned by Darwin (1862) and formally proposed by Müller (1881), is a solution prevalent in many plant species (Müller 1881; 1883; Vallejo-Marín et al. 2010).

Research has supported the division of labour hypothesis within the family Melastomataceae (Trevizan et al. 2023). For example, in heterantherous flowers of species such as *Microlicia cordata*, some anthers attract and feed bees, whereas others are dedicated to reproduction (Velloso et al. 2018). Velloso et al. (2018) found that pollinating stamens involved in reproduction had larger anthers than feeding stamens, which were clearly visible to pollinators because of their contrast colour. This supports the division of labour hypothesis, as the anthers' conspicuous size and colour align with their distinct roles. Similarly, Brito et al. (2021) found that the number of pollen grains and stamen morphology affect pollen release dynamics, reinforcing the division of labour among stamens in *Pleroma* species.

Research on *Melastoma* spp. further supports this hypothesis. For instance, *Melastoma malabathricum* exhibits distinct roles among its stamens, with feeding stamens being involved in feeding and pollination stamens in fertilisation. Pollination stamens have been found to produce more abundant and fertile pollen (Luo et al. 2008), and primary pollinators transfer pollen predominantly from these stamens to the stigma. However, patterns vary within the family, such as alternative strategies exhibited by *Pterolepis glomerata* (Telles et al. 2020), whereas in *Melastoma affine*, bees collect pollen from both feeding and pollination stamens (Gross 1993; Gross & Kukuk 2001). These variations highlight the need for further investigation of the division of labour hypothesis across different Melastomataceae species to better understand its applicability and stamen roles.

Understanding the biomechanics of stamen-pollinator interactions is crucial for elucidating the division of labour between stamen types. Mesquita-Neto et al. (2018) observed three types of bee behaviour in buzz-pollinated plants: flower buzzing, anther buzzing, and pollen-thieving. Flower buzzing bees vibrate the entire flower, and are the most effective pollinators, while anther-buzzing bees are slightly less effective (Mesquita-Neto et al. 2018). The biomechanics involved in these behaviours, including the force and frequency of vibrations, are the key to understanding how bees interact with different stamen types. Exploring these biomechanical interactions can reveal how the structure and function of stamens influence their pollination efficiency and their role in the plant's reproductive strategy.

In this study, we investigated *Melastoma candidum*, a species with two distinct stamen types. Previous research identified *Bombus* and *Xylocopa* bees in Taiwan (Liu et al. 2008) and *Amegilla* bees in the Ryukyu Islands (Kato 2000) as pollinators of *M. candidum*. However, these studies did not explore differences in bee behaviour, their contribution to pollination, or hypotheses regarding the division of labour. We examined pollen morphology and fertility, bee behaviour, stamen preference, and the relationship between insect weight and stamen strength to understand the biomechanics of stamen-pollinator interactions. We aimed to identify the pollinators of *M. candidum*, assess stamen function specialisation, and understand how biomechanical factors influence pollinator behaviour and the effectiveness of different stamen types.

MATERIALS AND METHODS

MELASTOMA CANDIDUM AS THE TARGET SPECIES

Melastoma candidum is a shrub belonging to the family Melastomataceae, which is widely distributed across the Ryukyu Islands, Taiwan, southern China, Indochina, and Philippines (Hatusima 1975). In the Ryukyu Islands, *M. candidum* is the only species of the genus *Melastoma* (Hatusima & Amano 1994). It typically grows in sunny environments and produces flowers approximately 8 cm in diameter, featuring five feeding stamens with yellow anthers and five pollinating stamens with purple anthers (Fig. 1). Feeding stamens were positioned at the centre of the flowers (Fig. 1Aa), whereas the pollinating stamens have anthers that extend downward from the centre (Fig. 1Ab) and have yellow appendages that resemble the yellow anthers of the feeding stamens. The flowers have a single pistil extending downward from the centre and arranged parallel to the pollinating stamens (Fig. 1Ac).

Melastoma has historically been one of the focal species for studies on the division of labour hypothesis in pollination biology because of the distinct floral morphology, which features separate stamens for feeding and pollination (Gross 1993; Gross & Kukuk 2001; Luo et al. 2008). Large flowers of *M. candidum* are particularly suitable for experimental manipulation to investigate the role of stamen morphology and function in pollination biomechanics. Given its common occurrence, ease of observation, and suitability for manipulation, *M. candidum* was chosen as the target species for our study, which aimed to elucidate the role of stamen strength in the biomechanics of pollination. The prominence of this species in the Ryukyu Islands, where our research was conducted, further justifies its selection as the target species for this investigation.

PLANT COLLECTION AND BEE OBSERVATION SITE

In this study, we collected plant material and recorded bee behaviour on Okinawajima Island, located in the middle of the Ryukyu Islands (Fig. 2), from 2015 to 2016 and from 2020 to 2023. The survey sites on Okinawajima Island are listed in Table 1. The Ryukyu Islands, a group of approximately 140 subtropical islands, are located between Japan's Kyushu Island and Taiwan. The unique subtropical climate of these islands fosters a diverse array of ecosystems, which are conducive for the growth of *M. candidum* and a variety of pollinators.

POLLEN MORPHOLOGY

In June 2022, we collected nine flowers, with three flowers from each of the following three sites: Genka, Nago, and Onna, ensuring that each flower was taken from a different individual plant. Pollen

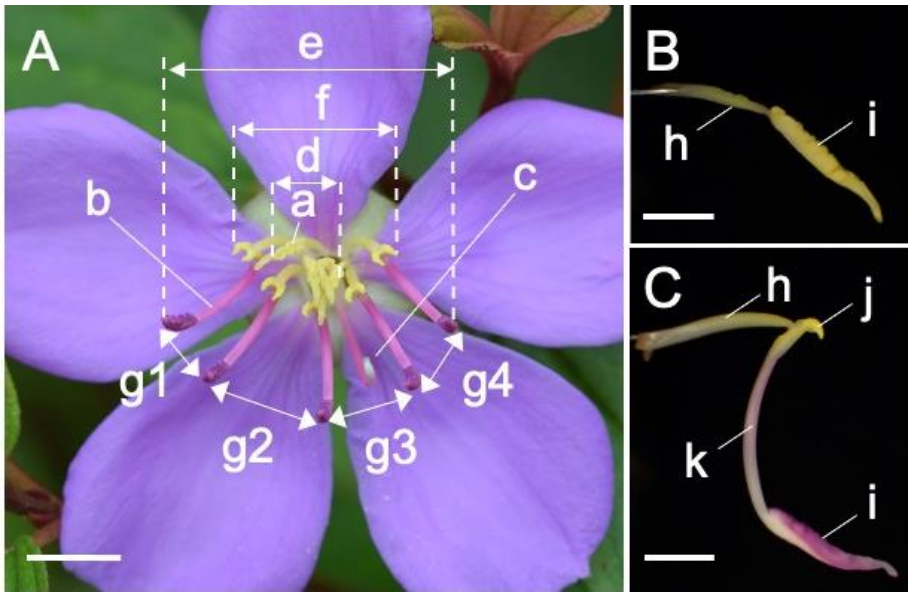

Figure 1. Flower and dimorphic features of stamens of *Melastoma candidum*. A, measured parts of the flower; B, feeding stamen; C, pollinating stamen. a, feeding stamens; b, pollinating stamens; c, pistil; d, distance between outermost anthers of the feeding stamens; e, distance between outermost anthers of the pollinating stamens; f, distance between outermost anther appendages of the pollinating stamens; g1-4, distance of neighbouring anthers of the pollinating stamen; h, filament; i, anther; j, anther appendage; k, anther connective. Scale bars are 1 cm in A, 0.5 cm in B and C.

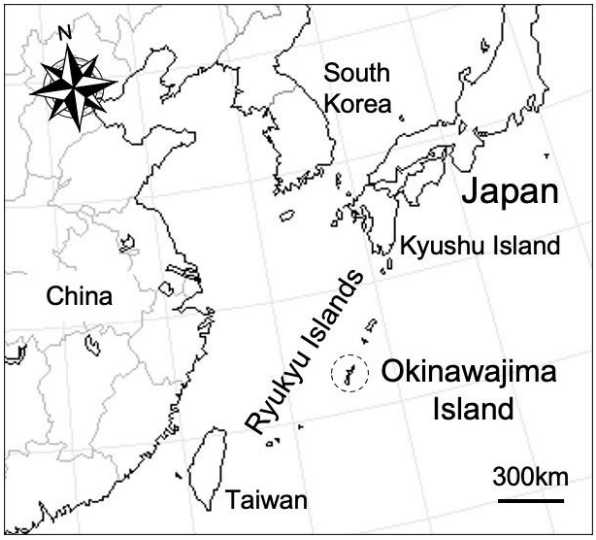

Figure 2. Ryukyu Islands and adjacent regions. Okinawajima Island, the location of the present study, is encircled by the broken line. This blank map was provided by CraftMAP (http://www.craftmap.box-i.net/).

was subsequently extracted from the flowers onto glass slides, stained with an improved Alexander's solution (Peterson et al. 2010), and examined and photographed under an optical microscope. We measured the polar and equatorial diameters of 20 pollen grains from each feeding and pollination stamen of each flower. Nine flowers were examined, with one feeding and one pollination stamen sampled from each flower, yielding a total of 180 pollen grains measured using ImageJ software (version 1.53k) (Schneider et al. 2012). Additionally, the volume of each pollen grain was calculated from the measured polar diameter (a) and equatorial diameter (b) using the following formula according to Pinheiro-Costa et al. (2018): $v = 4/3 \times \pi(a/2)^2 \times (b/2)$ (μm^3).

