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RESEARCH ARTICLE

First record of bat-pollination in *Merremia* (Convolvulaceae)

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ABSTRACT

- While insects are the dominant pollinators of angiosperms, vertebrate pollinators such as bats and birds play a crucial role in pollinating plants that require cross-pollination. *Merremia platyphylla* (Convolvulaceae) exhibits floral traits typical of bat pollination (i.e., chiropterophily), including white bell-shaped flowers with crepuscular anthesis and abundant nectar production. These traits suggest that bats are likely the most important pollinators for this species.
- We investigated the nectar traits, volatile organic compounds, and the pollination and mating system of *M. platyphylla* to assess whether its floral characteristics align with the chiropterophily syndrome and determine if bats are the most effective pollinator.
- Although peak nectar production and volatile compounds did not entirely match those expected for bat-pollinated plants, *M. platyphylla* was effectively pollinated by bats, followed by diurnal pollinators, likely hummingbirds, but not honeybees, since they did not contribute to fruit production. This study provides the first record of bat pollination and floral volatile compounds not only in the genus *Merremia*, but also in the entire tribe Merremieae.
- We discuss whether this species is in a stable mixed pollination state or undergoing a transitional phase toward specialized bat pollination, given its generalized pollination system and the mismatch between some floral traits and those typically seen in chiropterophilous plants.

INTRODUCTION

Several angiosperms have evolved specific floral traits, such as morphology, colour, scent, anthesis timing, and nectar production, that increase the attraction of particular pollinators (Van der Pijl 1961). These trait combinations associated with specific functional groups of pollinators, known as pollination or floral syndromes (Fenster *et al.* 2004; Rosas-Guerrero *et al.* 2014), are considered adaptations to attract "the most effective pollinator" to maximize plant fitness (Stebbins 1970). While pollination syndromes are often used to predict pollinators of plant species, this concept has faced criticism as flowers may attract a broader spectrum of visitors than those expected by their floral traits (Waser *et al.* 1996; Ollerton *et al.* 2007). Nevertheless, a phylogenetic meta-analysis supported the pollination syndrome hypothesis, indicating that adaptations strongly drive convergent floral evolution to the most effective functional group of pollinators (Rosas-Guerrero *et al.* 2014). Therefore, empirical assessment of different floral visitors' contributions to plant fitness is crucial for testing the validity of the pollination syndrome concept (e.g., de Santiago-Hernández *et al.* 2019).

It is important to recognize that secondary pollinators that belong to different functional groups than the most effective pollinators can play crucial roles in floral evolution when the primary pollinators are temporarily absent or scarce (Rosas-Guerrero *et al.* 2014), resulting in mixed floral phenotypes. Thus, plants with mixed floral phenotypes are expected to have generalized pollination systems. For instance, Dar *et al.* (2006) found that both bats and hummingbirds carried pollen of the cactus *Marginatocereus marginatus* and showed strong pollen limitation when either pollinator guild was absent, concluding that this mixed pollination system ensures the plant reproduction given the low abundance of hummingbirds and competition with other cacti for bat pollinators. Several studies have shown that hummingbirds and bats frequently visit the same species (e.g., Young 2002; Muchhala 2003; Dar *et al.* 2006; Fleming & Muchhala 2008; Martén-Rodríguez *et al.* 2009; Muchhala & Thomson 2010), with bats generally being more effective pollinators due to their fur, which can retain more pollen than the feathers of hummingbirds (Muchhala *et al.* 2009; Muchhala & Thomson 2010; Queiroz *et al.* 2016).

In general, plants pollinated by bats (i.e., chiropterophilous plants) display large (>30 mm long), white, green, or dark red flowers that open at dusk, typically feature exposed anthers and stigma, abundant nectar and pollen, and are often bell-, brush-, dish-, or gullet-shaped (Rosas-Guerrero *et al.* 2014: Table S1). Although morphological traits are often evaluated in syndrome studies, floral scent remains understudied despite its potential

