POLLINATION, MATING SYSTEM, PHENOLOGY AND CHARACTERISATION OF *MEDINILLA MULTIFLORA* MERR. (MELASTOMATACEAE) ON MT MAKILING, PHILIPPINES

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ABSTRACT

An investigation into the reproductive biology of *Medinilla multiflora* Merr. (Melastomataceae) from Mt Makiling, Luzon, is presented. This includes a morphological and distributional examination of the population on the mountain, the documentation of reproductive phenological patterns, a study of the mating system and observations of biotic interactions. Measurements were made of trait variability, reproductive phenology was characterised from field and herbarium observations, stigmatic receptivity was tested by counting pollen germination, insect exclusion and hand-pollination experiments helped determine the mating system and field observations recorded the identity and behaviour of floral visitors. Significant reproductive morphological differences were found between described populations. This identified a need for the recognition of this diversity and further delimitation of the *Medinilla multiflora* species complex. Although *Medinilla multiflora* produced flowers and fruit year-round, the population also exhibited cycles of increased reproduction most likely initiated by seasonal low temperatures. *Medinilla multiflora* was found to require pollination but not cross-pollination. Two major peaks in stigmatic receptivity occurred throughout the day and the majority of pollen was found to be viable. Generalist bees proved to be the primary pollinators of the study population and were most active in the morning depending on weather conditions. Generalist pollinators and self-compatibility are advantageous traits for establishment and persistence in isolated mountain habitats; however, losses of this habitat due to climate change could have profound consequences for the future success of *Medinilla multiflora*.

INTRODUCTION

Medinilla Gaudich. ex DC. is a large palaeotropical genus spanning from West Africa (*M. manii* Hook.f.) to American Samoa (*M. samoensis* (Hochr.) Christoph.). Its three greatest centres of diversity are Madagascar, the Philippines and New Guinea (Regalado, 1995; Bodegom & Veldkamp, 2001). *Medinilla* inflorescences and fruit are often quite showy, and several species are commonly cultivated. Despite the widespread distribution of the genus, its prominence in many floras and it being of ornamental interest, relatively little research has focused on the group.

One of the most common *Medinilla* species found on Mt Makiling, an isolated mountain on the largest Philippine island of Luzon, is *M. multiflora* Merr. There, the species can be distinguished from other *Medinilla* species by its opposite, sessile leaves and pyramidal inflorescences which lack large bracts (Fig. 1). Mt Makiling's population

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of *M. multiflora* was originally described as *M. myriantha* Merr. but it and five other taxa (*M. camiguinensis* Merr., *M. canlaonensis* Merr., *M. negrosensis* Merr., *M. confusa* Merr. and *M. vulcanica* Merr.) from different localities within the Philippines were proposed as synonyms of *M. multiflora* by Regalado (1995). Other morphologically similar taxa are numerous and include *M. apayaoensis* Merr., *M. brevipes* Merr., *M. calelanensis* Elmer, *M. cumingii* Naudin, *M. formosana* Hayata, *M. hayatana* H.Keng, *M. intermedia* Blume, *M. pendula* Merr., *M. speciosa* Blume and *M. tayabensis* Merr., likely making *M. multiflora* representative of a sizable portion of the genus. Characterisation of the study population was performed to describe the traits present in this particular study, which will be helpful if similar studies are carried out in the future. Local distribution of the taxon was determined to help define the niche and inform the success of the species under future climate predictions.

The sole published study on *Medinilla* seasonality took place over five years on Mt Kinabalu, Borneo (Kimura *et al.*, 2009), and followed eight species, including *M. speciosa*, a species morphologically quite similar to *M. multiflora*. It showed that the number of species flowering at a given time increased biannually, indicating that two seasons a year were favourable for reproduction in some taxa (including *M. speciosa*). The study also suggested that flowering of *Medinilla* species was regulated by seasonal low temperatures. This study investigated whether these observations were also found on Mt Makiling.

Fig. 1 *Medinilla multiflora* in the mossy forest on Mt Makiling. Photo: Peter Quakenbush.

With regard to phenology, Clausing (2000) found that, in the related genus *Pachycentria* Blume, one to several flowers opened per day on an inflorescence, depending on the species. *Pachycentria constricta* Blume flowered for several months with inflorescences lasting two to six weeks. Fruit ripening took between four and eight weeks. The flowering system resulted in unripe fruit on the same inflorescence as buds, but fruit did not ripen until flowering was completed on the inflorescence. Observations of phenology in *Medinilla* are lacking, and this study addressed this.

Stigmatic receptivity is also an aspect of floral phenology and indicates when a flower is ready for pollination. Additionally, it can help determine the target pollinators, since they should also be active during this period. Such data has not previously been reported for *Medinilla* and was investigated as part of this study.

The particular mating system of a species affects its population size and genetic structure (Paggi *et al.*, 2015). The mating system can also be useful in the study of systematics (Anderson *et al.*, 2002; Andrade *et al.*, 2007), evolutionary theory, conservation and ecology. For instance, according to Baker's law, species like *Medinilla multiflora*, with isolated populations and a wide distribution, are often self-compatible (Baker, 1955). Self-incompatibility and deleterious genes would have been selected against in these populations and, though the populations are likely to be inbred, they may not suffer greatly from inbreeding depression. Baker's law is perhaps overly simplistic but it still holds some predictive value (Cheptou, 2012). Also, most self-compatible species have a mixed mating system, especially animal-pollinated species (Paggi *et al.*, 2015). The mating system of *Medinilla multiflora* is documented here and further informs these theories.

Pollen viability can also be an indication of inbreeding depression and population genetic health (Melser *et al.*, 1999). Pollen viability has not previously been reported for any *Medinilla* species and the results from a small, isolated mountaintop population of plants likely susceptible to inbreeding would be of special interest. These are presented here.