The differences in the surface texture of pollen from feeding and pollination stamens were assessed using a scanning electron microscope (SEM; JEOL JSM-6060LV). In July 2020, flower buds were collected just before anthesis from three individual plants in Katsuu, Motobu-cho. We collected all the anthers of the feeding and pollination stamens from each flower bud, dissected them in 1.5 mL microtubes containing 70% ethanol to prepare a pollen suspension, and then removed the anthers. The pollen suspension was dehydrated with 100% ethanol. Subsequently, 100% ethanol was replaced sequentially with a mixture of ethanol and t-butanol (ethanol : t-butanol = 2:1, 1:1, 1:2), and finally with 100% t-butanol. The pollen samples were then freeze-dried and mounted on SEM stubs, sputter-coated with gold-palladium using an ion coater. Ten pollen grains from each flower were observed using SEM, and their surface structures were compared.

Pollen fertility

In June 2021, we collected one flower from each of 10 individuals in Kitazato immediately after blooming. From each flower, we harvested one feeding and one pollinating stamen. The pollen grains from each stamen were placed onto separate glass slides, each coated with a 2 mm-thick layer of

Table 1. Location information of survey sites. All survey sites are located on Okinawajima Island, Okinawa Prefecture, Japan.

Survey site	Latitude	Longtitude
Hentona, Kunigami-son	26° 44'48.8" N	128° 10'19.4" E
Kitazato, Motobu-cho	26° 41'32.5" N	127° 54'12.1" E
Katsuu, Motobu-cho	26° 41'07.4" N	127° 54'41.4" E
Genka, Nago-shi	26° 36'31.3" N	128° 05'11.1" E
Nago, Nago-shi	26° 35'23.3" N	128° 00'06.3" E
Onna, Onna-son	26° 28'16.3" N	127° 50'37.4" E
Tancha, Onna-son	26° 28'16.2" N	127° 50'67.6" E
Katsuren-haebaru, Uruma-shi	26° 19'13.9" N	127° 52'59.4" E
Toma, Nakagusuku-son	26° 15'30.2" N	127° 47'43.1" E
Senbaru, Nishihara-cho	26° 14'58.4" N	127° 45'59.6" E

agar medium (1% agarose, 10% sucrose), and incubated for an hour in a moist chamber at 25 °C. After incubation, the slides were stained with improved Alexander's solution. We then observed and recorded the number of germinated pollen grains, counting 1,000 pollen grains (100 per slide from 10 slides) to determine the percentage of pollen grains with germinated pollen tubes for each stamen type.

To investigate potential differences in pollen fertility between feeding and pollinating stamens, we conducted a crossing experiment in Kitazato and Katsuu from June to July 2020 and 2021. The flowers were covered with fine-meshed bags early in the morning before opening to avoid insect visitation. Subsequently, flowers were emasculated. The five treatments included a control treatment without emasculation (Po-A: 101 flowers), cross-pollination with pollen from the feeding stamen (Po-B: 106 flowers) or pollinating stamen (Po-C: 106 flowers) of a different individual, and self-pollination with pollen from the feeding stamen (Po-D: 107 flowers) or pollinating stamen (Po-E: 106 flowers). For these treatments (Po-A to Po-E), five flowers from each individual were used, and each flower was subjected to one of the five treatments. Flowers for these treatments were selected from different individuals, ensuring that each flower came from a unique individual plant, thus maintaining the independence of the samples. Additionally, for the Po-F treatment (35 flowers), a separate set of 35 plants was selected in 2021 only, where flowers were bagged without any treatment to confirm the presence or absence of autonomous self-pollination. After pollination, all flowers, except those in the Po-A treatment, were bagged again to exclude the effects of visiting insects. Approximately 20 days after the treatments, fruits were harvested, and the fruit set (%) was calculated as the number of fruits divided by the number of treated flowers multiplied by 100.

Flower morphology

The flower parts used for measurements are shown in Fig. 1A. We measured morphological traits, including the distance between anthers of the outermost feeding stamens (Fig. 1Ad), the distance between the anthers of the outermost pollinating stamens (Fig. 1Ae) and the distance between the outermost anther appendages of the pollinating stamens (Fig. 1Af) of the 69 flowers collected from Nago and Onna in June 2023. Each of these 69 flowers was collected from a different plant to ensure independence of the samples. Additionally, we measured the distance between adjacent pollinating stamen anthers (Fig. 1Ag) for 20 flowers collected from Kitazato in 2022. All measurements were conducted immediately after field collection.

Pollinator behaviour on the two types of stamens

Over the course of 159 h, we observed and recorded the species of flower-visiting insects and their behaviour at four different sites: Katsuu, Nago, Tancha, and Senbaru during 2015, 2016, 2020, and 2021. The observations at each survey site were opportunistic, with approximately equal observation times. These observations were conducted only during the daytime because *M. candidum* flowers are one-day blooms that open in the morning and wilt by evening. Insect species that briefly landed on the corolla without contacting stamens or pistils were excluded. For unidentified species, specimens were collected for species identification and stored in the zoological collection room of the Faculty of Science at the University of the Ryukyus.

Flower visitation and stigma contact frequencies among native bee species were assessed using video recordings of 29 flowers from different individual plants immediately after blooming in Kitazato, Katsuu, and Nago in 2020 and 2021. Each flower was observed for 3.0 h between 8:00 and 12:00 (Japan Standard Time). We recorded the position and number of bee visits to individual flowers and calculated the frequency of flower visits for each bee species as visits per hour per flower (v/h/f). The data from these 29 flowers were averaged for each bee species. In addition, we recorded the stigma contact rate for each bee species as the percentage of visits that resulted in stigma contact. Finally, we determined the stigma contact frequency as contacts per hour per flower (c/h/f) for each bee species by multiplying flower visit frequency by stigma contact rate.

To further investigate the influence of different stamens on primary pollinator behaviour, we conducted an emasculation experiment following the methodology described by Luo et al. (2008). Surveys were conducted at Kitazato, Katsuu, and Nago between June and July 2020 and 2021. On

each survey day, flower buds that were about to bloom were covered with bags. After blooming, we conducted the following five treatments, each applied to five flowers from 29 different individual plants: no treatment (Em-A, control); the removal of pollinating or feeding stamens, leaving only feeding or pollinating stamens, respectively (Em-B and Em-C, respectively); the removal of both feeding and pollinating stamen anthers, leaving only pollinating stamen appendages (Em-D); and the removal of all stamens (Em-E). Each flower from each individual plant was subjected to one of the five treatments, ensuring that the treatments were consistently applied across different flowers. Flower-visiting insects were prevented from accessing the flowers by covering them with bags after the treatment until observations began. Observations were conducted for 3 h per flower, between 8:00 and 12:00. The number of visits and positions of the primary pollinators on the treated flowers were recorded. Data from the 29 flowers in each treatment group were averaged for analysis.

Pollen removal by the primary pollinator

To quantify pollen loss during visitation by the primary pollinator, we evaluated the pollen remaining in anthers after visitation. Initially, we determined the number of pollen grains per intact anther in both the feeding and pollinating stamens using the method described by De Luca et al. (2013). In July 2021, we collected one flower bud from each of the 10 individuals at Katsuu. For each of these 10 flower buds, we removed all the anthers. The collected anthers of the feeding and pollinating stamens were then separately dissected in 1.5 mL microtubes containing 700 μL of 10% sucrose solution to prepare a pollen suspension, which was stirred to disperse the pollen evenly. Subsequently, the pollen count per microlitre of suspension was determined using a haemocytometer (Sunlead Glass, modified Neubauer counting chamber). This value was multiplied by the original suspension volume (700 μL) to calculate the number of pollen grains in each feeding and pollinating stamen per individual flower and then averaged across 10 flowers.

Next, we assessed the amount of pollen grains remaining after a single visit by the primary pollinator. In July 2021, we enclosed 12 flower buds, each from a different individual plant on the verge of blooming in Kitazato, within bags. The bags were gradually removed after the flowers had completely bloomed. Once the primary pollinator visited each flower, we collected the flowers and removed the anthers. Using the same method as that used for intact anthers, we quantified the pollen grains from the collected anthers and averaged the counts across 12 flowers.

Additionally, we calculated the percentage of pollen grains remaining on each anther after visitation by the primary pollinator using the number of pollen grains in the intact feeding and pollinating stamens as the baseline counts (100%).

Bee size and stamen strength

To compare the behaviour of bee species during flower visitation, we assessed the body size of native bee species and the support strength provided by feeding and pollinating stamens. Thoracic width was measured for 22 *Xylocopa flavifrons*, 13 *Amegilla urens*, 20 *Amegilla dulcifera*, and 24 *Ceratina okinawana* specimens stored in the Zoological Collection Room of the Faculty of Science, University of the Ryukyus, according to the taxonomy of Tadauchi & Murao (2014). Additionally, we collected 18, 17, and 20 numbers of *X. flavifrons*, *A. dulcifera*, and *C. okinawana*, respectively, from various locations on Okinawajima Island, between May and December 2021. Some of the bees were preserved as specimens, whereas others were released after weighing. The force (N) exerted by the weight of the bee was calculated using the formula proposed by Kobayashi et al. (2018): Force (N) = body weight (g) × 0.0098. Because of the limited number of specimens, the weight and force of *A. urens* could not be determined.