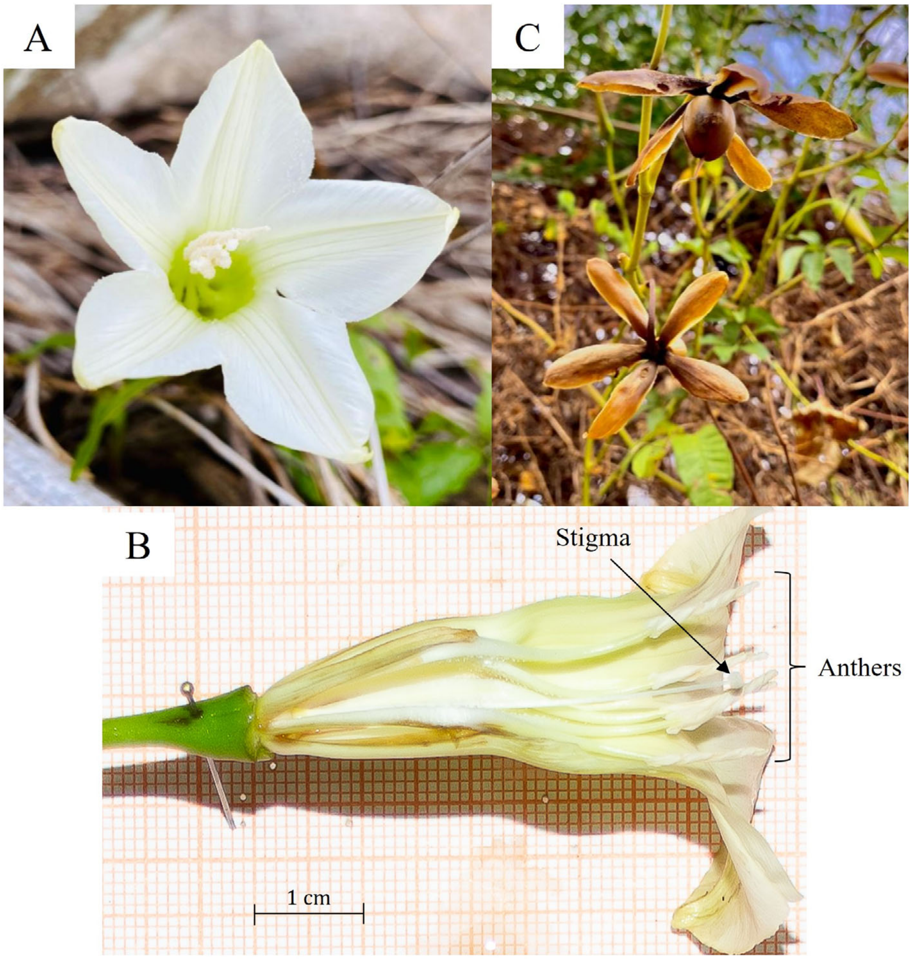

Fig. 1. Morphological features of *Merremia platyphylla*. (A) Frontal view of the corolla. (B) Longitudinal cut of the flower showing the stigma position and the five twisted anthers. Note the lack of herkogamy with some anthers. (C) Mature fruits with persistent calyces.

importance, particularly in bat-pollinated flowers, where bats can detect volatiles over long distances (Raguso 2004; Gonzalez-Terrazas *et al*. 2016). Bat-attracting floral volatiles typically include sulfur compounds, such as dimethyl disulfide (Von Helversen *et al*. 2000), and aromatic compounds, like methyl benzoate (Gonzalez-Terrazas *et al*. 2016).

The high cost of producing large flowers and abundant rewards in chiropterophilous plants may be offset by the advantageous pollination services performed by bats, which are capable of carrying large pollen loads over long distances (Fleming *et al*. 2009). This is particularly valuable in self-incompatible species that require outcrossing for fruit production, as demonstrated in several *Agave* species (Arizaga *et al*. 2000; Borbón-Palomares *et al*. 2018). Indeed, it has been found that the fruit and seed production of many bat-pollinated plants fell, on average, 83% when their vertebrate pollinators were excluded (Ratto *et al*. 2018).

Even when the climbing plant *Merremia platyphylla* exhibits characteristics associated with chiropterophily, including large white bell-shaped flowers with abundant nectar and crepuscular anthesis (Rosas-Guerrero, personal observation; Fig. 1), bat-pollination has never been reported in this genus. In the present study, we investigated the nectar traits, volatile organic compounds, pollination and mating system of *M. platyphylla*, to determine whether its floral traits align with the chiropterophily syndrome and to assess whether bats are its most effective pollinator. Given its crepuscular anthesis and high nectar volume, this species is expected to be visited by a variety of animals, with bats being the most effective pollinators. Finally, we expected this plant to exhibit a self-incompatible system making it highly dependent on bats for fruit production and likely to experience pollinator limitation.

MATERIAL AND METHODS

Study species

Merremia platyphylla Fernald O'Donell (Convolvulaceae), is an annual climbing plant with solitary white bell flowers (Fig. 1A), with a corolla diameter of 67.5 ± 1.7 mm, floral tube length of 38 ± 1.18 mm, and floral tube width of 15 ± 0.73 mm (n = 30 in all cases, means ±1 SE here and hereafter, unpublished data). The five stamens are slightly inserted with helically twisted anthers of 10–13 mm in length (O'Donell 1941; Fig. 1B). The fruits are capsule-shaped (Fig. 1C), accompanied by enlarged and separated sepals, with one or two (up to four) dark seeds with fine, velvety pubescence (O'Donell 1941; Austin & Pedraza 1983). In Mexico, the species is distributed across the states of Guerrero, Puebla, Morelos, and Oaxaca. In our study populations, flowering occurs from November to April, which is within the dry period of the region. Before this study, basic aspects of floral visitors, pollination system, floral volatiles, and mating system, remained unknown.

Study site

Fieldwork was conducted at three locations in Tecpan de Galeana, Guerrero, Mexico: "El Súchil" (17°12′52″ N, 100°38′50″ W, 47 m asl), "Ventarrón" (17°12′25″ N, 100°38′59″ W, 26 m asl) and "20 de Noviembre" (17°13′47″ N, 100°48′25″ W, 40 m asl). The maximum distance among sites was 16 km. This region has a warm sub-humid climate (Aw) with a mean annual temperature of 27°C, a maximum of 32°C in April–May and a minimum of 18°C during December–January, with precipitation of 950 mm in the rainy season (June to November), and <70 mm during the dry season (December to May; INEGI 2009). The native vegetation consists primarily of tropical dry forest (INEGI 2009).

Nectar traits

Nectar production and its concentration were assessed during January–March 2023 and 2024 to determine whether they aligned with chiropterophilous patterns and if peak nectar production coincided with peak bat activity. Using the "standing harvest" method (Corbet 2003), we sampled 20 flowers from different individuals each year. Nectar was extracted at full anthesis (17:00–17:30 h) and every 2 h until 02:00 h, when most flowers wilted. Additionally, in January 2024, accumulated nectar volume was quantified in 20 flowers bagged from anthesis to 02:00 h. Nectar volume was measured using 20 µL capillary tubes, and sugar concentration was determined with a portable refractometer (Atago 0%–50%, Japan).

