Natural History Note

Bimodal Pollination Systems in Andean Melastomataceae Involving Birds, Bats, and Rodents

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abstract: Floral adaptation to a single most effective functional pollinator group leads to specialized pollination syndromes. However, adaptations allowing for pollination by two functional groups (bimodal pollination systems) remain a rarely investigated conundrum. We tested whether floral scent and nectar traits of species visited by two functional pollinator groups indicate specialization on either of the two pollinator groups or adaptations of both (bimodal systems). We studied pollination biology in four species of *Meriania* (Melastomataceae) in the Ecuadorian Andes. Pollinator observations and exclusion experiments showed that each species was effectively pollinated by two functional groups (hummingbirds/bats, hummingbirds/rodents, flower-piercers/rodents), nectar composition followed known bird preferences, and scent profiles gave mixed support for specialization on bats and rodents. Our results suggest that nectar-rewarding *Meriania* species have evolved stable bimodal pollination strategies with parallel adaptations to two functional pollinator groups. The discovery of rodent pollination is particularly important given its rarity outside of South Africa.

Keywords: mixed pollination systems, buzz pollination, nectar, floral scent, pollinator effectiveness, rodent pollination.

Introduction

Specialization between plants and pollinators is regarded as an integral process in angiosperm evolution driven by selection for adaptation to a plant species' most effective pollinator (Stebbins 1970; Fenster et al. 2004). Pollinator effectiveness is generally understood as the product of pollinator "quantity" (visitation frequency) and "quality" (efficiency in conspecific pollen transfer). These ideas are essential in the concept of pollination syndromes, assuming convergent floral evolution in adaptation to a specific (most effective) functional pollinator group (Faegri and van der Pijl 1979; Fenster et al. 2004; Rosas-Guerrero et al. 2014). Although specialization on the most effective pollinator is generally assumed, generalization by floral adaptation to several relatively ineffective pollinators in addition to the most effective pollinator can evolve if this results in an overall fitness gain (Aigner 2001, 2006) and if pollinator-mediated adaptive trade-offs are minimal (Thomson 2003; Castellanos et al. 2004; Muchhala 2007). Bimodal pollination systems, defined as systems effectively pollinated by two different functional groups and intermediate in adaptation between two pollination syndromes, are particularly interesting in the specialist-generalist continuum (Manning and Goldblatt 2005).

In this context, it is important to distinguish between bimodal pollination systems with two almost equally effective pollinators and pollination systems with one primary (most effective) and one secondary (less effective) pollinator (Waser 1996; Manning and Goldblatt 2005). The latter often includes a plant lineage's ancestral, presently quite ineffective pollinator (Rosas-Guerrero et al. 2014). In the New World tropics, for example, a small number of mixed hummingbird-bat systems have been described (e.g., Muchhala et al. 2009 and references therein; Amorim et al. 2013; Queiroz et al. 2016). Bat pollination is believed to have evolved from hummingbird pollination in these systems (Rosas-Guerrero et al. 2014; but see also Fleming et al.

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2009). Hence, either hummingbirds could represent ancestral, less effective pollinators or these systems could constitute bimodal pollination systems, with adaptations to both bats and hummingbirds.

Given obvious differences in morphology, activity patterns, and sensory abilities between birds and bats, these systems are ideal for testing hypotheses on floral adaptation and pollinator effectiveness. Crucial features of (bimodal) hummingbird-bat systems would include both diurnal and nocturnal anthesis, attractor cues for and morphological fit with both pollinator groups, and accessibility and continuous availability of nectar rewards (Muchhala et al. 2009). For example, visual attractiveness is generally associated with diurnal pollination (e.g., red corollas in bird systems; Faegri and van der Pijl 1979). Floral scent, on the other hand, is regarded as a particularly important attractant for nocturnally active pollinators and is less important in diurnal bird pollination systems (Raguso et al. 2003; Dobson 2006; but note that scent can also function as a deterrent, e.g., of herbivores). Furthermore, specific scent bouquets have been related to different functional pollinator groups in some plant lineages (Knudsen and Tollsten 1995; Dobson 2006; Knudsen et al. 2006). Strong associations between nectar sugars and pollinator group/specificity have also been documented (e.g., Baker and Baker 1983; Dupont et al. 2004; Johnson and Nicolson 2008). Flowers pollinated by specialized nectar-feeding birds, for example, present nectar rich in sucrose, while hexose dominates in nectar of generalist bird-pollinated flowers (Baker et al. 1998; Johnson and Nicolson 2008). Bat-pollinated flowers in the New World are intermediate in sucrose and hexose levels (Abrahamczyk et al. 2017).