Subsequently, we evaluated the force supported by stamens of *M. candidum* using 12 flowers from 7 individuals collected in Senbaru. The pedicels were immersed in water immediately after collection, and within 20 min, the force supported by each flower was measured (Fig. 3). One feeding stamen and one pollinating stamen were retained from each flower, and the remaining stamens and pistils were removed. The flowers were then fixed on a table. A digital force gauge (Imada, Japan; DS2-5 N; resolution: 0.001N) with a gauge length of 5 mm was used to measure the force exerted by the anthers of both feeding and pollinating stamens, as well as the appendage of

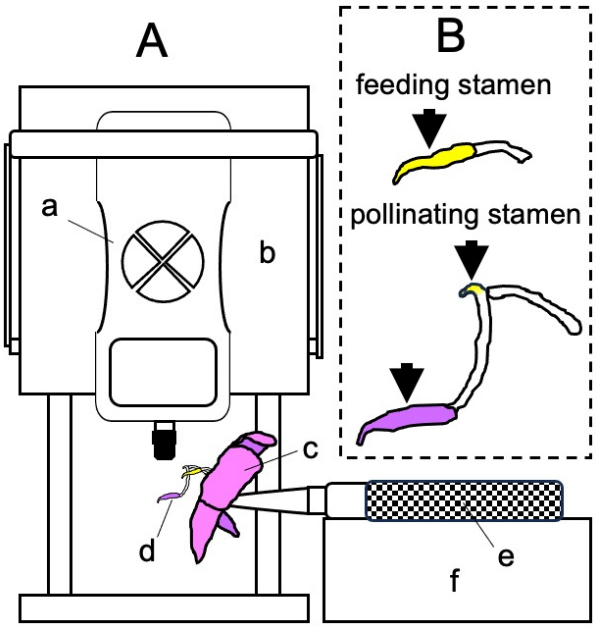

Figure 3. Strength measurement for the stamens of *Melastoma candidum* using a digital force gauge (IMADA, DS2-5). A, installation status of digital force gauge and flower during measurement; B, specific points on each stamen where the force gauge was applied during measurement (arrows). a, digital force gauge; b, digital force gauge stand; c, flower of *M. candidum*; d, stamens; e, plier for fixing a flower; f, pedestal.

the pollinating stamen when pushed back to prevent contact with petals.

STATISTICAL ANALYSIS

All statistical analyses were conducted using R (version 4.3.3). The following packages were utilised: 'lme4' for linear mixed-effects models (LMM), 'lmerTest' for a type III analysis of variance (ANOVA), 'FSA' for Dunn's test, and 'stats' for general statistical tests including Wilcoxon rank-sum test, Kruskal-Wallis test, Fisher's exact test, Holm correction, and Bonferroni correction.

1) *Statistical analyses considering sample independence*

POLLEN MORPHOLOGY: We analysed the effect of stamen type on pollen size (polar diameter, equatorial diameter, and volume) using LMM. The model included pollen size as the response variable and stamen type (feeding or pollinating) as fixed effects. Random intercepts were included for both individuals and flowers to account for variability among individuals and flowers. The model was fitted using the restricted maximum likelihood (REML) method. The significance of the fixed effects was assessed using ANOVA with Satterthwaite's approximation for degrees of freedom.

BEE SIZE AND STAMEN STRENGTH: To assess differences in force among the three stamen types (feeding stamen, pollinating stamen, and the appendage of the pollinating stamen), we employed LMM analysis with the model specified as "force ~ stamen-type + (1 | individual)". The significance of the stamen-type effect was evaluated using an ANOVA performed on the fitted LMM model. Post hoc pairwise comparisons were conducted using Dunn's test, with p-values adjusted for multiple comparisons using the Bonferroni correction. Bonferroni correction was applied using the p.adjust function from the 'stats' package.

2) *Statistical analyses not considering sample independence*

POLLEN FERTILITY: Due to the small sample size and non-normal distribution of the pollen germination rate data, the Wilcoxon rank-sum test was used to compare germination rates between feeding and pollinating stamens. For the crossing experiment, Fisher's exact test was used to analyse differences in fruit set percentages among the various treatments. The Holm correction was used to adjust for multiple comparisons.

POLLINATOR BEHAVIOUR ON THE TWO TYPES OF STAMENS: We compared flower visitation frequencies among bee species using the Kruskal-Wallis test to determine overall differences. If significant differences were found ($P < 0.05$), the Steel-Dwass multiple comparison test was used for pairwise differences. Stigma contact rates were analysed using Fisher's exact test. The Holm correction was applied to adjust for multiple comparisons.

Statistical analyses comparing flower visit frequencies among the five emasculation treatments used the Kruskal-Wallis test to determine overall differences, followed by the Steel-Dwass multiple comparisons test for pairwise differences if significant results were found. The methods applied were similar to those used for analysing differences in the flower visiting frequencies among bee species.

POLLEN REMOVAL BY THE PRIMARY POLLINATOR: The Wilcoxon rank-sum test was employed to compare pollen counts in intact anthers of feeding stamens

with those in pollinating stamens and to compare pollen counts in these stamens after visitation by the primary pollinator. This approach assessed the significant differences in pollen removal.

Results

Pollen morphology

The pollen grains of both feeding and pollinating stamens of *M. candidum* were oblong in shape (Fig. 4A, B). The feeding stamens exhibited mean dimensions (± standard error (SE)) of 21.90 ± 1.07 μm for equatorial diameter, 27.07 ± 1.91 μm for polar diameter, and a mean volume of 8476.32 ± 395.23 μm^3. In contrast, pollinating stamens had mean ± SE dimensions of 21.64 ± 1.01 μm for equatorial diameter, 26.49 ± 1.42 μm for polar diameter, and a mean volume of 7980.64 ± 272.11 μm^3.

Both LMM and ANOVA revealed that the measured dimensions of feeding stamens were significantly greater than pollinating stamens across all traits (Fig. 5). Specifically, the LMM analysis showed that feeding stamens had a larger equatorial diameter (Estimate = 0.2531, SE = 0.1005, t = 2.517, P = 0.0123), polar diameter (Estimate = 0.5879, SE = 0.1536, t = 3.828, P = 0.000153), and pollen volume (Estimate = 495.674, SE = 113.207, t = 4.378, P = 1.58e-05). The ANOVA results confirmed these findings, with F-values of F(1, 350) = 6.337, P = 1.23e-02 for equatorial diameter, F(1, 350) = 14.651, P = 1.53e-04 for polar diameter, and F(1, 350) = 19.171, P = 1.58e-05 for pollen volume. These results highlight the significant influence of stamen type on these morphological characteristics in *M. candidum*. Additionally, the surface texture of pollen of both feeding and pollinating stamens exhibited a wrinkled pattern on the surface, with no obvious differences in morphology between them (Fig. 4C, D).

Pollen fertility

Pollen germination rates were comparable between the feeding (median, 64.2%; interquartile range, 4.7%) and pollinating stamens (median: 63.0%, interquartile range: 5.4%) (Wilcoxon rank-sum test; $P > 0.05$). In the cross-breeding experiment, the fruit set (%) was as follows: 54.5% in the control Po-A treatment group, 50.0 and 58.5% in the cross-pollination Po-B and Po-C treatment groups, 35.5 and 53.8% in the self-pollination Po-D and Po-E treatment groups, respectively, and 0.0% in the autonomous self-pollination group (Po-F) (Fig. 6). Fruit set (%) was lower in the Po-D treatment group, wherein pollen from its feeding stamen was used, than in the Po-C treatment group, wherein pollen from an external pollinating stamen was used. However, no significant differences were observed between the other treatments, except for the Po-F treatment (Fisher's exact test; $P < 0.01$).

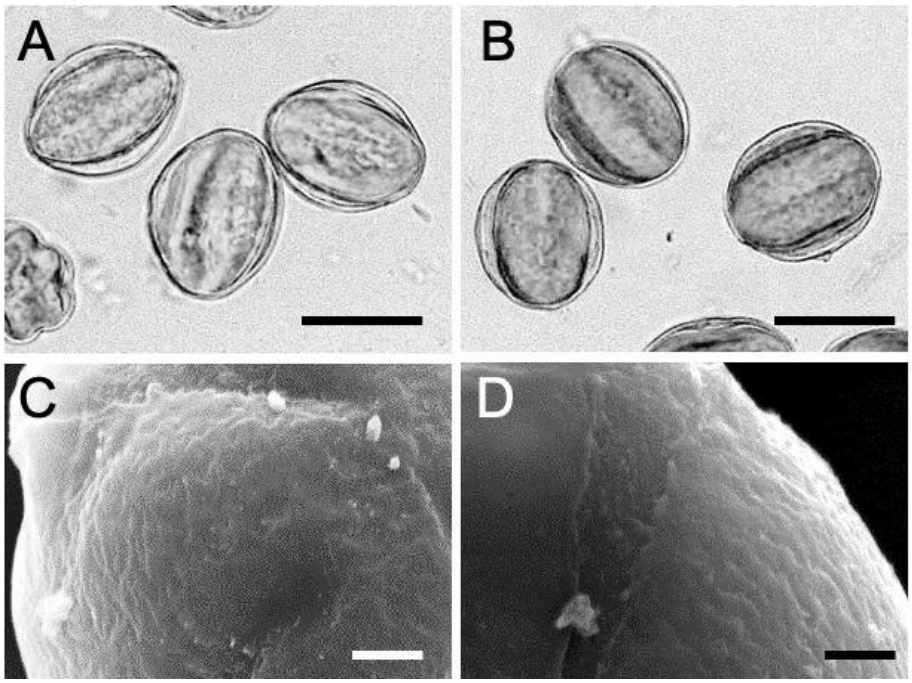

Figure 4. Morphology of pollen collected from feeding and pollinating stamens of *Melastoma candidum*. A and B, light microscopic images of pollens from feeding and pollinating stamens, respectively; C and D, scanning electron microscope images of the surface texture of pollen from feeding and pollinating stamens, respectively. Scale bars in A and B are 20 μm, and in C and D are 2 μm.