Floral volatile composition

Floral volatile organic compounds from flowers and nectar were analysed during January–February 2023. Extractions were performed at anthesis and midnight to assess whether volatiles vary across flower longevity, performing three to six replicates per time point and 4-h extraction periods. The floral stems were cut and placed in a container with water for 1 h before the extraction. This method has been proven not to affect emission of floral volatiles (Kishimoto & Shibuya 2021). Following protocols from Gervasi & Schiestl (2017) and Martínez-Díaz *et al.* (2024), the scent was collected in adsorbent traps containing 150 mg Porapak Q (mesh size 80/60; Supelco, USA). The traps were eluted with 2 mL HPLC hexane, and the samples concentrated to 50 µL with a gentle flow of nitrogen gas.

Floral nectar was collected from several flowers because a 2 mL volume is required to perform the analysis (from 18:30 to 22:00 h). The nectar (6 replicates, 2 mL each) was placed in separate borosilicate glass vials and stored in low-temperature (2–3°C) containers until extraction. The nectar was extracted using 20 µL capillaries. The extraction was carried out as previously described. As a control, air was collected from empty glasses to rule out contaminants in the nectar and floral volatile samples.

From each sample, 3 µL were analysed with an Agilent 6890 gas chromatography equipped with a HP-5MS capillary column (30 m × 0.25 mm with 0.25 µm film thickness), coupled to an Agilent 5973 N selective mass detector. The analyses were carried out following Kantsa *et al.* (2017). The compounds were identified by comparing the mass spectra with the NIST 2011 database, and Kovats retention indices were calculated (alkane mix C8–C20; Sigma-Aldrich, MO, USA). The peak area in the total ion chromatogram was used to estimate the relative content of each compound.

Floral visitors

Floral visitation was monitored using night vision video cameras (Sony FDR-AX700) during February–March 2022 and January–March 2023. Recordings began at flower anthesis (17:00–17:30 h) and continued until 02:00 h, with 30-min sampling periods every 2 h. Flowers monitored by cameras were 1–2 m above ground level and cameras were set up ~1 m apart from the flowers (1–2 flowers focused by each camera). Total observation time was 39 h in 2022 and 38 h in 2023. Visitor type, time of visit, and contact with the floral reproductive structures were recorded. Since hummingbirds often visited flowers out of reach of cameras (3–4 m above the ground), specific direct observations of hummingbirds were conducted during February 2024 (4 days, 8 h total).

To identify bat visitors and verify pollen transfer, mist nets were placed near *M. platyphylla* plants from 18:00 to ~23:00 h during January–February 2023. The faces of captured bats were sampled for pollen using fuchsin-stained gelatin (Beattie 1971). The gelatin was transferred to microscope slides, heat-fixed, covered with coverslips, and examined under an optical microscope to compare them with reference pollen collected directly from *M. platyphylla* fresh flowers.

Pollination treatments

Seven pollination treatments were carried out during January–February 2023, using flowers from three populations to assess pollinator limitation (treatments 1 and 4), pollinator dependence (treatments 2 and 4), compatibility system (treatments 3 and 4) and pollinator effectiveness (treatments 5, 6, and 7): (1) *natural pollination* (n = 30) – flowers were exposed to all floral visitors; (2) *autonomous self-pollination* (n = 45) – flowers were bagged from anthesis until senescence; (3) *manual self-pollination* (n = 30) – pollen was deposited on receptive stigmas of the same flower and then bagged; (4) *manual cross-pollination* (n = 30) – pollen from different individuals was deposited on receptive stigmas of bagged and emasculated flowers; (5) *diurnal cross-pollination* (n = 93) – emasculated flowers were exposed to diurnal visitors (from 17:00–17:30 h, depending on flower anthesis) and were bagged from dusk (~ 19:00 h) until flower senescence; (6) *nocturnal cross-pollination* (n = 64) – emasculated flowers were covered from anthesis (17:00–17:30 h) until dusk (~19:00 h) then exposed to nocturnal visitors and bagged before sunrise; (7) *honeybee pollination* (n = 30) – flowers bagged prior to anthesis were exposed exclusively to honeybees (*Apis mellifera*), which visit them mainly at flower anthesis. On average, each flower in this last treatment received 5–10 honeybee visits. After bee visitation, each flower was bagged. All flowers were tagged and monitored across treatments until either abscission or fruit production, ca. 40 days later. To test for differences in fruit set among treatments, a generalized linear model (GLM) was performed with a binomial distribution and a logit link function with treatment as independent variable and fruit set as dependent variable, followed by a Tukey test to perform multiple comparisons. This analysis was performed using the *glm* function built into the "stats" package in R software v. 4.3.0 (R Core Team 2023).

RESULTS

Floral anthesis began ~17:00–17:30 h, about 80–110 min before sunset. Diurnal flower visitors such as bees and hummingbirds were the first to have access to the flowers, and ~80 min later, bats and hawkmoths arrived (see "Flower visitors" below). Flowers lasted an average of 9 h, with some persisting up to 15 h.

Nectar production

Peak nectar production occurred at 18:30 h in both years (~50 min before the bats began to visit the flowers at dusk) when hummingbirds and bees were visiting flowers. In 2023, individual flowers produced up to 99.3 ± 2.9 μL (mean ± 1 SE) of nectar with a sugar concentration of 25.7 ± 0.4 °Brix. (Fig. 2A). In 2024, nectar volume reached 106.7 ± 4.6 μL with a sugar concentration of 26.0 ± 0.2 °Brix (Fig. 2B), while accumulated nectar production was 328 ± 24.5 μL with a concentration of 24.0 ± 0.1 °Brix.