The plant family Melastomataceae (ca. 5,000 sp.) is functionally specialized on bee-buzz pollination and characterized by nectarless flowers, anthers opening by small apical pores, and pollen as sole reward (Buchmann 1983; Renner 1989). However, nectar secretion and concomitant pollinator shifts from pollen-collecting bees to nonbuzzing insects or vertebrates have been documented in ca. 100 Neotropical Melastomataceae species scattered across four tribes (e.g., Lumer 1980; Vogel 1997; Kriebel and Zumbado 2014; Wester et al. 2016; Brito et al. 2017). Although ambiguity remains as to where and how nectar is secreted (Stein and Tobe 1989; Varassin et al. 2008), the shift from pollen to nectar rewarding clearly opened up the specialized bee-buzz pollination syndrome to multiple functional pollinator groups (Brito et al. 2017; Dellinger et al. 2019*a*). In the tribe Merianieae, for example, all investigated nectar-rewarding species were visited by different combinations of one diurnal and one nocturnal functional pollinator group (e.g., hummingbirds and bats or hummingbirds and rodents; Dellinger et al. 2019*a*). Using multivariate analyses of floral traits, it was impossible to distinguish flowers based on these different combinations of pollinators; instead, results suggested pooling them into a single "mixed vertebrate" syndrome (Dellinger et al. 2019*a*). This syndrome is characterized by the visitors' shared interest in the nectar reward and their ability to cause pollen release via a "saltshaker" mechanism. Pollen release is activated when the visitors insert their mouthparts into the pendant, pseudocampanulate corollas to take up nectar and thereby push against the thecae (fig. 1*A*–1*D*, 1*I*, 1*J*). It remains unclear, however, whether the different functional pollinator groups are equally effective and whether the mixed vertebrate syndrome actually comprises a variety of truly bimodal systems. Alternatively, these systems could represent transitions (shifts) between two functional pollinator groups that differ in pollination effectiveness.

In this study, we selected four *Meriania* species of the mixed vertebrate syndrome to test differences in pollinator efficiency by assessing quantity (visitation rate) and quality (in terms of pollen deposition on stigmas) of diurnal and nocturnal pollinators. We demonstrate that nectar rewards are easily accessible to all functional pollinator groups involved and test whether nectar and scent composition show adaptations to a single pollinator group or adaptations for bimodal pollination systems.

Methods

Taxon Sampling and Study Design

The four selected *Meriania* species stem from two independent shifts from ancestral bee-buzz pollination to alternative pollinators (shift 1: *Meriania furvanthera*, *Meriania tomentosa*; shift 2: *Meriania* aff. *sanguinea*, *Meriania sanguinea*; Dellinger et al. 2019*a*). The exact taxonomic status of *M.* aff. *sanguinea* is unclear; this taxon occurs in an isolated population in northern Ecuador, while *M. sanguinea* is restricted to southern Ecuador and northern Peru (Wurdack 1967). The northern population has generally been treated as *M. sanguinea*, but given clear morphological and molecular differences (Dellinger et al. 2019*a*), we treat it as a separate taxon in this study.

Meriania species are shrubs or treelets, mostly growing in small, isolated populations in montane rainforests (1,500–3,200 m) of the tropical Andes, the world's richest biodiversity hotspot (Myers et al. 2000). Extensive field studies were conducted in Ecuador in October/November 2016 and 2017 (*M.* aff. *sanguinea*: Guanderas Reserve; *M. furvanthera* and *M. sanguinea*: Podocarpus National Park; *M. tomentosa*: Bellavista Reserve). We aimed at locating the maximum number of accessible flowering individuals along different trails at each forest site, with the total sampling area spanning a minimum air line distance of 500 m at