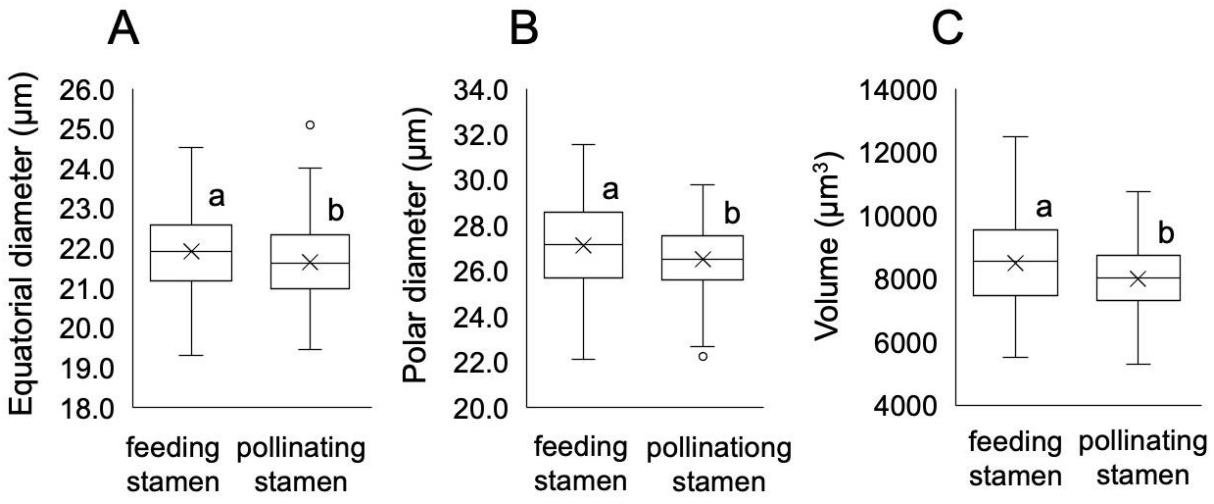

Figure 5. Box plots comparing the morphological traits of pollen grains between feeding and pollinating stamens of *Melastoma candidum*. The traits analysed include (A) equatorial diameter, (B) polar diameter, and (C) pollen volume. The plots display the median, quartiles, and outliers (○) for each trait. The mean is shown as an '×', and lowercase letters above the boxes indicate statistical significance (LMM; P < 0.05, ANOVA; P < 0.05).

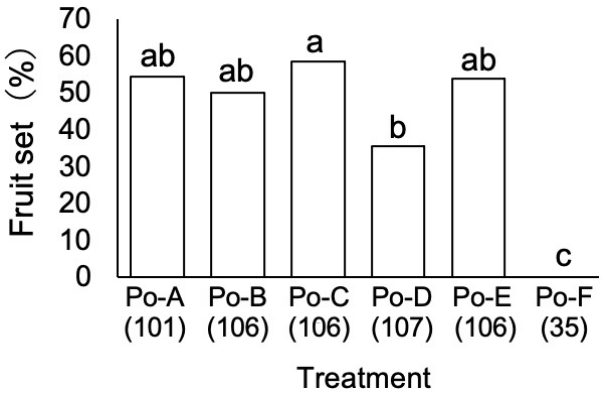

Figure 6. Fruit set (%) of *Melastoma candidum* across six treatments of artificial crossing experiments. Po-A, control experiment without any treatment; Po-B, cross-pollination with pollen from another individual's feeding stamen; Po-C, cross-pollination with pollen from another individual's pollinating stamen; Po-D, self-pollination with pollen from the same plant's feeding stamen; Po-E, self-pollination with pollen from the same plant's pollinating stamen; Po-F, autonomous self-pollination. Numbers in the parentheses indicate the number of flowers used for each treatment. Different lowercase letters above bars denote significant differences between them (Fisher's exact test; *P* < 0.05).

Flower morphology

The distance between the outermost anthers of the feeding stamens averaged 7.62 ± 0.32 mm (mean ± standard error, S.E.), whereas, in the case of the pollinating stamens, it was 32.45 ± 0.62 mm, with 9.24 ± 0.5 mm between adjacent pollinating stamen anthers. The width between the outermost appendages of the pollinating stamens measured 15.01 ± 0.29 mm (Table 2).

Pollinator behaviour on the two types of stamens

The observed flower-visiting insects included five bee species from the family Apidae: *Amegilla dulcifera*, *A. urens*, *Apis mellifera*, *Ceratina okinawana*, and *Xylocopa flavifrons*. Since *A. mellifera* is an invasive species and cannot buzz-pollinate, it was excluded from subsequent observations. Distinct flower visitation behaviour was observed among the bee species (Table 3; Fig. 7). *Xylocopa flavifrons* primarily targeted feeding stamens (including pollinating stamen appendages) and was engaged in buzzing (Fig. 7A), with visitation frequencies of 99.8 and 0.2% for feeding and pollinating stamens, respectively (Table 3). Conversely, when *X. flavifrons* visited pollinating stamens, they attempted to grasp them, but consistently slipped without buzzing. *Amegilla urens* also targeted and buzzed several feeding stamens (Fig. 7B), with visitation frequencies of 95.1 and 4.9% for feeding and pollinating stamens, respectively (Table 3). In contrast, *A. dulcifera* preferred pollinating stamens, with 68.0, 31.2, and 0.8% visitation frequencies for pollinating stamens, feeding stamens, and petals, respectively, often holding one or two pollinating stamens alongside several feeding stamens during buzzing (Fig. 7C, D). When these

Table 2. Distance between the outermost anthers of feeding and pollinating stamens and distance between adjacent anthers of pollinating stamens.

	Distance between	
Stamen type	Outermost anthers (mm)	Adjacent anthers (mm)
feeding stamen anther	7.62 ± 0.32	-
pollinating stamen anther	32.45 ± 0.62	9.24 ± 0.5
pollinating stamen appendage	15.01 ± 0.29	-

Numerical values represent the mean ± standard error.

three bee species buzzed on feeding stamens, pollen from the pollinating stamens spread and adhered to their dorsal surface (Fig. 7A). Unlike other species, *C. okinawana* did not buzz, but probed for pollen with its proboscis by biting a hole in the anther with its mandible, primarily targeting the pollinating stamens (Fig. 7E, F). The visitation frequencies of *C. okinawana* were 1.5, 17.1, and 81.4% for petals, feeding stamens, and anthers of pollinating stamens, respectively (Table 3).

Among the four native bee species, *X. flavifrons* demonstrated the highest frequency of flower visitation, averaging 7.1 ± 0.9 v/h/f (mean ± S.E.), followed by *C. okinawana* at 2.3 ± 0.5 v/h/f. The *Amegilla* bee species *A. dulcifera* and *A. urens* exhibited lower visitation frequencies of 1.4 ± 0.4 v/h/f and 0.9 ± 0.5 v/h/f, respectively (Fig. 8). Analysis using the Kruskal-Wallis test showed significant differences in flower visit frequencies among the four bee species ($H = 44.666$, degrees of freedom, $DF = 3$, $P < 0.01$). Subsequent Steel-Dwass multiple comparison tests indicated significant differences in flower visit frequencies between *X. flavifrons* and the other three species ($P < 0.01$). Additionally, a statistically significant difference was observed between *C. okinawana* and *A. urens* ($P < 0.05$) but not between *C. okinawana* and *A. dulcifera* or *A. dulcifera* and *A. urens* ($P > 0.05$).

Stigma contact rates varied among bee species, with *X. flavifrons* showing the highest rate (98.5%) (Table 4; Fig. 9). *Amegilla urens*, *A. dulcifera*, and *C. okinawana* exhibited stigma contact rates of 53.7, 14.4, and 1.0%, respectively (Table 4; Fig. 9). Significant differences were observed in all pairwise comparisons between the four bee species (Fisher's exact test; $P < 0.01$). Moreover, *X. flavifrons* demonstrated the highest frequency of stigma contact at 7.0 c/h/f, followed by *A. urens* at 0.5 c/h/f, *A. dulcifera* at 0.2 c/h/f and *C. okinawana* at 0.02 c/h/f (Table 4).

The flower visitation frequency of *X. flavifrons*, which exhibited the highest flower visitation frequency and stigma contact rate among the four native bee species for flowers subjected to emasculation treatment, is shown in Fig. 10. The Kruskal-Wallis test revealed significant differences among the emasculation treatments ($H = 63.053$; $DF = 4$; $P < 0.01$). Subsequent pairwise multiple comparisons using the Steel-Dwass test indicated no significant difference between untreated flowers (Em-A) (7.1 ± 0.9 v/h/f) and flowers in which only the pollinating stamens were removed and only feeding stamens were retained (Em-B) (7.3 ± 1.3 v/h/f) ($P > 0.05$). However, flower visitation frequency significantly decreased (2.4 ± 0.4 v/h/f) for treatments retaining only pollinating

Table 3. Percentage of visitation places on *Melastoma candidum* flowers by four different bee species.