Floral volatile composition

Nine floral volatile compounds were identified in total (Table S1). The flowers emitted nine compounds in the afternoon, eight at night, and six in the nectar (Fig. 3). Benzaldehyde and limonene dominated the afternoon emissions, while 1-ethenyl-4-ethylbenzene and 4-ethylacetophenone, dominated at night and in the nectar.

Floral visitors

Five groups of floral visitors were recorded during 2022, including the red-tailed stingless bees (*Trigona fulviventris*; Fig. 4A), honeybees (*Apis mellifera*; Fig. 4B), cinnamon hummingbirds (*Amazilia rutila*; Fig. 4C), skippers (a diurnal lepidopteran of the family Hesperiidae; Fig. 4D), and bats (likely *Glossophaga*; Fig. 4G,H). In 2023, additional visitors included a wasp of the family Vespidae (Fig. 4E) and a hawkmoth of the family Sphingidae (Fig. 4F).

Visitation rates varied among years. In 2022, the stingless bee *Trigona fulviventris* and the bats showed the highest rates (0.40 and 0.39 visits flower^{-1} h^{-1}, respectively), while the honeybee, the skipper, and the cinnamon hummingbird exhibited lower rates (Fig. 5). On the other hand, in 2023, honeybees had the highest visitation rate, followed by bats (0.80 and 0.1 visits flower^{-1} h^{-1}, respectively; Fig. 5). Direct observation in 2024 revealed two hummingbird species, *A. rutila* and *Cynanthus doubledayi*, visiting at 0.4 flowers h^{-1}. Video recordings and direct observations confirmed that both bee species, hummingbirds, wasps, and bats contacted the floral reproductive structures of *M. platyphylla*, while diurnal lepidopterans and hawkmoths did not.

Five of the 18 captured nectarivorous bats carried *M. platyphylla* pollen on their bodies, which belonged to the following species: *Glossophaga soricina, G. morenoi*, and *G. commissarisi*.

Pollination treatments

Pollination treatments revealed that *M. platyphylla* had: (1) pollinator limitation, since flowers from manual cross-pollination had higher fruit set than flowers after natural pollination (Fig. 6); (2) complete pollinator dependence, since no flower from the autonomous self-pollination set fruit; (3) complete self-incompatibility, since manual self-pollination did not produce any fruit; and (4) effective pollination by diurnal and nocturnal floral visitors, although not by honeybees, skippers or moths, since these do not produce fruits (honeybees) or never touch the anthers and stigmas (skippers and hawkmoths).

Significant differences were detected among the fruit set of all pollination treatments ($\chi^2 = 36.3$, $P < 0.001$). Manual cross-pollination produced the highest fruit set (80%), followed by nocturnal cross-pollination (50%), natural pollination (30%) and diurnal-cross pollination (24%). Diurnal cross-pollination was significantly lower than nocturnal cross-pollination, indicating that bats were the primary effective pollinators, whereas the hummingbirds likely act as secondary effective pollinators of *M. platyphylla*.

DISCUSSION

To our knowledge, this study provides the first evidence of bat pollination, not only in the genus *Merremia*, but also in the entire tribe Merremieae, which includes around nine genera (see Simões & Staples 2017). Furthermore, our findings support the "most effective pollinator principle" and the floral syndrome hypothesis, as bats were identified as the most effective pollinators and aligned with the functional group predicted by most floral traits. However, this species exhibits a mixed pollination system, with bats serving as the primary effective pollinators and hummingbirds as secondary effective pollinators. These findings underscore the importance of identifying the most effective pollinators to gain deeper insights into floral evolution and plant–pollinator interactions. The relationships between its pollination system and nectar traits, floral volatile compounds, pollinator limitation, pollinator dependence, and incompatibility system are discussed below.

Although *M. platyphylla* exhibits several floral traits associated with chiropterophily (e.g., robust bell-shaped white flowers, abundant nectar production, and crepuscular anthesis), its nectar production peak occurs before bat activity, and its floral volatile profile differs from those of typical New World bat-pollinated flowers (see Pettersson *et al.* 2004). One possible explanation is that chiropterophily is a recent evolutionary adaptation in this species or that phylogenetic constraints limit a closer alignment with bat activities or preferences. Additionally, environmental factors, such as