Most species in Melastomataceae have the buzz-pollination syndrome. Flowers are visited by bees that vibrate (or sonicate) the poricidal anthers to expel pollen and gather it as a food source (Renner, 1993; Larson & Barrett, 1999). Therefore, bees are cited in the literature as the probable pollinators of *Medinilla* species (Regalado, 1990; Tobe *et al.*, 1989). Kimura *et al.* (2009) reported seeing stingless bees (*Trigona sp.*) infrequently on *Medinilla* flowers on Mt Kinabalu but Renner (1983) found these small bees to be pollen thieves rather than pollinators of melastome flowers. Observations as part of this study provide information on *Medinilla* pollinators and their methods.

All these aspects of the reproductive biology of a population of *Medinilla multiflora* provide a knowledge base that helps understand patterns and interactions of a representative *Medinilla* taxon. Beyond autecology, these can inform conservation actions related to this large genus. The data can also be compared with other taxa to test for the generality of these findings and help develop questions and hypotheses to be further investigated.

MATERIALS AND METHODS

Site and timetable

All experiments and observations were carried out on healthy, mature, naturally occurring individuals of *Medinilla multiflora*, a species common in the mossy forest of Mt Makiling, Laguna, Philippines (as documented by Merrill, 1906; Brown, 1919; Gruezo, 1997 and Pancho & Gruezo, 2006). Observations and collections were primarily conducted from Peak 2 (14°08'09.5"N 121°11'37.9"E), at an elevation of about 1,060 m asl, or from the trail leading up to Peak 2, from February 2015 to February 2017.

Population description

Measurements of the hypanthium, petals, stamens and style were performed on at least 30 fresh flowers – ten flowers each from three individuals. These measurements should be comparable to previous measurements since reproductive material such as petals and anthers is delicate and must be measured when fresh or rehydrated (pers. obs.; Regalado, 1990). Other measurements and characterisations were largely attained by non-destructive methods in the field. *Quakenbush 40* (LBC) was collected as a voucher specimen.

Local distribution

Local distribution of the *Medinilla multiflora* population on Mt Makiling was determined by recording the elevation of the first occurrence of an individual along the trail to Peak 2, the general frequency of encountered individuals at particular elevations and the elevation at which a reproductive individual was first noted.

Reproductive phenology

Regular field observations along with herbarium specimens helped determine the seasonality and flowering and fruiting patterns of *Medinilla multiflora* on Mt Makiling. Photographs were taken of the same stand of *Medinilla multiflora* on Peak 2 at least monthly to document changes during the study period. Collections were examined for the presence of flowers or fruit from the Philippine National Herbarium (PNH) and the University of the Philippines Los Baños Museum of Natural History (CAHUP and LBC), as well as photographs of specimens held at the Royal Botanic Gardens, Kew (K), the New York Botanical Garden (NY) and the United States National Herbarium (US). Anthesis was determined through hourly photographs (04:00–18:00) of an inflorescence on Peak 2 and stigmatic receptivity testing (described below).

Stigma receptivity

Stigma receptivity was first tested by placing the stigma in hydrogen peroxide following Dafni (1992). Bubbling indicated receptivity. This was performed on three stigmas at 08:00 and 14:00, and on one-day-old stigmas. Again, Dafni was followed to determine the time the stigma was most receptive. Flowers were sampled per hour, from 06:00 to 17:00 over the course of three days. Six flowers were sampled each hour from 06:00 to 14:00. From 15:00 to 17:00, only three flowers were sampled. Semi-solid agar medium was prepared a day in advance. A stigma was removed and mashed on a microscope slide, then mixed with the gel. Pollen from the anthers was released into the mixture by buzzing the anthers with a tuning fork. The pollen was allowed to germinate for at least one day. Stigma receptivity was determined by counting the number of germinated pollen grains in five views under a microscope. In order to ensure that views of slides were in areas with adequate pollen and stigma mixing, germinated pollen was first located and then counts were performed in the same view.

Mating system

To test for self-pollination or agamospermy in *Medinilla multiflora*, six mature inflorescences from different plants were selected. Flowers and developing fruits were removed to leave only buds. Each inflorescence was bagged to exclude insects and observed weekly until completion of the experiment. Bags were 60 cm long and 30 cm in diameter and were constructed from 1 mm mesh size tulle fabric.

Three additional bagged inflorescences were induced to self-pollinate. Upon each visit, open flowers were hand-pollinated using a tuning fork (A-440 Hz). Once struck, the vibrating tool was held against the anthers several times to ensure sufficient amounts of pollen expulsion. Care was taken to wipe the instrument between uses. Counts of flowers pollinated and developing fruit were recorded weekly. An unbagged inflorescence was observed as the control.

Pollen viability

Dafni (1992) was followed for the pollen viability assessment. Three plants were chosen from each of which ten flowers were collected. These were kept cool or frozen until examination. Two anthers from each flower were crushed with a drop of lactophenol aniline blue on a slide and observed under a microscope. Viable pollen turned blue, while non-viable pollen remained transparent. Counts from five views on each slide were recorded.

Floral visitors

Floral visitor observations were performed at least weekly from September 2015 to November 2015. One overnight visit covered the earliest and latest hours of the day.

The presence of a particular species engaged in pollinating activities was noted for each observation session within a time period (hour of the day). Video and photographs (Canon PowerShot SX700 HS, 4.5–135.0 mm) aided the documentation.