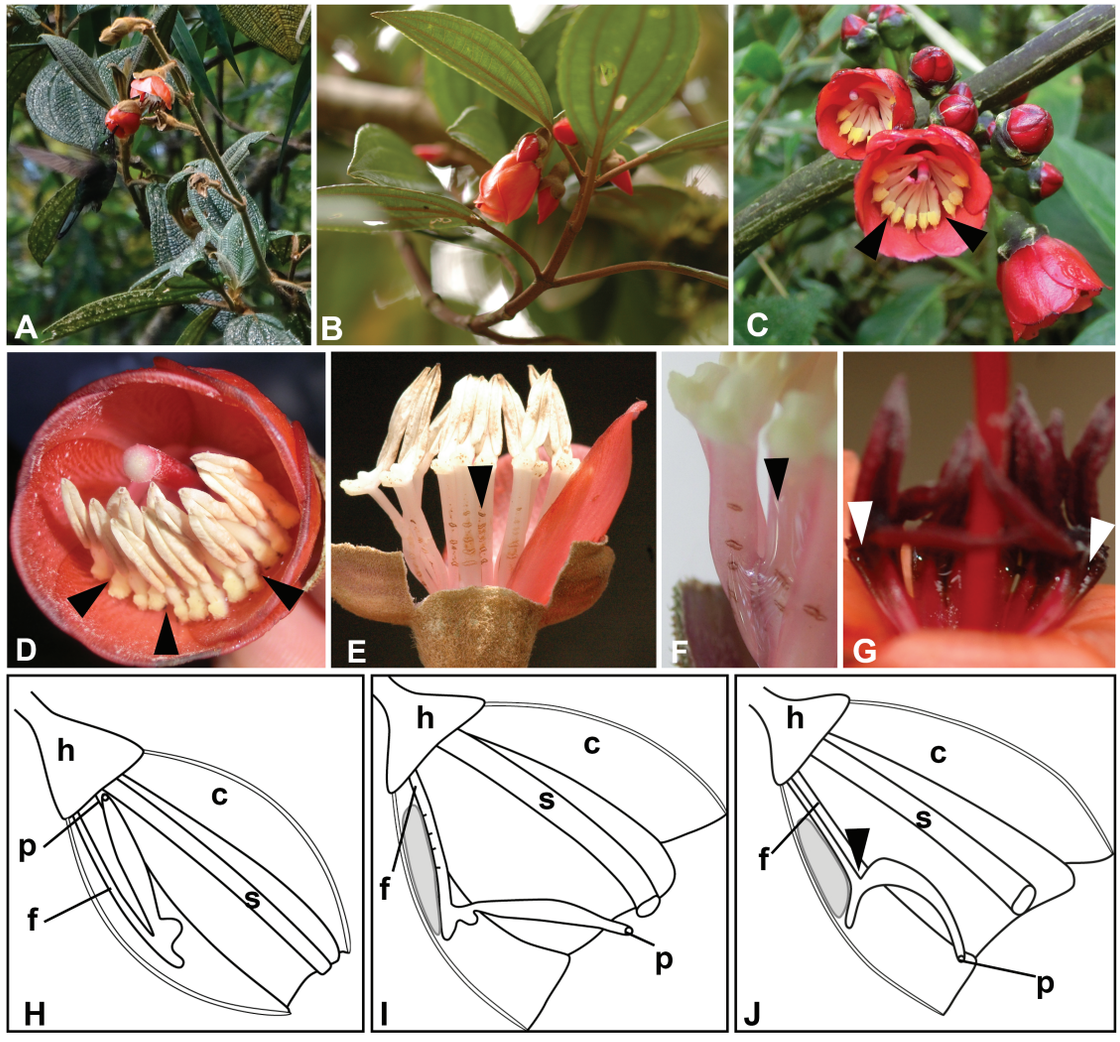

Figure 1: Inflorescences, flowers, and nectar secretion in *Meriania* species of the mixed vertebrate syndrome. *A*, *Meriania tomentosa* with protruding inflorescence and flame-throated sunangel visiting a flower. *B*, *Meriania furvanthera* with flowers arranged in a simple dichasium, allowing flowerpiercers and rodents to perch close to flowers. *C*, Multiflowered inflorescence on a procumbent branch of *Meriania sanguinea*, allowing access for hummingbirds and rodents; arrowheads indicate site of nectar aggregation. *D*, Fully anthetic flower of *M. tomentosa* with reflexed stamens, pores, and stigma positioned at corolla opening; arrowheads indicate location of nectar aggregation. *E*, *M. tomentosa*, anthetic flower seen from the side with petals partly removed, showing dorsal side of filaments with ruptures secreting nectar (arrowhead). *F*, Nectar drop (arrowhead) on filament ruptures in *M. tomentosa* (type a). *G*, Stamens of *M. furvanthera* with nectar visible on ventral side of filament-connective joint (arrowheads; types b, c). *H*, Generalized schematic drawing of a nectar-secreting *Meriania* flower at the beginning of anthesis; stamen is bent with anther tip pointing toward the style base; no nectar secretion yet. *I*, Schematic drawing of an anthetic *M. tomentosa* flower; stamens are erect with the anther tip and the pore close to the stigma; nectar-secreting filament ruptures are indicated (type a); shaded area indicates position of nectar aggregation on corolla. *J*, Schematic drawing of an anthetic *M. sanguinea* flower; stamens are erect and the anthers are distinctly curved; anther tip is close to the stigma; arrowhead indicates location of nectar secretion on ventral side of filament-connective joint (type b); shaded area indicates position of nectar aggregation on corolla. Type of nectar secretion is given as described in the main text. c = corolla; f = filament; h = hypanthium; p = pore; s = style.

each site to buffer known effects of small-scale differences in pollinator activity (e.g., Akter et al. 2017; number of individuals studied: *M.* aff. *sanguinea*: 7; *M. furvanthera*: 3; *M. sanguinea*: 19; *M. tomentosa*: 7; for details, see table A1; tables A1–A13 are available online).

Pollinator Quantity and Quality

To assess visitation rates (quantity), flowers of multiple individuals (two to 10) per species were monitored using video cameras (Sony HDR-CX190 camcorder; table A3 de-

tails sample sizes). Cameras were placed on tripods approximately 2 m away from the plants, and single inflorescences were filmed during daytime (06:00–18:00) and nighttime (18:00–00:00). In each video, a minimum of three 30-min intervals (beginning/middle/end of video) were replayed, yielding a total of 108 reviewed hours (table A3). Floral visitors were scored as pollinators if their morphology fitted with the flower and their behavior could cause pollen ejection. Visitation rates were calculated as pollinator visit per flower per hour (table 1). Most inflorescences presented more than one open flower so that it was possible to monitor multiple flowers simultaneously (yielding a total of more than 390 flower observation hours; for a similar approach, see Muchhala et al. 2009). Pollinators were identified with the help of literature (Ridgely and Greenfield 2001; Tirira 2017).