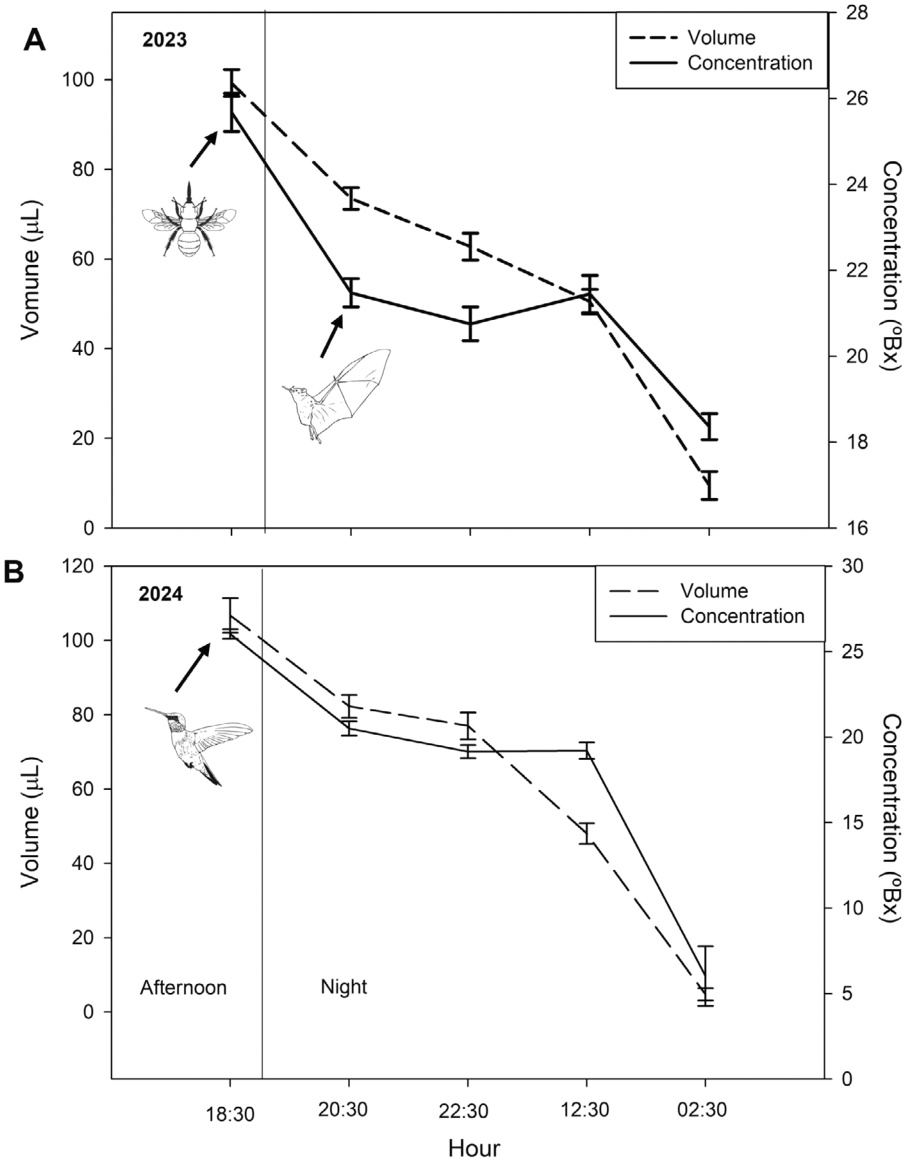

Fig. 2. Nectar production and concentration during the flower lifespan of *Merremia platyphylla* during (A) 2023 and (B) 2024. Vertical lines show the time of dusk. Means ±1 SE are shown. The arrows indicate the time of peak visitation by honeybees, hummingbirds, and bats. Drawings obtained from http:/divulgare.net.

humidity and temperature, may influence nectar production, potentially affecting its alignment with bat pollination.

Even though the floral volatile profile of *M. platyphylla* lacks the typical sulfur compounds commonly observed in bat-pollinated plants (Knudsen & Tollsten 1993; Knudsen 1999; Von Helversen *et al.* 2000; Pettersson *et al.* 2004), it contains other volatiles (i.e., α-pinene, benzaldehyde, limonene, and β-ocimene) which are present in some bat-visited species (Gonzalez-Terrazas *et al.* 2016; Farré-Armengol *et al.* 2020). This pattern resembles that of *Parkia pendula*, a species visited by bats, where β-ocimene dominates in the floral scent (84%) and does not produce sulfur compounds (Piechowski *et al.* 2010), although in *M. platyphylla* this compound did not exceed 10%. On the other hand, β-ocimene and benzaldehyde have been reported in flowers visited by moths (Miyake *et al.* 1998; Albuquerque-Lima *et al.* 2020). For instance, in the orchid *Platanthera chlorantha*, which is pollinated almost exclusively by the hawkmoth *Sphinx pinastri*, β-ocimene was one of the main volatile compounds (Steen *et al.* 2019). Interestingly, in *M. platyphylla*, this compound was only detected in afternoon extractions (17:00–19:00 h), corresponding to the hawkmoths' peak activity. However, the hawkmoth visitation rate was low (<10%), and they likely do not contribute to the pollination of *M. platyphylla*, as they did not contact the reproductive structures. Other species of hawkmoths could serve as effective pollinators of *M. platyphylla*, as in their sister species, *M. palmeri* (Willmott & Búrquez 1996), but could be absent or were beyond the reach of cameras.

It is worth mentioning that bats have also been reported to visit flowers that do not emit sulfur compounds (Knudsen & Klitgaard 1998; Pettersson *et al.* 2004; Gonzalez-Terrazas *et al.* 2016), so the attraction of bats to these plants may be