RESULTS

Medinilla multiflora *population description*

Terrestrial or epiphytic shrub to 4 m tall; main stem to 8 cm diameter; bark reddish brown, deeply grooved; young stems terete to slightly ridged, green; nodes setose, often becoming glabrous. Leaves opposite or very rarely in threes, sessile or rarely subsessile, ovate, elliptic or orbicular, 5–7-plinerved, sometimes with very faint additional nerves at the base. Leaf to 21.5 × 12.5 cm, averaging 12 × 7 cm; transverse veins visible but not prominent. Inflorescence a pyramidal determinate thyrse, semi-erect, both axillary (42%) and terminal (58%; n=300), 11–32 × 6–32 cm, averaging 18 × 14 cm, bearing several hundred flowers (up to 670 buds observed); peduncle 4–11 cm long, branches usually in whorls of four, though sometimes more, inflorescence typically seven-tiered; bracts and bracteoles typically deciduous and minute, sometimes to 4 × 2 cm, persistent; young inflorescence green, turning whitish pink with flowers, then magenta or reddish with fruit. Flowers 4-merous, though some 5-merous (4.3%; n=415). Pedicel 3.5–11.5 mm long, usually 7 mm long. Hypanthium 4–5 × 3–4 mm, campanulate; limb c. 1.5 mm long. Petals rotate, 6.5–13 × 5.5–10 mm, usually c. 9 × 7.5 mm, pale to dark pink, obliquely obovate (butterfly wing shaped), imbricate in the floral bud, not observed to produce nectar. Stamens twice as many as petals, epipetalous and alternipetalous, homomorphic, c. 7 mm long; filaments 3–5 mm long, usually 4 mm, whitish, flattened; anthers with an apical pore, crowded together below the style, 2–4 mm, usually 3 mm, violet; appendages yellow, consisting of two thick ventral appendages and a slight dorsal spur, all 1 mm or less. Style filiform, sigmoid, 6–7.5 mm long, typically 6.5 mm, whitish; stigma punctate. Developing fruit magenta to reddish, ripe fruit subglobose, slightly wider than long, 5.5–10 × 5.5–9 mm, soft, juicy, purplish black, pericarp thin; stalks 5–14 mm long, averaging 8.5 mm. Seeds numerous (hundreds per fruit), minute, placentation axile.

Distribution on Mt Makiling

On the trail to Peak 2, the first observed occurrence of *Medinilla multiflora* was an individual at about 670 m asl. It did not flower during the period of observation. The next occurrence, and first occurrence of a flowering individual, was at about 850 m asl. Above about 900 m asl, occurrences became nearly continual. The peak area (about 1,060 m asl) was covered in dense stands of *Medinilla multiflora*.

Reproductive phenology

Medinilla multiflora is not distinctly seasonal on Mt Makiling, as evidenced by 35 available collections made from 1906 to 2012. Collections with both flowers and fruit existed for every month of the year except September. Personal observations confirmed the presence of flowers and fruit in every month between February 2015 and February 2017. However, there were noticeable reproductive cycles. Fig. 2 shows a stand of *Medinilla multiflora* on Peak 2 of Mt Makiling in June and September. Inflorescences are present at both times but in greatly different quantities. A definite peak in flowering occurred in May and June 2016 after the dry season, an exceptionally strong El Niño year (NOAA, 2017), which was also several months after the seasonal low temperatures typically reached in January and February (Brown, 1919). Another peak of reproductive activity was seemingly reached in November and December.

Flowers showed visible signs of opening before 04:00. Petals were open enough to allow access to the anthers by dawn, about 05:30, though were not fully open until 06:30. In the afternoon, flowers showed visible signs of closing by 17:00 and were closed again by 18:00. Essentially, flowers were open throughout the daylight period.

A flower lasted a single day. If not pollinated, the whole flower abscised within two days, as did the rachis until the nearest point of the inflorescence with viable flowers or fruit. If pollinated, the petals, stamens and style fell off within two days, and the fruit began to develop. The inflorescences took over one month to process from the first to last open flowers, with up to 39 new flowers observed to open on a single day (still less than 6% of the total). Fruit did not ripen until all flowers were spent. Fruit also ripened in a staggered pattern, taking another couple of months for the last berries to ripen.

Stigma receptivity

Stigmas were first receptive at 07:00 and were still receptive at 17:00. There were distinct peaks in receptivity mid-morning and again in the late afternoon (Fig. 3). When

Fig. 2 *Medinilla multiflora* on Mt Makiling was found flowering and fruiting throughout the year but still underwent reproductive phases. A Peak of flowering in June 2016 at Peak 2. B Little reproductive activity at the same location in September. Photos: Peter Quakenbush.

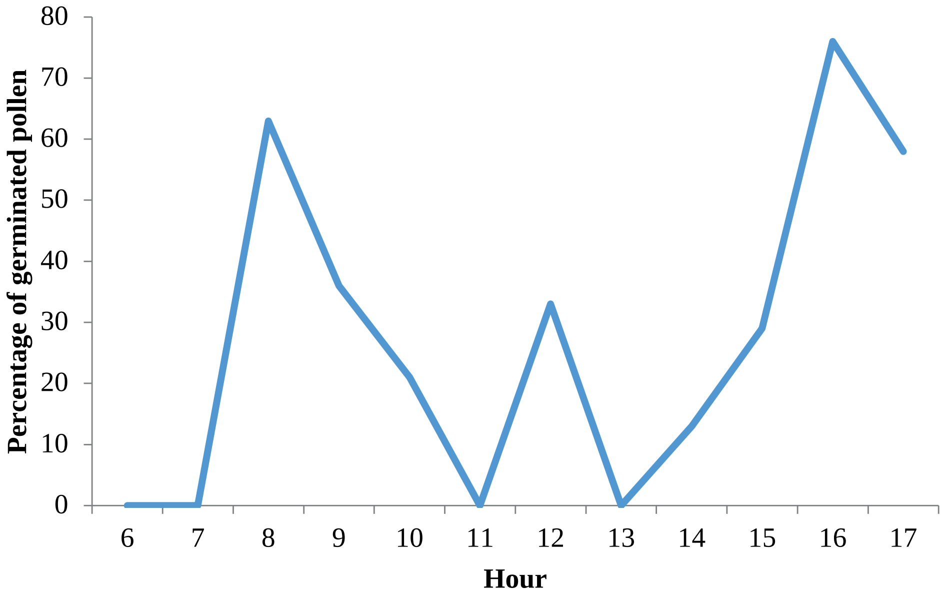

Fig. 3 Hourly stigmatic receptivity for *Medinilla multiflora* on Mt Makiling by percentage of germinated pollen per sample set.

placed in hydrogen peroxide, the stigma bubbled at 08:00 and 14:00, and on day-old stigmas, indicating receptivity at these times.