To understand whether the different functional pollinator groups differed in their quality (measured by pollen deposition on stigmas), we conducted a pollinator exclusion experiment over a 7-day period in *M.* aff. *sanguinea*, *M. sanguinea*, and *M. tomentosa* (tables A1, A4; *M. furvanthera* was excluded from this experiment as too few individuals with accessible flowers were available). As each species was visited by one functional group during daytime and another functional group during nighttime, we excluded either daytime or nighttime visitors by bagging inflorescences with bridal veil (mesh density < 1 mm) at the respective other time interval (daytime visitors excluded from 05:45 to 18:00; nighttime visitors excluded from 18:00 to 05:45; for details on sample sizes, see table A4; total flowers, $n = 80$). From day 1 to day 4, consecutively opening virgin flowers within each inflorescence were added to the exposure trials; flowers opening on days 5–7 were not considered. After 3 days or nights of pollinator exclusion (which also marks the end of the flower's life span), styles were collected in 70% ethanol. Unmanipulated control flowers from inflorescences not used in exclusion experiments or neighboring individuals (when not enough inflorescences were present on individuals used for exclusion trials; table A4) were used to assess stigma pollen loads under natural conditions. In the lab, stigmas were cut from styles, placed into a drop of lactic acid on microscope slides, squashed with a coverslip to flatten out the tissue, and viewed under a fluorescence microscope (Kearns and Inouye 1993). The entire squashed stigmatic area was measured at ×10 magnification, and pollen grains were counted at ×20 (entire field of view) in three areas from the edge to the center of the stigma. Pollen grain sizes of all species had been measured previously (17.3–19.9 μm), and pollen grains of sizes different from those of *Meriania* were excluded from counting. Total pollen grain number was calculated by multiplying total stigma area by mean pollen grain number per μm^2. For each species, a generalized linear mixed effects model (GLMM) was used to test for differences between diurnal

Table 1: Pollinator assemblages and visitation rates per flower per hour of the four *Meriania* species and total number of flower observation hours in parentheses

Species	Group	Diurnal pollinators	Diurnal visitation rate/flower/hour	Nocturnal pollinators	Nocturnal visitation rate/flower/hour
Meriania aff. *sanguinea*	HB	*Eriocnemis derbyi* (black-thighed puffleg); *Metallura tyrianthina* (Tyrian metaltail); *Schistes geoffroyi* (wedge-billed hummingbird)	1.7 (50)	*Anoura* cf. *peruviana* (bat)	.88 (45.2)
Meriania tomentosa (Cogn.) Wurdack	HB	*Coeligena torquata* (collared inca); *Adelomyia melanogenys* (speckled hummingbird); *Ocreatus underwoodii* (booted racket-tail); *Urosticte benjamini* (purple-bibbed whitetip)	3.49 (15.5)	*Anoura* sp. (bat)	.73 (20.5)
Meriania sanguinea Wurdack	HR	*Heliangelus micraster* (flame-throated sunangel)	.25 (52)	*Thomasomys* sp. (rodent)	.03 (63.7)
Meriania furvanthera Wurdack	FR	*Diglossa cyanea* (masked flowerpiercer)	.11 (94)	Rodent, unidentified	.46 (43.6)

Note: For details, see table A3, available online. Pollinator groups: FR = flowerpiercer/rodent; HB = hummingbird/bat; HR = hummingbird/rodent.

and nocturnal stigma pollen loads and between controls and exclusion trials, including plant individual as a random effect (lmerTest package in R; Kuznetsova et al. 2017). We can rule out possible confounding effects of pollen deposition on stigmas by our own disturbance when bagging/unbagging inflorescences, as pollen is retained within the poricidal anthers. Also, unmanipulated control flowers did not show significantly different stigmatic pollen loads than the median diurnal plus nocturnal stigmatic pollen loads from the exclusion experiment (Kruskal-Wallis χ^2: 0.428, df = 1, $P = .513$).

Localization of Nectaries

To provide a better understanding of the evolution of nectar-rewarding flowers from pollen-rewarding ancestors in the family, we compared nectar-secreting structures of the four study species plus six additional nectar-secreting species (table A2). Note that there is no underlying expectation related to nectar-secreting strategies and the different mixed pollinator assemblages. Ethanol-preserved floral material was studied with SEM or LM to localize areas of nectar secretion. For SEM, hypanthia and stamens were dehydrated over an ethanol series, transferred to acetone, critical-point dried (Autosamdri-815), mounted on stubs, sputter-coated with gold (SCD 050), and scanned in a JEOL JSM-6390 at 10 kV. For producing serial thin sections, material was dehydrated, infiltrated (Technovit 7100, hardener I; Heraeus Kulzer, Wehrheim, Germany), embedded in 2-hydroxyethyl methacrylate (Technovit 7100, hardener II), and sectioned at 5 μm with a Microm HM rotary microtome 355 (Walldorf, Germany). Sections were stained with 0.2% ruthenium red and 0.5% toluidine (RT stain). Images of selected sections were taken with a Nikon digital sight DS-Fi1 camera (Nikon, Tokyo) on an Olympus BX50 system microscope (Olympus, Tokyo).