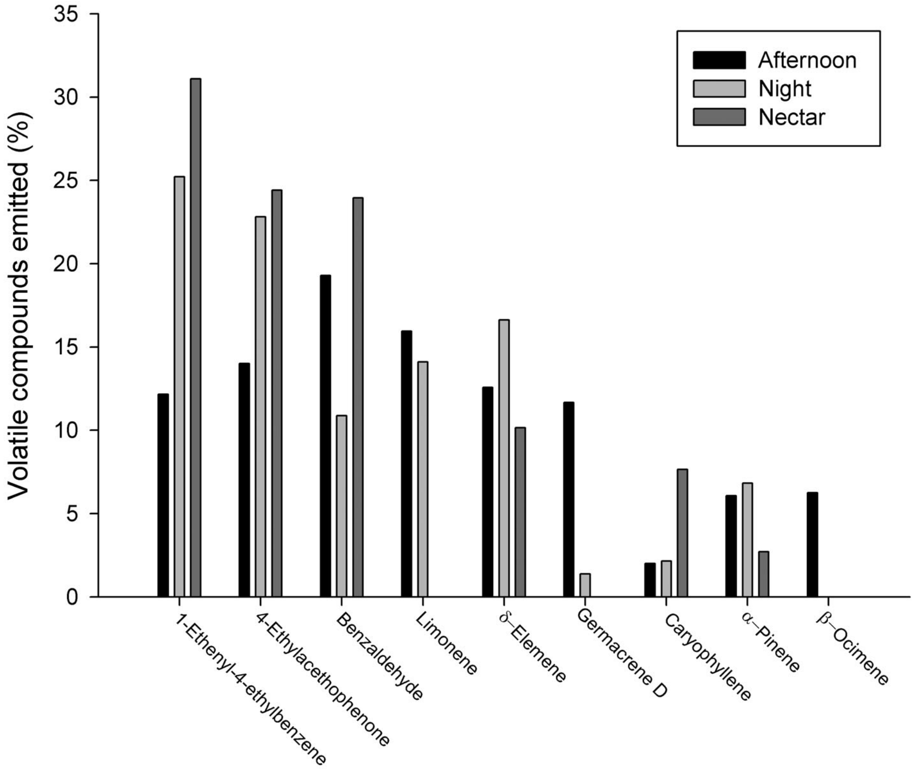

Fig. 3. Percentage of volatile organic compounds detected in flowers and nectar of *Merremia platyphylla* in 2023. The extractions were made in the afternoon (18:00 h) and at midnight (00:00 h) in the corolla, and at night (20:00 h) in the nectar.

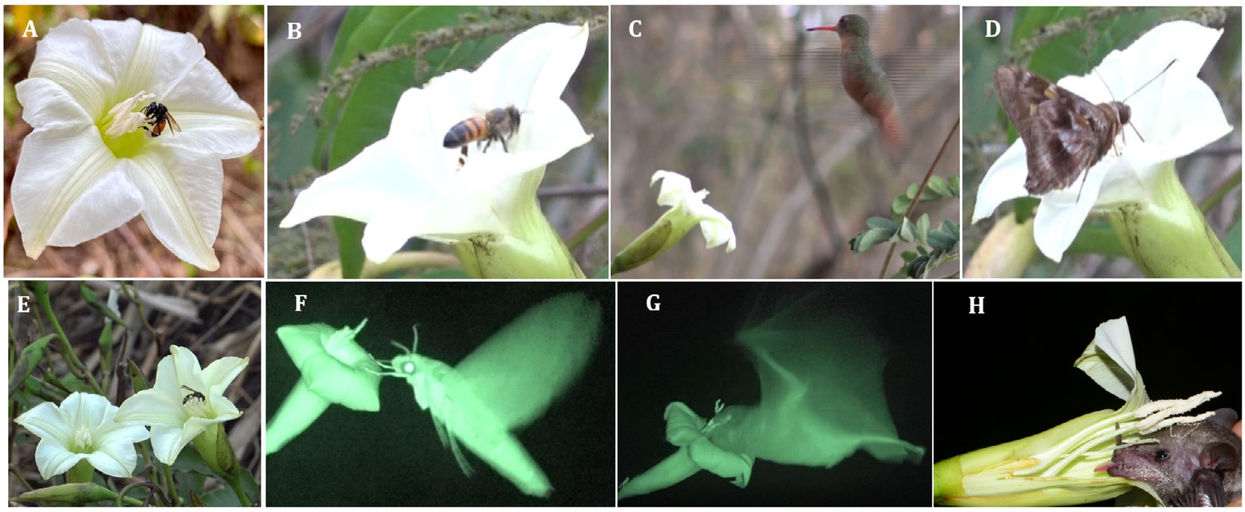

Fig. 4. Floral visitors recorded in *Merremia platyphylla* in 2022 and 2023. Diurnal visitors: (A) red-tailed stingless bee (Apidae: *Trigona fulviventris*), (B) honeybee (Apidae: *Apis mellifera*), (C) cinnamon hummingbird (Throchilidae: *Amazilia rutila*), (D) skipper (Hesperiidae), (E) wasp of the family Vespidae. Nocturnal visitors: (F) hawkmoth (Sphingidae), (G) nectarivorous bat (Phyllostomatidae: Glossophaginae), (H) head of *Glossophaga soricina* forced to entry into a flower with a longitudinal cut to appreciate its fit with the reproductive parts of the flower.

related to other volatiles or other characteristics of bats such as echolocation, which plays an essential role in locating food sources (Von Helversen & Von Helversen 2003; Gonzalez-Terrazas *et al.* 2016). Recently, it was shown that smell in birds also plays a role in foraging (Caro *et al.* 2015; Kim *et al.* 2021). Unfortunately, there is still little information on the subject, and it is unknown whether the volatile compounds found in *M. platyphylla* may participate in attracting hummingbirds.

The diversity of floral volatiles, combined with the considerable nectar volume in the afternoon and crepuscular anthesis of *M. platyphylla*, may reflect an adaptive strategy to attract a