	Percentage of flower-visiting places (%)		
Bee species	Feeding stamen anthers and pollinating stamen appendages	Pollinating stamen anthers	Petal
Xylocopa flavifrons	99.8	0.2	0.0
Amegilla urens	95.1	4.9	0.0
Amegilla dulcifera	31.2	68.0	0.8
Ceratina okinawana	17.1	81.4	1.5

Figure 7. Pollen collecting behaviour of four native bee species on the flower of *Melastoma candidum*. A, *Xylocopa flavifrons* on feeding stamens and pollinating stamen's appendages; B, *Amegilla urens* on feeding stamens and pollinating stamen's appendages; C, *Amegilla dulcifera* on pollinating stamens; D, *A. dulcifera* on feeding stamens and pollinating stamen's appendages; E, *Ceratina okinawana* on pollinating stamens; F, holes on the anther of the pollinating stamen made by *C. okinawana*. Arrows indicate the stigma.

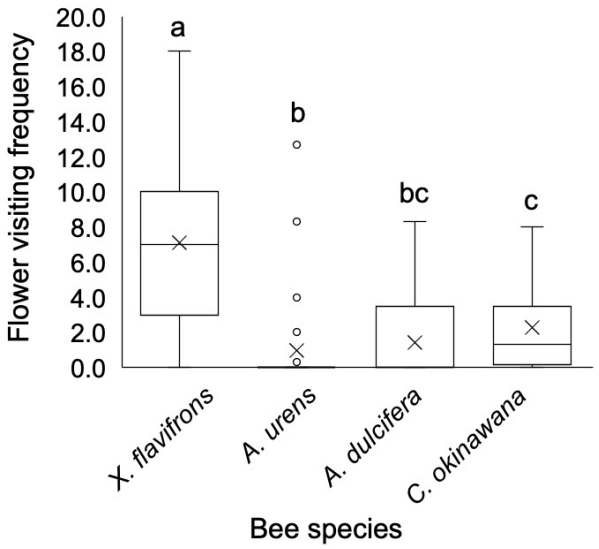

Figure 8. Box plots showing the flower visiting frequency of four native bee species to the flowers of *Melastoma candidum*. The plots display the median, quartiles, and outliers (○) for each group. The mean is indicated with an ×, and lowercase letters above the boxes indicate statistical significance (Steel-Dwass multiple comparison test, $P < 0.01$).

stamens (Em-C), and for treatments retaining only the appendages of pollinating stamens (Em-D, 2.3 ± 0.5 v/h/f) compared with that for the Em-A and Em-B treatments ($P < 0.01$). The Em-E treatment, with all stamens removed, exhibited the lowest flower visitation frequency of 0.4 ± 0.1 v/h/f. Treatments that retained feeding stamens (Em-A and Em-B) showed higher visitation frequencies than those in which feeding stamens were removed (Em-C, Em-D, and Em-E) ($P < 0.01$).

The flower visitation behaviour of *X. flavifrons* for each emasculation treatment is shown in the bottom part of Fig. 10. In untreated flowers (Em-A), bee visits to feeding stamens were 99.8%, with only 0.2% visits to pollinating stamens. For the treatments in which only pollinating stamens were removed (Em-B), all bee visits (100%) were on feeding stamens. In treatments that removed only feeding stamens (Em-C), 98.1% of visitations were on the appendages of pollinating stamens, 1.4% on the anthers of pollinating stamens, and 0.5% on the petals. In treatments where only the appendages of pollinating stamens remained (Em-D), 99.0% of visitations were on the appendages, 0.5% on the petals, and 0.5% on the pistil. For treatments in which all stamens were removed (Em-E), 54.8% of visitations were on the petals, and 45.2% were on the pistil.

Pollen removal by the primary pollinator

Pollen removal by the primary pollinator *X. flavifrons* was assessed by examining the number of pollen grains per anther and post-visitation count (Table 5). Pollinating stamens harboured significantly more pollen grains than feeding stamens, with counts of 273,887 ± 27,216 and 167,307 ± 18,530 (mean ± S.E.), respectively (Wilcoxon rank-sum test; $P < 0.01$). After a single visit by *X. flavifrons*, the counts were 180,293 ± 11,342 for pollinating stamens and 62,997 ± 8,585 for feeding stamens (Wilcoxon rank-sum test; $P < 0.01$). The residual pollen on the anthers of the pollinating stamens was 65.8%, and that on feeding stamens was 37.7%.

Bee size and stamen strength

The thoracic width and weight of each bee species is listed in Table 6. The width of *X. flavifrons* was 8.02 ± 0.09 mm (mean ± S.E.), whereas *A. dulcifera*, *A. urens*, and *C. okinawana* exhibited widths of 4.88 ± 0.09 mm, 5.18 ± 0.08 mm, and 2.30 ± 0.04 mm, respectively. The weights of *X. flavifrons*, *A. dulcifera*, and *C. okinawana* were 0.540 ± 0.021 g, 0.132 ± 0.010 g, and 0.019 ± 0.001 g, respectively. The force (N) values for *X. flavifrons*,

Table 4. Contribution of flower visiting bees to pollination of *Melastoma candidum*.

Bee species	Flower visiting frequency【A】 (visits/hour/flower)	Contact rate to stigma【B】 (%)	Stigma contact frequency【A×B】 (contacts/hour/flower)
Xylocopa flavifrons	7.1 ± 0.9	98.5	7.0
Amegilla urens	0.9 ± 0.5	53.7	0.5
Amegilla dulcifera	1.4 ± 0.4	14.4	0.2
Ceratina okinawana	2.3 ± 0.5	1.0	0.02

Numerical values for flower visiting frequency represent the mean ± standard error.

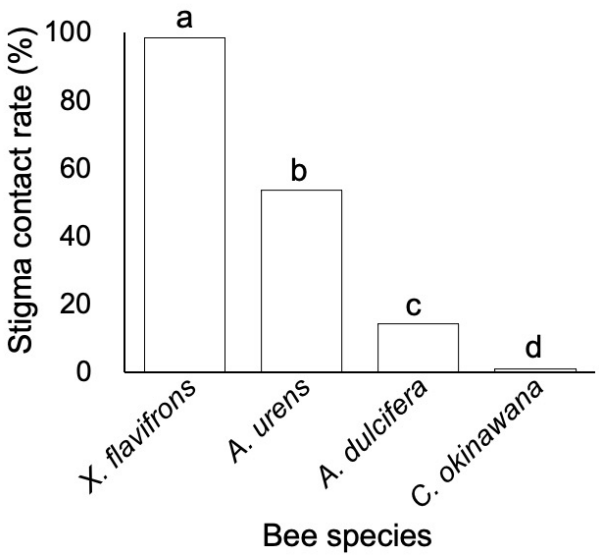

Figure 9. Stigma contact rate (%) of four native bee species. Different lowercase letters above bars indicate there are significant differences in stigma contact rates between them (Fisher's exact test; *P* < 0.01).

A. dulcifera, and *C. okinawana* were 0.0053 ± 0.0002 N, 0.0013 ± 0.0001 N, and 0.0002 ± 0.00001 N, respectively, and were calculated based on their weights.

According to the LMM analysis, pollinating stamen appendages exhibited the highest strength at 0.0123 ± 0.001 N (mean ± S.E.), compared to the strengths of the feeding stamen anthers (0.0057 ± 0.0005 N) and pollinating stamen anthers (0.0027 ± 0.0005 N) (Table 6).

Stamen strength among the three stamen types differed significantly according to the ANOVA performed on the fitted LMM ($F(2, 28.91) = 78.313$, $P < 0.001$). Following ANOVA, pairwise comparisons were conducted using Dunn's test with Bonferroni correction to identify specific differences between stamen types. Significant differences were observed between the