Nectar Collection and Analyses

To assess differences in nectar properties between day and night, flowers of any age were bagged in the early morning (05:30–07:00) or early evening (17:30–18:45) after removing all nectar if present (table A6). The age of a flower was scored as "first day," "second day," or "old" by the degree of petal spreading and anther reflexion to document nectar secretion through anthesis. Twelve hours after initial bagging, the presence of nectar, its volume, and its concentration were recorded. Nectar was extracted with 10-μL microcapillaries, and its concentration was measured using an Eclipse Refractometer 45-81 (Bellingham and Stanley). Its volume was estimated from the number of filled 10-μL capillaries per flower. A subset of flowers was rebagged to assess nectar replenishment at 12-h intervals (table A6). For *M. furvanthera*, the nectar volume could not be measured due to small sample sizes, and the concentration was measured from unbagged flowers. Summary statistics were calculated for all species from all measurements. GLMMs were used to assess significant differences in nectar concentration and volume between day and night measurements in *M. tomentosa* and *M. sanguinea*, setting treatment (day/night) as a fixed factor and flower ID as a random effect (*M.* aff. *sanguinea* was excluded due to small *n*). This model was chosen to specifically account for the effects of repeated sampling of individual flowers. Another GLMM was run on all measurements on nectar concentration ($n = 105$) to assess significant differences between species and day/night, treating plant individual as a random effect to account for the repeated sampling of different flowers on the same plant individual.

Ten microliters of the nectar collected at day/night sampling times was stored in 70% ethanol for sugar analyses using high-performance liquid chromatography (HPLC; a total of 87 samples; table A6). Nectar sugar samples were dried in a vacuum concentrator centrifuge to remove ethanol and redissolved in 500 μL of water. For HPLC, an aliquot from each sample was further diluted 1 : 100 with water and analyzed on an ICS300 HPLC (Dionex) using anion-exchange chromatography coupled with pulsed amperometric detection. Sugars were separated on a CarboPac PA1 column (two 250-mm separation columns and two 50-mm guard columns) using isocratic separation with 80 mM NaOH and a flow rate of 0.25 mL min^{-1}. Authentic standards were separated for calibration to ensure proper quantification of each sugar. For each sample, the percentage of glucose, fructose, and sucrose was calculated for day and night (Baker and Baker 1983). Bray-Curtis dissimilarity matrices were calculated in R package vegan (Oksanen et al. 2018), and a two-way crossed permutational MANOVA (PERMANOVA) was run with pairwise comparison and a Bonferroni correction to test for significant differences in nectar composition between species, day/night, and individuals (pairwiseAdonis; Martinez Arbizu 2017). Disparity in sugar composition was calculated (betadisper function), and ANOVA was used to test for significant differences in disparity between species.

Volatile Collection and Analyses

Floral volatiles were collected in situ during daytime (06:00–08:00) and nighttime (18:00–21:00) using dynamic headspace methods (Dötterl et al. 2005; total $n = 113$). Individual anthetic flowers (age and pollination status not considered) were enclosed in polyester oven bags (10 cm × 15 cm; Toppits, North Rhine-Westphalia, Germany), and volatiles were collected for 10–30 min (depending on the strength of the perceived scent) through small adsorbent tubes (Varian Chro-

matoProbe quartz microvials; length: 15 mm, inner diameter: 2 mm) using a membrane pump (G12/01 EB; Rietschle Thomas, Puchheim, Germany; flow rate: 200 mL/min). The tubes contained 1.5 mg Tenax TA (60–80 mesh) and 1.5 mg Carbotrap B (20–40 mesh; both Supelco) fixed by glass wool plugs (Mitchell et al. 2015). In addition, three leaf scent samples were collected at approximately 5 m distance from the flowers for each species (negative controls). Trapped volatiles were analyzed by gas chromatography/mass spectrometry (GC/MS) using an automatic thermal desorption (TD) system (TD-20; Shimadzu, Kyoto, Japan) coupled to a Shimadzu GC/MS-QP2010 Ultra equipped with a ZB-5 fused silica column (5% phenyl polysiloxane; 60 m, i.d.: 0.25 mm, film thickness: 0.25 μm; Phenomenex). Samples were run with a split ratio of 1 : 1 and a consistent helium carrier gas flow of 1.5 mL/min. GC oven temperature started at 40°C, followed by an increase of 6°C/min to 250°C (held for 1 min). The MS interface worked at 250°C. Mass spectra were taken at 70 eV (electron ionization mode) from m/z 30 to 350. GC/MS data were processed using the GCMSolution package, version 4.11 (Shimadzu). Compound identification was carried out using the ADAMS, ESSENTIALOILS-23P, FFNSC 2, and W9N11 databases, as well as a database generated from synthetic standards available at the Plant Ecology Lab at the University of Salzburg. Only compounds not present in the negative controls (i.e., flower-specific compounds) were included in further analyses. For quantitative analysis of volatile organic compounds, known amounts of monoterpenes and aliphatic and aromatic compounds were injected into the GC/MS system and mean peak areas were used to determine the total amount of scent (Etl et al. 2016). Mann-Whitney *U*-tests were used to test for significant differences in scent release between day and night for each species separately. As for nectar composition, Bray-Curtis dissimilarities were calculated on the relative amounts of compounds and two-way crossed PERMANOVAs run with species and daytime as factors. Relative scent compositions were visualized by nonmetric multidimensional scaling (vegan) and stacked barplots. All data have been deposited in the Dryad Digital Repository: https://dx.doi.org/10.5061/dryad.jk673fq (Dellinger et al. 2019*b*).