Mating system

Of the six bags for the insect exclusion experiment, only two fruit developed (discussed later), indicating that *Medinilla multiflora* requires pollination for fruit development. The three bags in the hand-pollinated self-compatibility test averaged fruit sets of 57%. In the control, only 43% of buds set fruit. Seed set was very comparable for both the control and self-pollinated fruit (Table 1).

Pollen viability

Most pollen were stained (94% – 1,016 stained, 59 not stained), showing the presence of cytoplasm and likely high viability.

Floral visitors

A total of 57.25 hours of floral visitor observations were made from 04:00 to 18:00 (Table 2). Bees curled around the anther tips and vibrated, making a distinctive buzzing sound. This was audible, species-specific and often helped the observer locate the bee. A dust of

Inflorescence	Flowers pollinated	Fruit set	Percentage of fruit set	Seed set per fruit (self-pollen)	Seed set per fruit (control)
1	6	5	83	91	126
2	32	23	72	238	214
3	45	19	42	Average: 165	Average: 170
Total	83	47	57 (ave.)		
Control	670 (buds)	290	43		

Table 1 Fruit and seed development rates for hand-pollinated (self-pollen) and control flowers (open to regular pollinators) of *Medinilla multiflora* on Peak 2 of Mt Makiling. NB: the total number of buds was counted on the control inflorescence since determining which flowers were pollinated was difficult; however, not all buds produce viable flowers, so the percentage of fruit set of viable flowers for the control was likely higher than shown here.

			Sessions in which species was present			
Time period	**Hours of observation**	**Observation sessions**	***Halictus* sp.1**	***Halictus* sp.2**	***Amegilla cingulata***	***Xylocopa***
4:00	1	1	0	0	0	0
5:00	1	1	0	0	0	0
6:00	1	1	0	0	0	0
7:00	1.75	3	1	1	1	0
8:00	3.5	4	4	1	0	0
9:00	5.5	6	1	1	0	0
10:00	6.5	8	0	2	0	0
11:00	8.75	10	4	3	0	1
12:00	8.5	10	2	0	0	0
13:00	8.25	10	0	0	0	0
14:00	6.75	9	0	0	1	0
15:00	2.75	5	0	0	1	0
16:00	1	1	0	0	0	0
17:00	1	1	0	0	0	0
Total	57.25	70	12	8	3	1

Table 2 Observation data for foraging bees on *Medinilla multiflora* flowers on Peak 2 of Mt Makiling.

pollen was thus expelled and caught in the hairs of the bee, from where it was groomed out and carried away. The anther appendages did not appear to be manipulated in any way.

Larger bees bundled all the anthers together, coming in contact with the stigma generally near the head. Smaller bees could not bundle all the anthers together and did not always contact the stigma. Bees travelled from one flower to the next, often on the same inflorescence. The smallest bees spent a couple of minutes on each flower, with buzzes lasting up to four seconds and repeated more than a dozen times. The medium-sized bees spent up to a minute on a flower and had buzzes of shorter duration, up to two or three seconds. The blue-banded bee, *Amegilla cingulata*, was observed to buzz about five times in four seconds on a flower. The carpenter bee, *Xylocopa sp*., buzzed for less than a second, once per flower, and quickly moved from flower to flower.

Various bees could be observed at almost any time of day (Fig. 4) but, during wet and windy periods, none would be seen. Inspecting the petals for a dusting of pollen grains or using a tuning fork on the anthers to see if much pollen remained effectively indicated whether pollinators had been active that day. Often flowers had already been