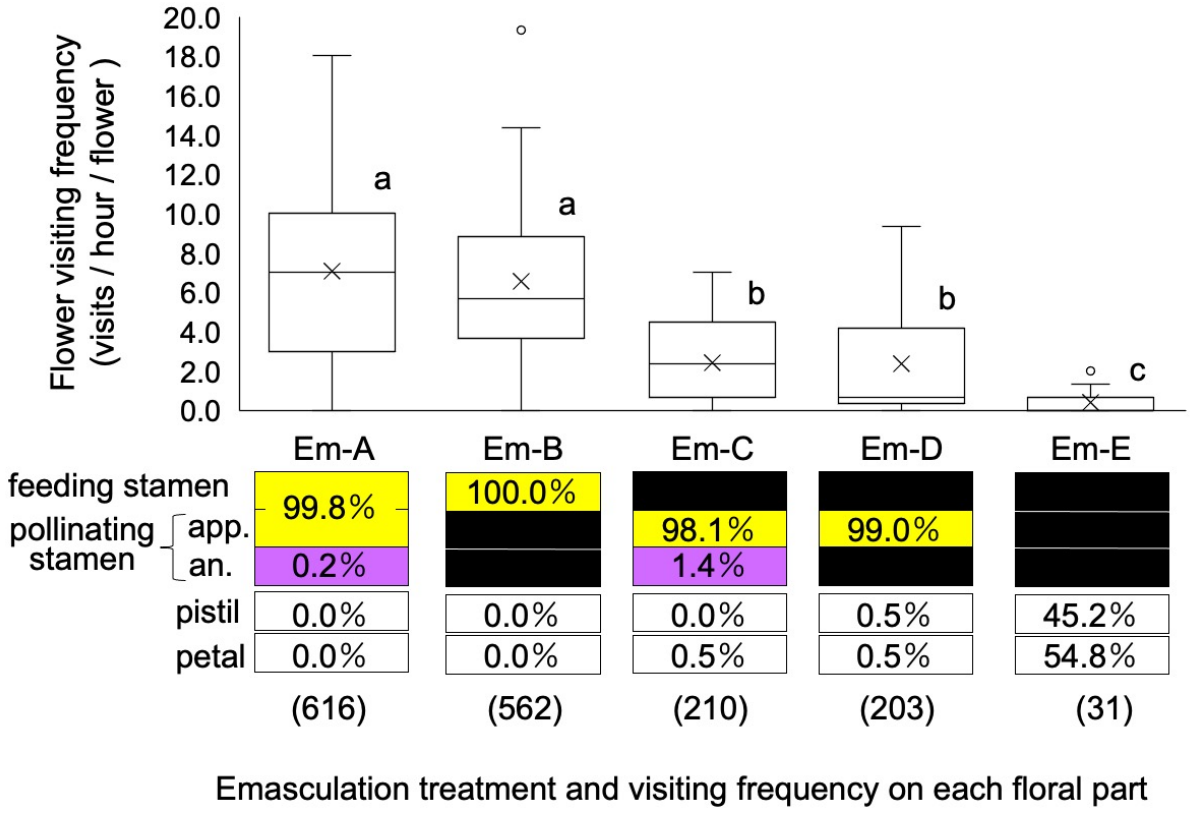

Figure 10. Box plots showing the flower visiting frequency of *Xylocopa flavifrons* for each of the five treatments in the emasculation experiments. The treatments are: Em-A, no-emasculation (control); Em-B, removal of pollinating stamens leaving only feeding stamens; Em-C, removal of feeding stamens leaving only pollinating stamens; Em-D, removal of both feeding and pollinating stamens leaving only the appendages of pollinating stamens; Em-E, removal of all stamens. The plots display the median, quartiles, and outliers (○) for each treatment. The mean is indicated with an '×', and lowercase letters above the boxes denote significant differences between treatments (Steel-Dwass multiple comparison test, p < 0.01). The percentage of visits to different flower parts and the number of visits observed for each treatment are tabulated below the graph. app. refers to appendages, and an. refers to anthers. In each treatment, black boxes indicate the removed parts.

Table 5. Number of pollen grains per anther before and after a single flower visit of *Xylocopa flavifrons*.

	Number of pollen per anther		Percentage of remaining pollen
Stamen type	Before a visitation	After a visitation	(%)
feeding stamen	167307 ± 18530	62997 ± 8585	37.7
pollinating stamen	273887 ± 27216	180293 ± 11342	65.8

Numerical values in the table represent the mean ± standard error.

pollinating stamen appendage and both the feeding stamen anther (Z = 2.833, *P_adj* = 0.0138) and the pollinating stamen anther (Z = 5.550, *P_adj* = 8.583e-08). Additionally, a significant difference was observed between the feeding stamen anther and pollinating stamen anther (Z = 2.716, *P_adj* = 0.0198).

DISCUSSION

Four native bee species from the family Apidae visited *M. candidum* flowers on Okinawajima Island, Japan. Among them, *X. flavifrons* exhibited the highest flower visitation frequency at an average of 7.1 ± 0.9 v/h/f, along with the highest contact rate with the stigma of 7.0 c/h/f, establishing it as the primary pollinator of *M. candidum* on Okinawajima Island. *Bombus* species often serve as primary pollinators of *Melastoma* (Luo et al. 2008; Peng et al. 2012; 2014) in other regions, but they are absent on Okinawajima Island (Tadauchi & Murano 2014). *Xylocopa* bees are the primary pollinators of other Melastomataceae species, with large flowers (Abe 2006; Luo et al. 2008; Peng et al. 2012; 2014). Although *Xylocopa* has been reported as a minor pollinator of *Melastoma* in certain regions (Huang et al. 2021), it is one of the most common pollinators of *Melastoma* species.

Xylocopa flavifrons collected pollen by holding onto all feeding stamens and vibrating their bodies. During this process, pollen from the anthers of the feeding stamens is deposited on the ventral side of the bee, while pollen from the pollinating stamens is deposited on the dorsal side, as the pollinating stamen appendages are also grasped by the bee while buzzing all feeding stamens. According to Mesquita-Neto et al. (2018), bee visitors to plants with poricidal anthers are categorised into three types, with *X. flavifrons* falling into the 'flower buzzing bee' category, where large bees grasp a single position on the flower and vibrate entire flower. In contrast, the other three bee species exhibited different behaviours. Similar to *X. flavifrons, A. urens* also visited feeding stamens and occasionally utilised pollinating stamens for buzz pollination. However, owing to its relatively small size and infrequent visits, the contact rate of *A. urens* with the stigma was less than that of *X. flavifrons,* and its contribution to the pollination of *M. candidum* was, therefore, relatively less. According to our observations, *A. dulcifera* frequently grasped and buzzed pollinating stamens, resembling the

Table 6. Thorax width, weight, bee strength estimated from body mass of the bees, and stamen strength.

Bee species and stamen type	Thorax width (mm)	Body mass (g)	Strength (N)
bee species			
Xylocopa flavifrons	8.02 ± 0.09	0.540 ± 0.021	0.0053 ± 0.0002
Amegilla urens	5.18 ± 0.08	-	-
Amegilla dulcifera	4.88 ± 0.09	0.132 ± 0.010	0.0013 ± 0.0001
Ceratina okinawana	2.30 ± 0.04	0.019 ± 0.001	0.0002 ± 0.00001
feeding stamen anther	-	-	0.0057 ± 0.0005
pollinating stamen anther	-	-	0.0027 ± 0.0005
pollinating stamen appendage	-	-	0.0123 ± 0.001

Numerical values in the table represent the mean ± standard error.

behaviour of 'anther buzzing bees' as recognised by Mesquita-Neto et al. (2018), but exhibited a low contact rate with the stigma, limiting its contribution to *M. candidum* pollination. Contrarily, based on a comprehensive survey of numerous plants, *A. dulcifera* was reported as a pollinator of *M. candidum* on Amami Oshima Island (Kato 2000). Although another *Amegilla* bee species is considered the primary pollinator of *M. affine* (Gross & Kukuk 2001), the visitation of *A. dulcifera* to *M. candidum* was mainly for obtaining pollen without contact with stigmas.

Foraging behaviour of *C. okinawana* differed from the other three bee species, as this species mostly visited pollinating stamens but did not buzz. Instead, it created a hole in the anther using its mandibles, inserted its proboscis, and collected the pollen. However, its low contact rate with stigmas indicated a minimal contribution to *M. candidum* pollination. Renner (1989) reported that specific vibrations of 420 Hz or higher generated by buzzing bees are needed to release pollen from the poricidal anthers of the family Melastomataceae. However, several cases of pollen theft by bees that do not perform buzz pollination have been reported (Renner 1983; Gross 1993; Luo et al. 2008). For example, *Trigona* bees probe for collecting pollen from the apical pores of poricidal anthers and cut off the top of these anthers to expose relatively more pollen (Renner 1983). *Ceratina okinawana* created a hole in the middle of the anther with its mandibles and probed for pollen with its proboscis. Despite its small size (< 1 cm) (Tadauchi & Murao 2014), *C. okinawana* excavates nests in the pith of broken or burnt twigs and stems (Sakagami & Laroca 1971; Negoro 1980), which enables it to bite holes in the anthers. Therefore, *C. okinawana* does not exert efficient pollination mechanisms of *M. candidum*.

When *X. flavifrons* collected pollen from the flowers of *M. candidum* by vibrating feeding stamens, the pollen from the pollinating stamens adhered to its dorsal surface, which was relatively less accessible during grooming. This mechanism was similar to that observed in *M. malabathricum* (Luo et al. 2008). In *M. malabathricum*, most of the pollen grains adhering to visiting insects and stigmas originated from pollinating stamens, a conclusion drawn from the difference in the pattern on the pollen surface of the feeding and pollinating stamens (Luo et al. 2008). Although there were a few differences in the pollen size of *M. candidum*, we could not identify sufficient distinctions to differentiate the origin of the pollen found on the bee bodies or on the stigmas. Direct evidence that the pollen used for pollination comes from the pollinating stamens is not available; however, it is believed that *M. candidum* employs the same pollination mechanism as *M. malabathricum* because the stigma comes into contact with the dorsal side of the abdomen of *X. flavifrons* covered with pollen from the pollinating stamens. Therefore, it can be assumed that a division of labour exists between the two types of stamens in *M. candidum*.

Pollen germination tests and cross-pollination experiments revealed no differences in pollen germination or fruit set rates between feeding and pollinating stamens of *M. candidum*, indicating comparable pollen fertility between the two stamen types. Variations in pollen fertility among dimorphic stamens have been documented for several plant species (Nepi et al. 2003; Paulino et al. 2016; Pinheiro-Costa et al. 2018). In the genus *Melastoma*, *M. malabathricum* displays lower pollen fertility for pollen in its feeding stamens than for those in its pollinating stamens (Percival 1965; Luo et al. 2008); however, *M. affine* and *Microlicia cordata* exhibit no disparity in pollen fertility between pollen in their feeding and pollinating stamens (Gross & Kukuk 2001; Velloso et al. 2018). In the case of *M. affine*, its primary pollinator, *Amegilla anomala*, collects the pollen from both feeding and pollinating stamens, with the pollen of both stamen types being utilised for pollination and reward, implying less clear division of labour between the two stamen types (Gross & Kukuk 2001). However, in this study, *X. flavifrons*, the primary pollinator of *M. candidum*, displayed a 99.8% probability of collecting pollen from feeding stamens. In addition, emasculation experiments confirmed the higher visitation rates of *X. flavifrons* to flowers with feeding stamens (Em-A and Em-B) than to flowers without feeding stamens (Em-C, Em-D and Em-E). Considering that the presence or absence of pollinating stamens did not affect the visitation frequency of *X. flavifrons* (Em-A vs. Em-B and Em-C vs. Em-D), it is evident that this species selectively utilises the feeding stamens of *M. candidum*. The consistent preference of *X. flavifrons* for collecting pollen from feeding

stamens across visits indicates that pollen collected from feeding stamens serves as a reward. Although it cannot be ruled out that some pollen from pollinating stamens may occasionally be groomed and used as a reward by the bees, pollen from pollinating stamens is primarily utilized for pollination. This establishes a division of labor in *M. candidum*.