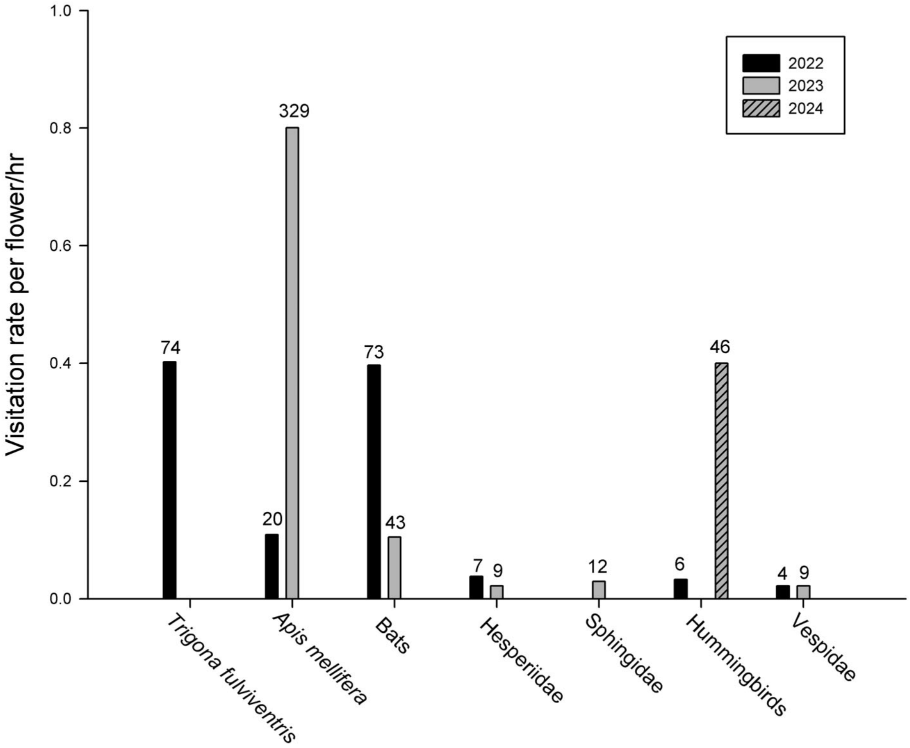

Fig. 5. Visitation rate of floral visitors of *Merremia platyphylla* during 2022–2024. The numbers above bars indicate the number of visits recorded. In 2024 only visitation rate by hummingbirds was estimated.

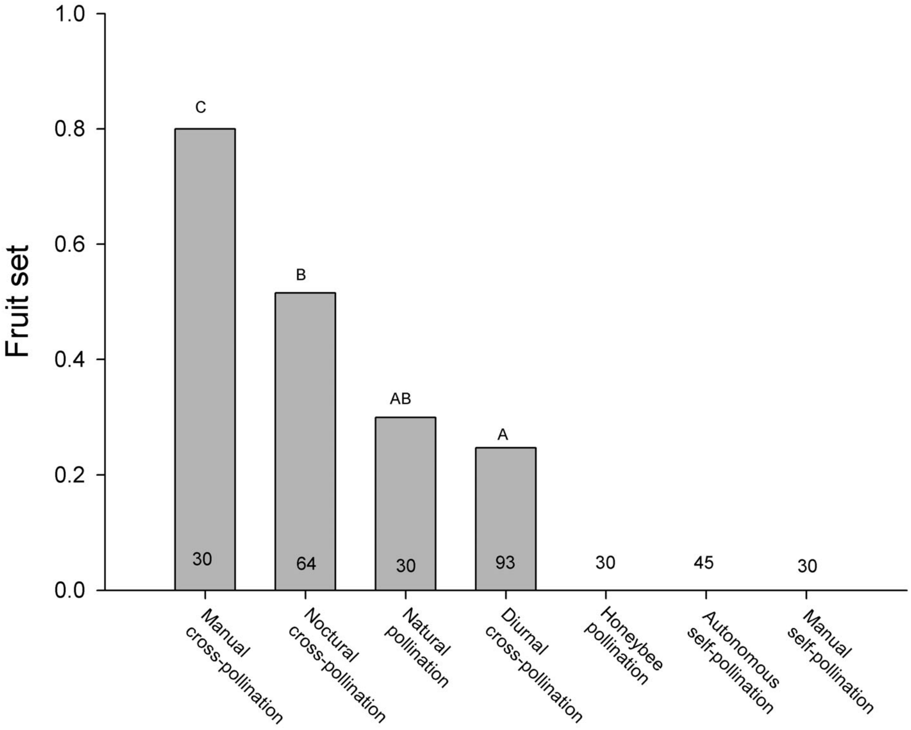

Fig. 6. Fruit-set obtained from different pollination treatments. Different letters above bars indicate significant differences among treatments after Tukey test. The numbers above treatments indicate the number of flowers used per treatment.

broad spectrum of pollinators, including both diurnal species, such as hummingbirds, as well as nocturnal species, such as bats or moths. Indeed, during the 2 years of observation, we recorded a diverse array of diurnal and nocturnal floral visitors, including not only bees, wasps, butterflies, hawkmoths, and hummingbirds, which have been described previously as floral visitors in other *Merremia* species (Maimonia-Rodella & Rodella 1986; Willmott & Búrquez 1996; Kiill & Ranga 2000; Neves *et al*. 2006; Raimúndez-Urrutia *et al*. 2008; Pick & Schlindwein 2011; Paz & Pigozzo 2013; Hassa *et al*. 2023), but also bats which have never previously been reported.

Despite the high diversity of functional groups of floral visitors and the high visitation rate of many of them (e.g., honeybees), this plant shows strong pollen limitation. At least four causes may elucidate this condition. First, not all floral visitors (e.g., skippers and hawkmoths) make contact with the reproductive parts of the flower and act as nectar thieves, decreasing the quantity of nectar, which could reduce the visitation rate and effectiveness of pollinators that may arrive later to the flower (Zhang *et al*. 2014). Second, this plant shows complete self-incompatibility and, thus, complete pollinator dependence. So, despite the proximity of anthers and stigma (Fig. 1) and the frequent contact of some floral visitors with these reproductive structures, seed production is only guaranteed if pollen from another individual is deposited in the stigma. Third, the lack of herkogamy may promote pollen–stigma interference through stigma clogging with self-pollen, which diminishes male and female fitness (Lloyd & Webb 1986). Finally, pollinator movement among flowers of the same or different plants is key to the reproductive success of self-incompatible plants. Honeybees show high floral fidelity (Eeraerts *et al*. 2019; Hung *et al*. 2023), which may cause fewer movements among plants than those performed by bats, and consequently, honeybees are expected to promote autogamous or geitonogamous pollination in this self-incompatible plant.