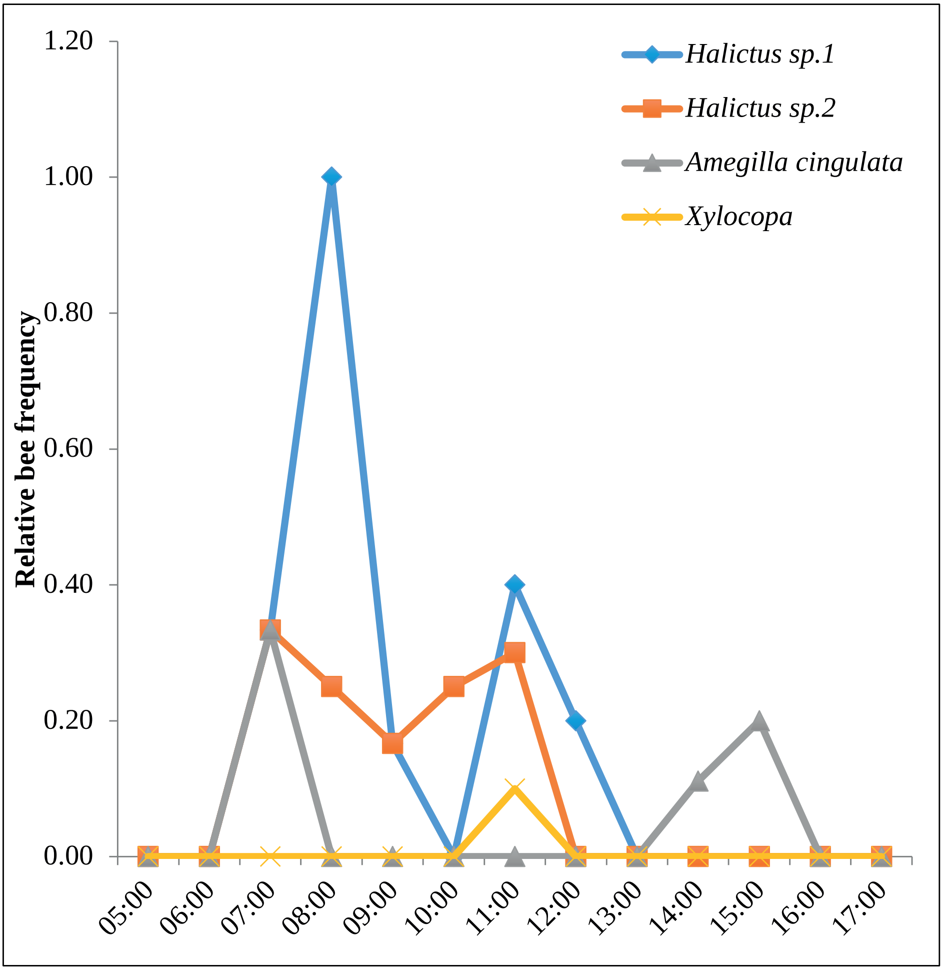

Fig. 4 Relative hourly frequency (observation sessions in which bee species were present divided by total number of observation sessions during that time period) of each bee species recorded foraging on *Medinilla multiflora* flowers on Peak 2 of Mt Makiling.

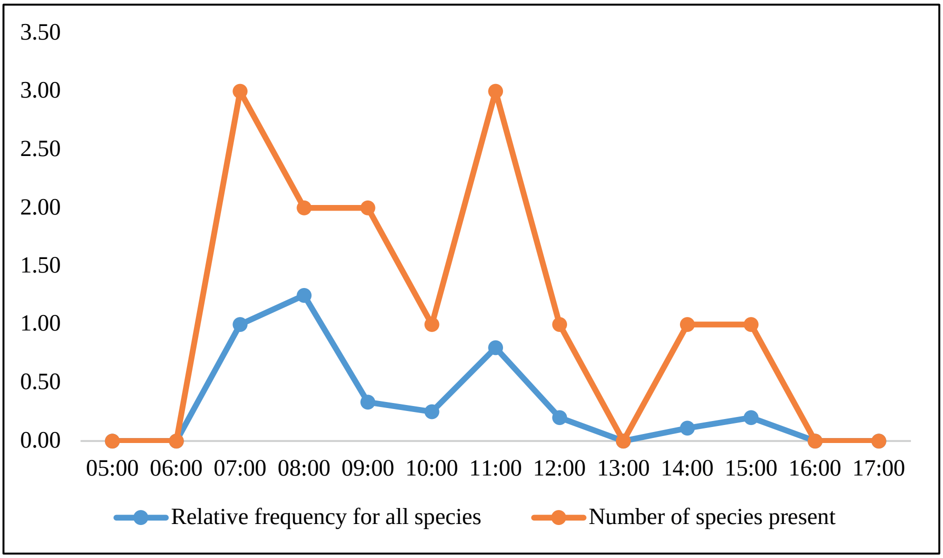

Fig. 5 Relative hourly frequency (observation sessions in which bee species were present divided by total number of observation sessions during that time period) for all bee species recorded foraging on *Medinilla multiflora* flowers on Peak 2 of Mt Makiling and total number of species present during each time period.

visited by the time of observation in the mid-morning, indicating early-morning visitations. The most visited times proved to be 07:00, 08:00 and 11:00 (Fig. 5) but only two hours of observation were performed earlier than 07:00, which happened to be a wet and windy day. The frequency of observing a bee species correlated with its size (Fig. 6), and all bee species were generalist pollinators observed at various other times in the area focusing on other flowers.

Unlike the bee pollinators, herbivorous beetles and other floral visitors could always be found on the flowers (Fig. 7). Beetles focused mainly on eating the anthers, spending long periods of time (even hours) on a single flower. Though they could have facilitated some pollen transfer via their mouth parts or by making contact with pollen on the petals left by the bees, the amount would probably be negligible in comparison to the pollen transferred by bees. *Metapocyrtus metallicus*, several other Curculionidae and Scarabidae were most commonly seen. Several arachnids inhabited the flowers and inflorescences, along with the occasional assassin bug and tiger beetle. These were not observed to manipulate the anthers and, being predatory species, were probably attracted to the other insects on the flowers. Ants were noticeably absent from buds and flowers as were caterpillars and butterflies.

DISCUSSION AND CONCLUSIONS

Understanding the range of variation within and between populations is important for defining species limits, which is beyond the scope of this study; however, significant

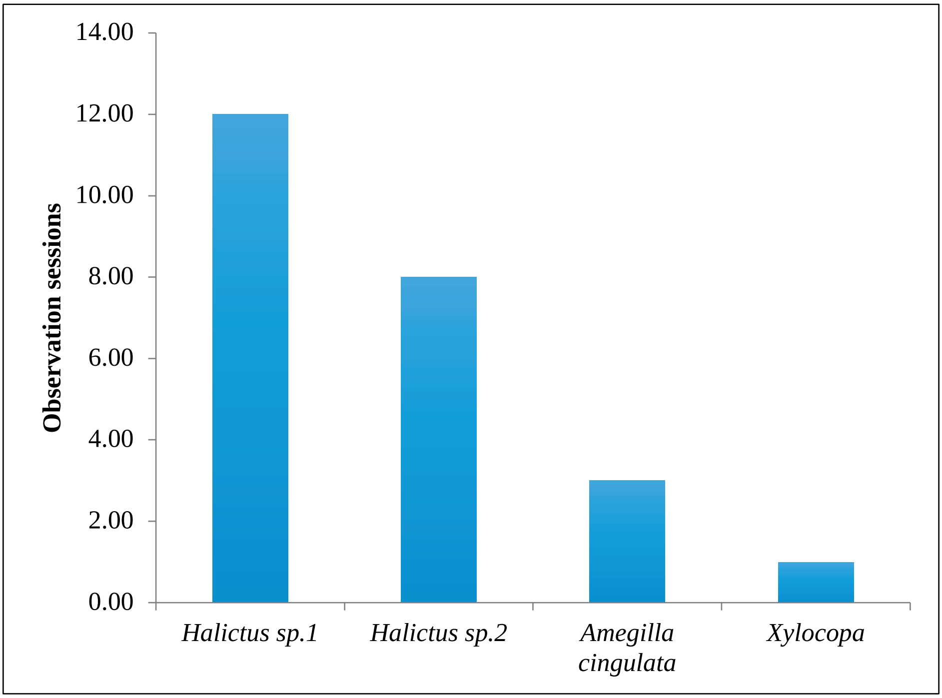

Fig. 6 Observation sessions in which each bee species was observed foraging on *Medinilla multiflora* on Peak 2 of Mt Makiling. Species arranged by size from smallest to largest.