These findings suggest that *X. flavifrons* prefers feeding on stamens with yellow anthers accompanied by yellow appendages of pollinating stamens when visiting *M. candidum*. Yellow anthers attract flower visitors to specific sites on the flower (Ushimaru et al. 2007; Telles et al. 2020). For instance, in *Microlicia cordata* (Melastomataceae), the strong contrast created by the yellow anthers of feeding stamens against purple petals renders them appealing to flower visitors (Velloso et al. 2018). In addition, it has been proposed that *Xylocopa* bees are drawn to feeding on stamens because of the yellow colour of the anthers in *M. malabathricum* (Luo et al. 2008). The removal of feeding stamens with yellow anthers in emasculation experiments (Em-C and Em-D) led to *X. flavifrons* visiting the yellow appendages of pollinating stamens relatively more frequently, suggesting the importance of yellow colouration in attracting bees. The yellow appendages of the pollinating stamens, devoid of pollen, attract *X. flavifrons*, supporting the idea that the yellow colour mimics pollen, thus attracting insects to the flowers (Buchmann 1983; Lunau et al. 2024).

Xylocopa flavifrons visited *M. candidum* frequently, averaging 7.1 v/h/f. After one visit, only 37.7% of the pollen remained in the feeding stamens, indicating the rapid depletion of pollen from the feeding stamens after blooming, serving as a reward for pollinators. In contrast, pollinating stamens retained a significant amount of pollen (65.8%) even after a visit of *X. flavifrons*. It seems feasible to utilise this remaining pollen for pollination by attracting bees with the yellow colour of both feeding stamen anthers and pollinating stamen appendages.

The poor contrast between the purple colour of the anthers and the petals in *Microlicia cordata* has been suggested to contribute to the avoidance of the pollinating stamens during bee visitations (Velloso et al. 2018). In this study, however, *A. dulcifera* and *C. okinawana* exhibited a stronger preference for the pollinating stamens of *M. candidum* than for the feeding stamens. Although *X. flavifrons* showed a notably low preference, it did not entirely avoid the exertion of pollinating stamens. However, when *X. flavifrons* attempted to visit the pollinating stamens, it consistently slipped off without effectively grasping the anthers. These observations suggest that in addition to colour, other factors may influence the behaviour of *X. flavifrons* to avoid utilising pollinating stamens, such as difficulties in grasping the pollinating stamens effectively during visitations.

The difference in the supporting force between feeding and pollinating stamens likely plays a crucial role in determining the consistent flower-visiting behaviour of *X. flavifrons* bees. Although a single pollinating stamen exerts insufficient force to support the weight of *X. flavifrons*, both feeding stamens and the appendages of pollinating stamens can withstand relatively more force. Due to their clustered arrangement around the flower centre, the wider thorax of *X. flavifrons* compared to other bee species allows it to grasp all feeding stamens at once. However, the widely spaced arrangement and weaker supporting forces of the pollinating stamens pose significant biomechanical challenges for *X. flavifrons* when utilising these stamens for pollen gathering. Consequently, the anthers of feeding stamens and the appendages of pollinating stamens provide a stable foothold for *X. flavifrons* for efficient pollen collection by buzzing, reinforcing the division of labour between stamens in *M. candidum*. These findings underscore the importance of biomechanical factors in influencing pollinator behaviour and stamen function, offering new insights into the mechanisms driving the division of labour in plant-pollinator interactions.

The inconsistent flower-visiting behaviour of *A. dulcifera* and *C. okinawana* is believed to be due to reasons other than those observed for *X. flavifrons*. The weights of these two bees were less, and the force of the pollinating stamens of *M. candidum* is therefore adequate to support these bees. These species can use feeding and pollinating stamens but prefer the latter, which contains relatively more pollen. Our results indicate that buzzing of feeding stamens by *X. flavifrons* leaves relatively more pollen in pollinating stamens for pollination.

It is speculated that *A. dulcifera* and *C. okinawana* visit pollinating stamens relatively more often because more pollen is available even after *X. flavifrons* bees have buzzed the feeding stamens. To validate this hypothesis, it would be necessary to investigate whether these bees probe the flowers to check if pollen is available. While these bees may occasionally contribute to the pollination of *M. candidum*, their low contact rate with the stigma suggests that their overall contribution is limited. Moreover, their tendency to collect a higher proportion of pollen from the pollinating stamens may potentially hinder *M. candidum* by reducing the efficiency of its pollination process.

ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to Professor Masatsugu Yokota, Associate Professor Atsuko Tanaka, and Assistant Professor Shun Kobayashi from the Faculty of Science at the University of the Ryukyus for their guidance and suggestions in conducting this study. We are also deeply grateful to Professor Yuichi Hirose from the Faculty of Science at the University of the Ryukyus for generously lending us the experimental equipment. We extend our sincere appreciation to Mr. Sadao Nakata and Mr. Hidezo Sakihama who kindly allowed us to conduct our surveys on their private properties. Finally, we express our gratitude to Dr. Yoko Yamaguchi, and Mr. Ryoma Ariyoshi for their invaluable advice and cooperation throughout the study.

AUTHOR CONTRIBUTION

SH and TD designed the study. SH and MU collected and analysed the data. SH and TD wrote first drafts of sections of the paper, all contributed to the final manuscript.

DISCLOSURE STATEMENT

The authors declare that they have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

DATA AVAILABILITY STATEMENT

The data used to write this article are available as Appendices.

APPENDICES

Additional supporting information may be found in the online version of this article:

Table S1. Diameters, and volume of pollen grains.

Table S2. Germination rate (%) of pollen grains.

Table S3. Number of pollen grains per intact anther.

Table S4. Number of pollen grains remaining after a single visit by *Xylocopa flavifrons*.

Table S5. Thoracic width of each bee species.

Table S6. Body weight of each bee species.

REFERENCES

Abe T (2006) Threatened pollination systems in native flora of the Ogasawara (Bonin) Islands. Annals of Botany 98: 317-334. https://doi.org/10.1093/aob/mcl117

Åstrand J, Knight C, Robson J, Talle B, Wilson ZA (2021) Evolution and diversity of the angiosperm anther: trends in function and development. Plant Reproduction 34: 307-319. https://doi.org/10.1007/s00497-021-00416-1

Barrett SC (2021) Heteranthery. Current Biology 31: R774-R776. https://doi.org/10.1016/j.cub.2021.03.040

Brito VLG, Fendrich TG, Smidt EC, Varassin IG, Goldenberg R (2016) Shifts from specialised to generalised pollination systems in Miconieae (Melastomataceae) and their relation with anther morphology and seed number. Plant Biology 18: 585-593. https://doi.org/10.1111/plb.12432

Brito VLG, Rech AR, Ollerton J, Sazima M (2017) Nectar production, reproductive success and the evolution of generalised pollination within a specialised pollen-rewarding plant family: a case study using *Miconia theizans*. Plant Systematics and Evolution 303: 709-718. https://doi.org/10.1007/s00606-017-1405-z

Brito VLG, Leite FB, Jorge LR, Sazima M (2021) Distinct pollen release dynamics between stamens generate division of labour in pollen flowers of two *Pleroma* species (Melastomataceae). Flora 285: 151961. https://doi.org/10.1016/j.flora.2021.151961

Buchmann SC (1983) Buzz pollination in angiosperms. In: Eugene CE, Little RJ (eds) Handbook of experimental pollination biology. Van Nostrand Reinhold Company Inc., New York, pp 73-114.

Buchmann SL, Buchmann MD (1981) Anthecology of *Mouriri myrtilloides* (Melastomataceae: Memecyleae), an oil flower in Panama. Biotropica: 7-24. https://doi.org/10.2307/2388066

Buchmann SL, Hurley JP (1978) A biophysical model for buzz pollination in angiosperms. Journal of Theoretical Biology 72: 639-657. https://doi.org/10.1016/0022-5193(78)90277-1

Cardinal S, Buchmann SL, Russell AL (2018) The evolution of floral sonication, a pollen foraging behavior used by bees (Anthophila). Evolution 72: 590-600. https://doi.org/10.1111/evo.13446

Darwin C (1862) Letter to Asa Gray. Darwin Correspondence Project, "Letter no. 3404". [online] URL: https://www.darwinproject.ac.uk/letter/?docId=letters/DCP-LETT-3404.xml

De Luca PA, Bussière LF, Souto-Vilaros D, Goulson D, Mason AC, Vallejo-Marín M (2013) Variability in bumblebee pollination buzzes affects the quantity of pollen released from flowers. Oecologia 172: 805-816. https://doi.org/10.1007/s00442-012-2535-1

Falcão BF, Schlindwein C, Stehmann JR (2016) Pollen release mechanisms and androecium structure in *Solanum* (Solanaceae): Does anther morphology predict pollination strategy? Flora 224: 211-217. https://doi.org/10.1016/j.flora.2016.08.001

Gross CL (1993) The breeding system and pollinators of *Melastoma affine* (Melastomataceae); a pioneer shrub in tropical Australia. Biotropica: 468-474. https://doi.org/10.2307/2388870

Gross CL, Kukuk PF (2001) Foraging strategies of *Amegilla anomola* at the flowers of *Melastoma affine* -no evidence for separate feeding and pollinating anthers. Acta Horticulturae 561: 171-178. https://doi.org/10.17660/ActaHortic.2001.561.25

Hatusima S (1975) Flora of the Ryukyus, added and corrected. Okinawa association of biology education (in Japanese).