The lack of herkogamy (i.e., spatial separation of anthers and stigma; Fig. 1) in *M. platyphylla* contradicts what should be expected in self-incompatible species. Probably, the self-incompatibility system of this species is a recent evolutionary event, or phylogenetic constraints could be limiting the evolution of herkogamy. A review of herkogamy in closely related sister species could help to clarify the evolution of this traits.

Honeybees frequently contacted the anthers and stigma of *M. platyphylla*; however, they did not contribute to the fruit set, likely because they visit multiple flowers on the same plant (Kobayashi *et al*. 2010; Gaffney *et al*. 2018), promoting self-pollination through autogamy or geitonogamy, as mentioned above. In addition, some studies showed that when honeybees groom their bodies and move the pollen to their scopae and corbiculae, pollen could deteriorate through physiological changes, and its adhesion to the stigmas could be reduced (Parker *et al*. 2015). So, honeybees should be considered as antagonists of *M. platyphylla*. Given that honeybees were recently introduced (in evolutionary time) to the Americas (~1600s; Zayed & Whitfield 2008), *M. platyphylla* probably has not had enough time to evolve mechanisms (e.g., deterrent nectar volatiles) to avoid their visits without impeding visitation by diurnal effective pollinators. The role of antagonists in plant fitness could be exacerbated if they affect the visitation rate or behaviour of the effective pollinators. This could be the case in *M. platyphylla*, given that fruit set from natural pollination is much less than the sum of the fruit set of diurnal and nocturnal cross-pollination treatments.

Based on our pollination treatments and observation of contact of reproductive parts of flowers by each floral visitor, only hummingbirds and bats may perform effective pollination, with the latter being significantly more effective than the former. The fit between the corolla of *M. platyphylla* and the head of bats is likely to be an essential factor in pollination effectivity, as the position of the stigma and the anthers may facilitate pollen deposition and removal by bats (Fig. 4G,H).

Only potential pollination by beetles (Raimúndez-Urrutia *et al*. 2008), bees (Kiill & Ranga 2000; Neves *et al*. 2006; Raimú ndez-Urrutia *et al*. 2008; Pick & Schlindwein 2011; Paz & Pigozzo 2013; Hassa *et al*. 2023), butterflies (Lakshminarayana & Solomon Raju 2018) and moths (Willmott & Búrquez 1996) had previously been documented in this genus. Almost all these **studies determined** the pollination systems of *Merremia* species through indirect metrics, such as contact with floral reproductive parts and pollen load. Although these measures provide a valid approximation, they may mislead the effective pollinators of the plants. Only the present study and that of Willmott & Búrquez (1996), have directly evaluated the pollination syndrome in *Merremia* through pollinator exclusion experiments.

The cinnamon (*Amazilia rutila*) and turquoise (*Cynanthus doubledayi*) hummingbirds are the diurnal visitors that may contribute to fruit production, given that honeybees did not lead to fruit set and the other diurnal floral visitors did not contact anthers and stigma (except for the stingless bee *Trigona fulviventris*). Detailed experiments are required to understand the specific contribution of each diurnal floral visitor to the fitness of *M. platyphylla*.

In several plant species, visits from hummingbirds in flowers with chiropterophilous syndrome have also been observed (e.g., Buzato *et al*. 1994; Sazima *et al*. 1994; Sahley 1996; Fleming *et al*. 2001; Muchhala 2003, 2007; Dar *et al*. 2006; Martén-Rodríguez *et al*. 2009; Borbón-Palomares *et al*. 2018). This common association has given rise to the idea that bat-pollinated flowers have evolved in most cases from hummingbird-pollinated flowers (Sahley 1996; Prather 1999; Rocha *et al*. 2005; Perret *et al*. 2007; Knox *et al*. 2008; Fleming *et al*. 2009; van der Niet & Johnson 2012; Rosas-Guerrero *et al*. 2014; but see Tripp & Manos 2008). Thus, *M. platyphylla* may be in a transitional stage toward an exclusive bat-pollination system.

On the other hand, the production of fruits recorded by diurnal floral visitors suggests that the mixed pollination system in *M. platyphylla* may represents a stable reproductive strategy. This system likely provides reproductive assurance, given the significant temporal variation in pollinator assemblages observed across years (e.g., bat visitation was four times more frequent in 2022 compared to 2023). Similar temporal variation in pollinator assemblages has been documented in other mixed pollination systems (e.g., Sahley 1996; Fleming *et al*. 2009). Indeed, mixed pollination systems have been argued to represent adaptations to unreliable pollinator services rather than transitional states (Amorim *et al*. 2012). It is worth mentioning that there is also some spatial variation in the pollinator assemblages, where bats were more commonly observed in places further away from human disturbances, such as highways. Nevertheless, further studies are required to explore whether proximity to roads influences the reproductive success of this plant.

In addition to the diurnal contribution to fruit production, the peak nectar production before dusk, the volatile profile different from typical bat-pollinated plants, and the pollinator limitation detected in this species, strengthen the idea that this mixed-pollination system may serve as a stable strategy that enhances the plant's reproductive success. This flexibility could also be advantageous in the face of current global change. Long-term pollinator limitation studies, as well as research on spatial variation in pollinator assemblages and comparison with sister species, are needed to explore this idea further.

AUTHOR CONTRIBUTIONS

Study design: EC and VR-G; field work and data analysis: EM and YM-D; manuscript writing and editing: EC, VR-G, CL and EM.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Percentage of floral volatile organic compounds emitted by *Merremia platyphylla*.

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