differences seem evident at present. For example, the type description of *Medinilla multiflora* (from Mt Arayat) gives a petal length of 5 mm and an inflorescence length of 35 cm (Merrill, 1905). In the present study (of what was originally described as *Medinilla myriantha* from Mt Makiling), no petals were less than 6.5 mm long, averaging about 9 mm, and no inflorescence reached 35 cm long, averaging about 18 cm. Another outlier among the current synonyms of *Medinilla multiflora* is *M. camiguinensis* Merr., which has a described inflorescence length of up to 50 cm long (Merrill, 1913). It has yet to be determined if these populations intergrade when more populations and traits are considered, but the conservation message with regard to diversity is clear: montane populations of *Medinilla* show considerable phenotypic variability and are likely genetically isolated. Therefore, widespread protection is recommended, and spreading non-local genotypes is ill-advised.

Medinilla multiflora on Mt Makiling was not distinctly seasonal but did undergo cycles likely initiated by seasonal low temperatures as suggested by Kimura *et al.* (2009). Flower and fruit counts were not performed on individuals at set intervals to determine actual peaks of reproductive activity in this study so observations were general and at population level. However, two peaks in flowering seemed noticeable four to five months after typical cool periods in the mossy forest (Brown, 1919), the

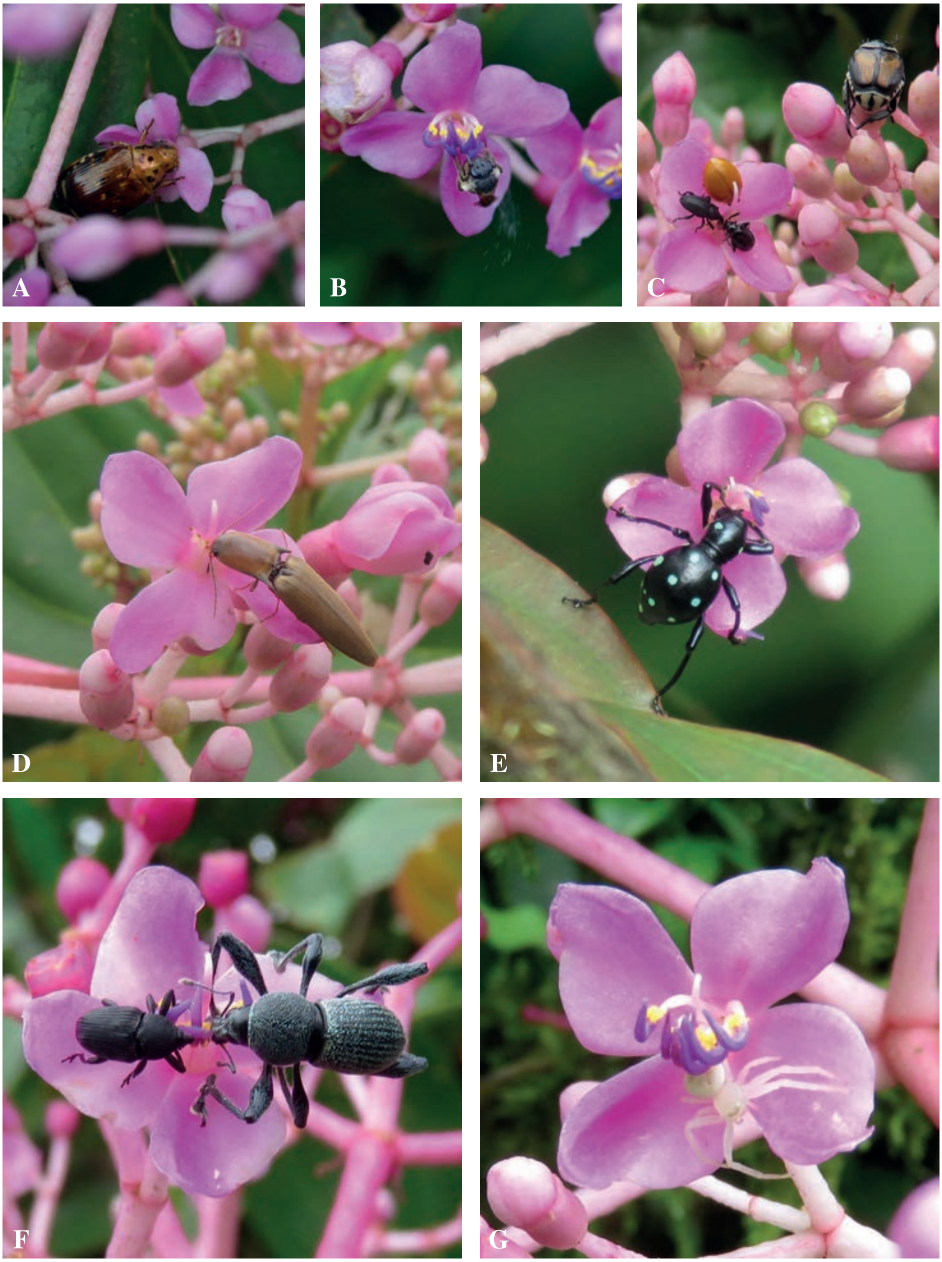

Fig. 7 Floral visitors of *Medinilla multiflora* on Peak 2 of Mt Makiling: A, C, D, E, F Beetles feeding on the anthers. B Helictid bee buzz-pollinating with expelled pollen visible. G Waiting spider. Photos: Peter Quakenbush.