Hatusima S, Amano T (1994) Flora of the Ryukyus, south of Amami Island. Biological Society of Okinawa (in Japanese).

Huang JCC, Hsieh YC, Lu SS, Yeh WC, Liang JY, Lin CJ, Tung GS (2021) Flower-visiting insects of genus *Melastoma* (Myrtales: Melastomataceae) at the Fushan Botanical Garden, Taiwan. Biodiversity Data Journal 9: e60315. https://doi.org/10.3897/BDJ.9.e60315

Islam MSU, Abid R, Qaiser M (2008) Anther types of dicots within flora of Karachi, Pakistan. Pakistan Journal of Botany 40(1): 33-41.

Kato M (2000) Anthophilous insect community and plant-pollinator interactions on Amami Islands in the Ryukyu Archipelago, Japan. Contributions from the Biological Laboratory, Kyoto University 29: 157-252.

King MJ, Buchmann SL (2003) Floral sonication by bees: mesosomal vibration by *Bombus* and *Xylocopa*, but not *Apis* (Hymenoptera: Apidae), ejects pollen from poricidal anthers. Journal of the Kansas Entomological Society 76: 295-305.

King MJ, Buchmann SL, Spangler H (1996) Activity of asynchronous flight muscle from two bee families during sonication (buzzing). Journal of Experimental Biology 199: 2317-2321. https://doi.org/10.1242/jeb.199.10.2317

Kobayashi S, Hirose E, Denda T, Izawa M (2018) Who can open the flower? Assessment of the flower opening force of mammal-pollinated *Mucuna macrocarpa*. Plant Species Biology 33: 312-316. https://doi.org/10.1111/1442-1984.12221

Koch L, Lunau K, Wester P (2017) To be on the safe site–Ungroomed spots on the bee's body and their importance for pollination. PLoS ONE 12: e0182522. https://doi.org/10.1371/journal.pone.0182522

Liu SC, Wen HH, Chen MY, Yang JT (2008) Study on pollination ecology of four species of Melastomataceae in Taiwan. Formosan Entomologist 28: 67-85 (in Chinese).

Lunau K, Piorek V, Krohn O, Pacini E (2015) Just spines - mechanical defense of malvaceous pollen against collection by corbiculate bees. Apidologie 46: 144-149. https://doi.org/10.1007/s13592-014-0310-5

Lunau K, Brito VLG, Camargo MGG (2024) Pollen, anther, stamen, and androecium mimicry. Plant Biology 26: 349-368. https://doi.org/10.1111/plb.13628

Luo Z, Zhang D, Renner SS (2008) Why two kinds of stamens in buzz-pollinated flowers? Experimental support for Darwin's division-of-labor hypothesis. Functional Ecology 22: 794-800. https://doi.org/10.1111/j.1365-2435.2008.01444.x

Marazzi B, Conti E, Endress PK (2007) Diversity in anthers and stigmas in the buzz-pollinated genus *Senna* (Leguminosae, Cassiinae). International Journal of Plant Sciences 168(4): 371-391. https://doi.org/10.1086/512105

Mesquita-Neto JN, Blüthgen N, Schlindwein C (2018) Flowers with poricidal anthers and their complex interaction networks - Disentangling legitimate pollinators and illegitimate visitors. Functional Ecology 32: 2321-2332. https://doi.org/10.1111/1365-2435.13204

Müller H (1881) Two kinds of stamens with different functions in the same flower. Nature 24: 307-308. https://doi.org/10.1038/024307c0

Müller F (1883) Two kinds of stamens with different functions in the same flower. Nature 27: 364-365. https://doi.org/10.1038/027364b0

Negoro H (1980) Ecological studies of *Ceratina* bees in Japan. I. Interspecific relationship of three *Ceratina* species on the campus of Kanazawa University. Japanese Journal of Ecology 30: 259-265.

Nepi M, Guarnieri M, Pacini E (2003) "Real" and feed pollen of *Lagerstroemia indica*: ecophysiological differences. Plant Biology 5: 311-314. https://doi.org/10.1055/s-2003-40797

Pacini E, Franchi GG (2020) Pollen biodiversity–why are pollen grains different despite having the same function? A review. Botanical Journal of the Linnean Society 193: 141-164. https://doi.org/10.1093/botlinnean/boaa014

Paulino JV, De Freitas Mansano V, Prenner G (2016) Evidence for division of labor and division of function related to the pollen release in Papilionoideae (Leguminosae) with a heteromorphic androecium. International Journal of Plant Sciences 177: 590-607. https://doi.org/10.1086/687351

Peng DH, Lan SR, Wu SS (2012) Studies on pollination biology of *Melastoma sanguineum* Sims (Melastomataceae). Journal of Tropical and Subtropical Botany 20: 618-625. https://doi.org/10.3969/j.issn.1005-3395.2012.06.016

Peng DH, Lan SR, Wu SS (2014) Pollination biology and breeding system of *Melastoma dendrisetosum*. Forest Research *27*(1): 11-16.

Percival MS (1965) Floral biology. Pergamon Press.

Peterson R, Slovin JP, Chen C (2010) A simplified method for differential staining of aborted and non-aborted pollen grains. International Journal of Plant Biology 1: e13. https://doi.org/10.4081/pb.2010.e13

Pinheiro-Costa BK, Mesquita-Neto JN, Rego JO, Schlindwein C (2018) Trade off between quantity and size of pollen grains in the heterandrous flowers of *Senna pendula* (Fabaceae). Acta Botanica Brasilica 32: 446-453. https://doi.org/10.1590/0102-33062018abb0132

Praz CJ, Müller A, Dorn S (2008) Specialized bees fail to develop on non-host pollen: do plants chemically protect their pollen. Ecology 89: 795-804. https://doi.org/10.1890/07-0751.1

Renner SS (1983) The widespread occurrence of anther destruction *Trigona* bees in Melastomataceae. Biotropica 15: 257-267. https://doi.org/10.2307/2387649

Renner SS (1989) A survey of reproductive biology in Neotropical Melastomataceae and Memecylaceae. Annals of the Missouri Botanical Garden 76: 496-518. https://doi.org/10.2307/2399497

Sakagami SF, Laroca S (1971) Observation on the bionomics of some neotropical Xylocopine bees, with comparative and biofaunistic notes (Hymenoptera, Anthophoridae). Journal of the Faculty of Science Hokkaido University series VI. Zoology 18: 57-127. [online] URL: https://eprints.lib.hokudai.ac.jp/dspace/bitstream/2115/27518/1/18(1)_P57-127.pdf

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9: 671-675. https://doi.org/10.1038/nmeth.2089

Tadauchi O, Murao R (2014) An illustrated guide to Japanese bees. Bun-ichi Sogo Shuppan (in Japanese).

Telles FJ, Klunk CL, Maia FR da, De Brito VLG, Varassin IG (2020) Towards a new understanding of the division of labour in heterantherous flowers: the case of *Pterolepis glomerata* (Melastomataceae). Biological Journal of the Linnean Society 131: 1-11. https://doi.org/10.1093/biolinnean/blaa107

Thorp RW (2000) The collection of pollen by bees. Plant Systematics and Evolution 222: 211-223. https://doi.org/10.1007/BF00984103

Trevizan R, Gaetano APS, Brito VLG, Oliveria PE, Telles FJ (2023) Stamen and pollen heteromorphism linked to the division of labour in Melastomataceae species. Flora Flora 305: 152315. https://doi.org/10.1016/j.flora.2023.152315

Ushimaru A, Watanabe T, Nakata K (2007) Colored floral organs influence pollinator behavior and pollen transfer in *Commelina communis* (Commelinaceae). American Journal of Botany 94: 249-258. https://doi.org/10.3732/ajb.94.2.249

Vallejo-Marín M, Manson JS, Thomson JD, Barrett SCH (2009) Division of labor within flowers: Heteranthery, a floral strategy to reconcile contrasting pollen fates. Journal of Evolutionary Biology 22: 828-839. https://doi.org/10.1111/j.1420-9101.2009.01693.x

Vallejo-Marín M, Da Silva EM, Sargent RD, Barrett SCH (2010) Trait correlates and functional significance of heteranthery in flowering plants. New Phytologist 188: 418-425. https://doi.org/10.1111/j.1469-8137.2010.03430.x

Velloso M de SC, Brito VLG de, Caetano APS, Romero R (2018) Anther specializations related to the division of labor in *Microlicia cordata* (Spreng.) Cham. (Melastomataceae). Acta Botanica Brasilica 32: 349-358. https://doi.org/10.1590/0102-33062017abb0358

Westerkamp C (1996) Pollen in bee-flower relations, some considerations on melittophily. Botanica Acta 109: 325-332. https://doi.org/10.1111/j.1438-8677.1996.tb00580.x

Westerkamp C (1997) Flowers and bees are competitors - not partners. Towards a new understanding of complexity in specialised bee flowers. Acta Horticulturae 437: 71-74. https://doi.org/10.17660/ActaHortic.1997.437.5

 ISSN 1920-7603