Results

Visitor Assemblages and Visitation Rates (Quantity)

Each *Meriania* species was visited by one diurnally active functional pollinator group and a nocturnally active one (table 1; hummingbirds [diurnal] and bats [nocturnal] in *Meriania* aff. *sanguinea* and *Meriania tomentosa*; hummingbirds [diurnal] and rodents [nocturnal] in *Meriania sanguinea*; video A1 [videos A1–A3 are available online]; flowerpiercers [diurnal] and rodents [nocturnal] in *Meriania furvanthera*; videos A2, A3). All flower visitors were foraging for nectar, which was taken up by inserting the head into the flower, thereby touching the thecae and activating the saltshaker mechanism of pollen release. While hummingbirds and bats mostly hovered, flowerpiercers (passerine birds) and rodents perched. Rodents were observed running along branches and spent up to 10 s on a single flower to drink nectar. Wasps and lepidopterans were seen as occasional nectar robbers in all species. On only a single sunny day, small bees were observed robbing pollen in *M. sanguinea*. The insects' contribution to pollination is likely negligible, as they either could not activate the saltshaker mechanism (wasps, lepidopterans) or did not touch the stigmas due to their small body size (bees). From here onward, the different pollinator assemblages are grouped as follows: hummingbird/bat (HB), hummingbird/rodent (HR), and flowerpiercer/rodent (FR).

Visitation rates between diurnal and nocturnal functional pollinator groups differed considerably in all species, with higher diurnal visitation rates in *M.* aff. *sanguinea*, *M. tomentosa* (both HB), and *M. sanguinea* (HR; table 1). In all species, both diurnal and nocturnal visitors occasionally visited more than one flower if multiple flowers were present (table A3).

Pollinator Efficiency (Quality)

There were no significant differences in pollen deposition efficiency between diurnal and nocturnal functional pollinator groups in *M. tomentosa* (HB: $t = 0.716$, df $= 27$, $P = .48$) and *M. sanguinea* (HR: $t = -0.343$, df $= 14$, $P = .737$), but nocturnal stigmatic pollen loads were higher in *M.* aff. *sanguinea* (HB: $t = 3.038$, df $= 11$, $P = .01$). Excluding either diurnal or nocturnal visitors did not significantly reduce total pollen loads compared to controls in *M. tomentosa* (HB) and *M. sanguinea* (HR) but did so in day samples of *M.* aff. *sanguinea* (HB; fig. 2; table A5).

Nectar Secretion: Location

Stamens were detected as nectar-secreting organs in all species. The exact location of nectar secretion differed between species, and three main types were distinguished: secretion by dorsal filament ruptures along the entire length of the filament (type a; figs. 1*E*, 1*F*, A1*A*, A1*B*; figs. A1–A4 are available online), secretion by small ruptures at the ventral side of the joint between filament and anther connective (type b; fig. A1*E*–A1*H*), and secretion by porous tissue on the proximal lateral sides of the filament (type c; figs. 1*G*, A1*C*, A1*D*). Accordingly, nectar droplets were found oozing out of dorsal filament ruptures (visible as dark necrotic cav-

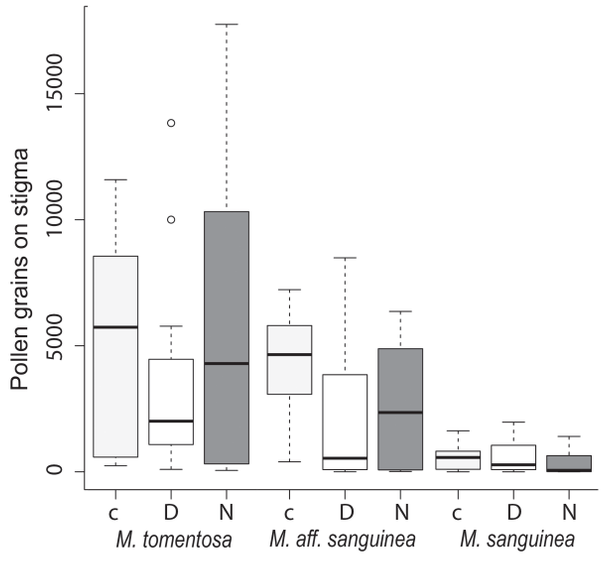

Figure 2: Boxplot showing pollen deposition loads on stigmas of pollinator exclusion experiments in *Meriania*: open flower access (c; light gray), day access only (D; white), and night access only (N; dark gray). There are no significant differences in *Meriania tomentosa* (hummingbird/bat [HB]) and *Meriania sanguinea* (hummingbird/rodent); control and night pollen loads are significantly higher in *Meriania* aff. *sanguinea* (HB).

ities) in *M. tomentosa* (HB; fig. 1*E*, 1*F*; type a) but sitting at the filament-connective joint/upper part of the filament in *M. furvanthera* (FR; fig. 1*G*; types b and c), *M.* aff. *sanguinea* (HB), and *M. sanguinea* (HR; type b). These patterns were confirmed by the study of six additional nectar-secreting *Meriania* species (table A2). Ruptures of type a and b formed only in the first hours of anthesis, while porous tissue of type c was already present in preanthetic flowers. Regardless of the exact site of secretion, nectar pooled between the stamens and petals and was freely accessible to all functional pollinator groups (fig. 1*I*, 1*J*).