coolest period occurring in about January, with a second dip in August, resulting in a biannual cycle similar to *Medinilla speciosa.* Interestingly, according to Brown's (1919) data from the mossy forest of Mt Makiling, September was the rainiest month, experienced the least amount of evaporation and had the most wind. On wet and windy days, pollinators were not observed, so the lull in flowering observed at this time could be beneficial. Conversely, May was the warmest and driest month, with December also dry. These are good pollinator conditions that corresponded with times of increased flowering in the study period. Brown's data is old but puts current plant patterns into the historical context they are likely adapted to. El Niño cycles may also trigger flowering events (Sakai *et al.*, 2006), which further helps to explain the huge display in the summer of 2016. Even so, *Medinilla* seasonality is a variable trait (Kimura *et al.*, 2009) and phylogenetic constraints could be investigated. There is also potential ecological significance to the reproductive cycle length. An abundant population of *Medinilla multiflora* flowering year-round would provide native bees with a reliable source of nutrition. Additionally, various beetle herbivores, frugivores and supported predators would benefit.

The methodology for determining the mating system in *Medinilla multiflora* could be improved. Stigmatic receptiveness varied, so times should be kept constant in the hand-pollination experiments. Manually cross-pollinated flowers would serve as better controls than uncovered flower buds, since some buds were found to abort before anthesis. Therefore, the rate of successful pollination and fruit set for the control would have been greater. Germination trials of seeds derived from cross and self-pollen would also be informative. Nonetheless, results showed a mixed mating system in *Medinilla multiflora* where it both out-crossed and was self-compatible. Two fruit did develop in the insect exclusion experiment; but these were next to or touching the mesh bag. Rubbing from the wind could have been a vector for self-pollination. Importantly, this mating system would allow a population in a new location to develop from a single propagule. As predicted, this trait likely aids the species in establishing in isolated habitats. Alternatively, self-pollination and inbreeding may be due to pollinator behaviour and be a non-adaptive cost to having a large floral display needed to attract pollinators (Paggi *et al.*, 2015). For example, the large inflorescences potentially encouraged bees in the present study to travel from one flower to the next, often on the same inflorescence, spreading self-pollen. However, *Medinilla multiflora* was common in the area and individuals appeared robust. Seed set was good and pollen was highly viable in the Mt Makiling population. These are all indicators of health. It should be noted that the particular pollen staining method used may overestimate viability when compared to other methods, but it is still informative (Dafni & Firmage, 2000).

It remains to be assessed if cryptic self-incompatibility exists. This would be an advantageous trait, since self-pollen could be the reproductive insurance; but out-crossed pollen could still be favoured. Such a mechanism has been reported in Melastomataceae (Dos Santos *et al.*, 2010). It would also be valuable to determine what other breeding systems exist in the genus and how they might relate to distribution, habitat, habit or speciation rates. A comparison of inbreeding levels in various populations would be noteworthy too, since inbreeding puts limits on adaptability and survivability due to the lack of genetic diversity (Paggi *et al.*, 2015).

The herkogamy of *Medinilla multiflora* flowers (stigma slightly above anther tips) could also lead to higher rates of selfing. Herkogamy is not very pronounced in this species, and the stigma is often bundled together with the stamens by larger bees. If medium- to smaller-sized bees are the major pollinators, this could be lessened. Some other *Medinilla* species have a much greater distance of anther-stigma separation, and selfing rates may be lessened considerably in these species (e.g. *M. magnifica* Lindl.). The anther-stigma configuration found in *Medinilla multiflora* is also present in *M. pendula* and *M. speciosa*. The more common form, with anthers positioned above the style, is found in *Medinilla magnifica*, *M. astronioides* Triana and *M. ternifolia* Triana. Sometimes there is also a set of smaller and larger anthers, as in *Medinilla teysmannii* Miq. or species similar to *M. quadrifolia* Blume. Additionally, *Medinilla myrtiformis* Triana and many Madagascan taxa have anthers surrounding the style in a more typical, solanid, buzz-pollination flower type (all based on pers. obs.). It would be interesting to investigate the role of flower size, target pollinator and breeding system in relation to these flower types and anther shapes.

Medinilla multiflora was pollinated by generalist bees capable of buzz-pollination. At least four species were observed visiting the flowers. Sizes and buzz frequencies of these bees and their behaviour varied greatly. Carpenter bees made quick visits to many consecutive flowers, while the smallest bees spent several minutes on a single flower. Though small bees were most frequently observed, the more efficient larger bees may still be more important pollinators. Interestingly, the blue-banded bee was observed to have two peaks of observation frequency similar to those of the stigmatic receptivity peaks, perhaps an indication of the importance of this species. General peak pollinator time was early morning (stigmas became receptive at 07:00), though it depended on the prevailing weather conditions. This may be the reason for the flowers being open all day (stigmas were still receptive at 17:00). Flowers of some species at lower elevations, such as *Medinilla venosa* Blume, *M. ternifolia* and *M. astronioides*, closed early in the afternoon (pers. obs.). More predictable weather conditions in the morning would be one possible explanation for this. Only the presence or absence of a bee species per observation session during a time period was noted in the present study: abundance data would have better characterised the significance of each pollinator. Observation sessions ranged from 15 to 60 minutes and were not always comparable either. It would have been better to make observations for each time period on the same days and the same number of times.