Nectar Secretion: Timing and Volume

Nectar secretion started within the first 6 h of anthesis in *M. tomentosa* (HB), while it started only after approximately 24 h in *M. sanguinea* (HR) and *M.* aff. *sanguinea* (HB; fig. A2). Nectar was secreted throughout anthesis from the first secretion onward and was replenished after removal. In all species, pollinators started visiting flowers at the beginning of anthesis even if there was no nectar present yet. Nectar volume was not significantly different between day and night (GLMM: *M. tomentosa* [HB]: $t = -1.82$, $\mathrm{df} = 31$, $P = .08$; *M. sanguinea* [HR]: $t = -0.52$, $\mathrm{df} = 28$, $P = .61$; table 2).

Nectar Concentration and Sugar Composition

Nectar sugar concentration ranged between 10.9° and 13.6° Brix in *M.* aff. *sanguinea*, *M. tomentosa* (both HB), and *M. sanguinea* (HR), while it was significantly higher (up to 20° Brix) in *M. furvanthera* (FR; tables 2, A7). Only *M. sanguinea* showed significant differences in nectar concentration between day and night (GLMM: *M. sanguinea* [HR]: $t = 3.56$, $\mathrm{df} = 17$, $P < .01$).

Sugar composition differed significantly among species ($F = 114$, $\mathrm{df} = 3$, $r^2 = 0.787$, $P = .001$; table A8), with sucrose being predominant in *M. tomentosa*, *M.* aff. *sanguinea* (both HB), and *M. sanguinea* (HR), while hexoses were dominant in nectar of *M. furvanthera* (FR; fig. 3). *Meriania furvanthera* differed significantly from all other species (table A9). Nectar sugar composition did not differ between day and night in any species or the interaction of species and day/night (table A8). Variability of nectar composition differed significantly between species ($F = 6.53$, $\mathrm{df} = 2$,

Table 2: Nectar volume, sugar content, and mean relative sugar proportions in day and night samples of the four *Meriania* species

	Mean nectar volume (μL)		Mean °Brix		Relative amount G (%)		Relative amount F (%)		Relative amount S (%)		S/(F+G)		F/G	
Species	N	D	N	D	N	D	N	D	N	D	N	D	N	D
Meriania aff. *sanguinea* (HB)	59.6	…	11.8	13.1	.8	1.0	.5	1.0	98.7	98.0	71.9	53.9	.7	1.5
Meriania tomentosa (HB)	124.5	82.7	12.2	12.43	10.7	5.2	9.6	7.8	79.7	87.0	35.5	38.3	1.3	1.5
Meriania sanguinea (HR)	73.7	46.8	10.9	13.6	1.6	.7	2.1	2.0	96.3	97.3	55.5	52.1	2.1	4.1
Meriania furvanthera (FR)	…	…	20	18.3	43.7	37.0	52.2	60.4	4.1	2.6	.04	.02	1.2	1.7

Note: Details on sample sizes are given in table A6, available online. D = measured after day; F = fructose; G = glucose; N = measured after night; S = sucrose. Pollinator groups: FR = flowerpiercer/rodent; HB = hummingbird/bat; HR = hummingbird/rodent.

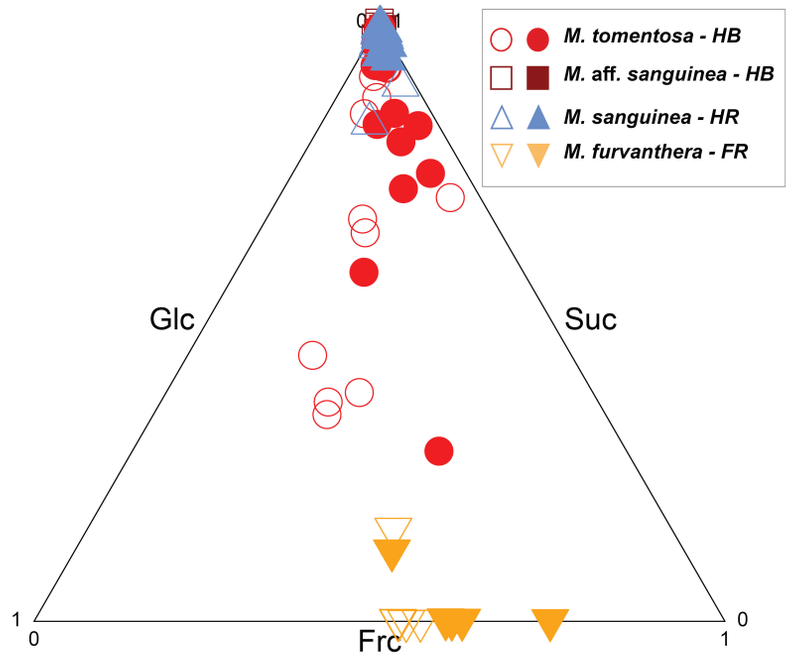

Figure 3: Triangle plot showing relative nectar sugar composition of day nectar (unfilled symbols) and night nectar (filled symbols) in the four *Meriania* species. Note the clear separation following bird pollinator preferences: sucrose prevalence in hummingbird-pollinated *Meriania sanguinea*, *Meriania* aff. *sanguinea*, and *Meriania tomentosa* and hexose dominance in flowerpiercer-pollinated *Meriania furvanthera*. Frc = fructose; Glc = glucose; Suc = sucrose. Pollinator groups: FR = flowerpiercer/rodent; HB = hummingbird/bat; HR = hummingbird/rodent.