Pale concealed pollen was the only floral reward, perhaps advertised by the yellow anther appendages. *Medinilla multiflora* did not produce nectar at the petal tips as is the case in *M. magnifica* (Tobe *et al.*, 1989). Small droplets of liquid and ants can readily be observed on buds of that species and do not appear to be related to pollination (pers. obs.). The trait may be unique to *Medinilla* (Tobe *et al.*, 1989) but it is not rare in the genus. Ants can be seen on other species such as *Medinilla venosa* Blume and *M. polillensis* C.B.Rob. (pers. obs.). Lack of this trait may be of phylogenetic importance.

The long-term survival of montane species such as *Medinilla multiflora* is of special concern, since climate change could displace many species (Costion *et al.*, 2015). A study from Taiwan reported an estimated average vegetation shift of 400 m uphill by 2100 (Hsu *et al.*, 2012). *Medinilla multiflora* only exists in the top 200 m of Mt Makiling. An Australian study modelled the future suitable climate niche of 19 plant species endemic to tropical mountain tops in NE Queensland. All scenarios predicted the loss of all suitable habitat for nine out of 19 species by 2080, with the remaining ten species having lost 46–99.9% of suitable habitat (Costion *et al.*, 2015). According to data from Brown (1919), it would only take a two-degree (Celsius) increase to bring Mt Makiling's temperature at its peak to about the same point where *Medinilla multiflora* was first found flowering in the present study (850 m asl), essentially leaving no habitat. According to RCP8.5, a high greenhouse gas concentration scenario, this could happen within the next 50 years (IPPC, 2014). Besides changes in plant community assemblages and possible extinctions, this could cause significant changes to the bee populations and other dependent native fauna. Monitoring the altitudinal distribution of *Medinilla* species could be one effective measure of local climate change.

In conclusion, the key findings of this study of a fairly representative *Medinilla* species are: *M. multiflora* exhibits seasonal reproductive phases that are likely initiated by seasonal low temperatures though the population is also somewhat reproductive under adequate conditions year round; flowers and fruit are provided in a steady state pattern similar to the related genus *Pachycentria*; the taxon is self-compatible and is perhaps not too adversely affected by inbreeding depression; there is an important relationship between *Medinilla* and buzz-pollinating bees; and, lastly, though *M. multiflora* is seemingly widespread, populations are likely isolated, unique and at risk due to climate change.

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SPECIMENS EXAMINED

Specimens from CAHUP, LBC and PNH were examined in person. Only photographs of specimens from K, NY and US were seen. T indicates type material.

PHILIPPINES Laguna: Mt Makiling, flowers, 17 Mar 1906, *Merrill 5129* (NY, US, K) (T); flowers, 17 Mar 1906, *Merrill 5133* (US); flowers, 18 Jan 1913, *Gates 5198* (CAHUP 2027); Jun–Jul 1917, *Elmer 17814* (PNH); fruit, 28 Aug 1945, *Sulit* s.n. (PNH 8136); flowers, 1,000 m asl, 11 Nov 1946, *Sulit 1197* (PNH 6883); flowers and fruit, 100 m asl, 15 Mar 1949, *Rivera 3* (LBC 399, PNH 09574); flower, 3 Apr 1955, *Pancho 1165* (CAHUP 4891); flowers and fruit, 1,000 m asl, 21 May 1958, *Canicosa 1931* (LBC 3345); fruit, summit, 22 Feb 1959, *Steiner 1725* (PNH 40017); fruit and flower, 1,000 m asl, 18 Dec 1960, *Gutierrez & Orlido 8* (CAHUP 10104); flowers and fruit, summit, 6 May 1961, *Curco* s.n. (CAHUP 10074); flowers, 10 Mar 1962, *Orlido* s.n. (CAHUP 14976 & 14977); flowers and fruit, 16 Dec 1962, *Orlido* s.n. (CAHUP 14978); flowers and fruit, peak, May 1963, *Alvaran* s.n. (CAHUP 10816); flower, summit, May 1963, *Desamparo* s.n. (CAHUP 10815); flowers and fruit, peak, May 1963, *Orlido* s.n. (CAHUP 10813 & 10814); flower, 7 Apr 1964, *Hernaez 697* (CAHUP 15638); flowers and fruit, peak, 21 Jun 1964, *Hernaez 834* (CAHUP 12400); flower, 800–1,000 m asl, 12 Feb 1968, *Callo 11* (CAHUP 16294); flower, summit, 17 Feb 1968, *Angeles* s.n. (CAHUP 16291); flowers and fruit, 17 Feb 1968, *Cunada 17* (CAHUP 16295); flowers, 17 Feb 1968, *Paunlagui 100* (CAHUP 18089); flowers and fruit, 920 m asl, 5 Dec 1968, *Price 190* (CAHUP 14995); flowers, cultivated, 2 Aug 1969, *Hernaez* s.n. (CAHUP 58178); fruit, 900 m asl, 27 Jul 1975, *Co 1209* (PUH 4808); flowers and fruit, 1,000 m asl, 8 Feb 1978, *Alcachupas* s.n. (LBC 6267); flower, 1,060 m asl, 29 Mar 1979, *Alcachupas* s.n. (LBC 6471); flowers and fruit, peak, 8 May 1983, *Regalado* s.n. (CAHUP 40441); flowers and fruit, peak, 11 Jun 1983, *Regalado 83-0059* (CAHUP 39751 & 39752); flowers and fruit, summit, 24 Mar 1984, *Gruezo 10890* (CAHUP 48203 to 48205); flowers and fruit, 26 May 1984, *Annalee & Joson* s.n. (CAHUP 57889); flowers, 800–1,000 m asl, 18 Jun 1988, *Regalado 297* (CAHUP 41975); flower, 800–900 m asl, 6–9 May 2011, *Fernando 478* (LBC 8359); flowers and fruit, summit, 1 Jul 2012, *Rayos 0 to 5* (CAHUP); flowers and fruit, summit, Apr ?, *Holman 52* (PNH 196138); flowers and fruit, s.d., *Pancho* s.n. (CAHUP 15640); flowers, *Quakenbush 40* (LBC).