$P < .01$; figs. 3, A3) and was significantly higher in *M. tomentosa* (HB) than in *M. sanguinea* (HR; table A10).

Scent Composition

Flowers of *M. sanguinea* (HR) released a strong solvent-like odor, and flowers of *M. tomentosa* (HB) produced weak flowery odors at all times. No odors detectable by the human nose were noted on flowers of *M.* aff. *sanguinea* (HB) and *M. furvanthera* (FR). The GC/MS analyses revealed flower-specific scent compounds in all species, however. Surprisingly, scent compounds were detected in only half or less of the samples in each species (table 3). *Meriania furvanthera* was the only species to release detectable floral scent compounds only during daytime, while both day- and nighttime samples of the other three species contained flower-specific compounds (table 3). Median total amounts of scent per flower per hour were significantly higher in daytime samples of *M. tomentosa* ($W = 110$, df $= 20$, $P < .01$), while differences were not significant in other species (table A11). Scent profiles were significantly different between species ($F = 10.8$, df $= 3$, $P < .001$; table A12). *Meriania tomentosa* (HB) was the only species where day and night scents differed significantly; *M. sanguinea* (HR) stood out as differing significantly from *M. tomentosa* (table A13). Scent samples of *M. sanguinea* (HR) contained aliphatic compounds only, with most diurnal and all nocturnal samples containing only 1-hexen-3-one. This compound was not detected in any other species. Scents of *M. furvanthera* (FR) also contained aliphatics, while scents of *M. tomentosa* and *M.* aff. *sanguinea* (both HB) also contained terpenoids such as sabinene and delta-3-carene and unknown compounds (figs. 4, A4).

Discussion

Our results suggest that the mixed vertebrate pollination syndrome in Merianieae comprises multiple bimodal pollination systems where different functional pollinator groups can act as equally effective pollinators. These systems overlap in their main traits—for example, often reddish flowers, day and night availability of nectar, easy reward access by widely open pseudocampanulate corollas, staminal nectar release and nectar aggregation beneath the stamens, and a common pollen expulsion mechanism (Dellinger et al. 2019*a*). On a finer scale, certain differences in adaptation to the distinct functional pollinator groups become

Table 3: Scent composition of the four *Meriania* species for day (D) and night (N)

		Meriania aff. *sanguinea* (HB)		*Meriania tomentosa* (HB)		*Meriania sanguinea* (HR)		*Meriania furvanthera* (FR)
Component	RI	D (n = 3/6)	N (n = 2/4)	D (n = 12/25)	N (n = 12/23)	D (n = 10/23)	N (n = 9/24)	D (n = 4/8)
Aliphatic components:								
methyl butanoate[a]	721					.0 (.0–.6)		
1-hexen-3-one[a]	775					100.0 (.0–100.0)	100.0	
3-hexanone[a]	785					.0 (.0–78.4)		
1-hexanol	866			.0 (.0–30.9)	.0 (.0–100.0)			.0 (.0–63.7)
pentanoic acid	869					.0 (.0–100.0)		.0 (.0–34.2)
3-octanol	995			2.1 (.0–36.1)				
1-octanol	1,069							.0 (.0–15.4)
octyl acetate[a]	1,210	.0 (.0–42.3)	35.0 (10.2–59.7)	.0 (.0–36.1)				
N-bearing compounds:								
2-methylbutanenitrile	723			.0 (.0–10.3)				
Terpenoids:								
sabinene[a]	979	57.7 (.0–90.2)	21.3 (13.6–29)					
δ-2-carene	1,006							.0 (.0–100.0)
δ-3-carene	1,017				54.6 (.0–100.0)			
(*Z*)-4,8-dimethyl-1,3, 7-nonatriene	1,099			.0 (.0–100.0)				
(*E*)-4,8-dimethyl-1,3, 7-nonatriene	1,119			81.6 (.0–100.0)				
p-1,3,8-menthatriene	1,134			.0 (.0–100.0)				
β-elemene	1,408				.0 (.0–90.7)			
(*E*)-β-caryophyllene[a]	1,437	.0 (.0–100.0)	15.0 (3.0–26.9)	.0 (.0–69.1)	.0 (.0–55.4)			
(*E*)-β-farnesene[a]	1,463		1.2 (.0–2.5)					
α-humulene[a]	1,481		5.8 (.1–11.6)					
Unknowns:								
m/z 40,41,55,69,107,119	1,084			.0 (.0–1.4)				
m/z 55,69,81,109,135,161	1,487			.0 (.0–100.0)				
m/z 40,41,69,94,120,135	1,493		8.7 (4.4–12.9)					
m/z 40,55,69,135,187,223	1,505		1.0 (.4–1.6)					
m/z 40,69,105,121,136,177	1,508		1.6 (.0–2.1)					
m/z 41,55,69,102,121,136	1,518		.9 (.0–18.6)					
Total amount of scent ng/flower/h		4.8 (2.8–8.5)	17.6 (16.3–18.9)	21.3 (2.8–78.7)	4.1 (.1–15.0)	31.9 (.9–184.1)	31.3 (2.0–87.4)	24.4 (2.2–58.6)

Note: n indicates the number of samples that contained detectable scent compounds out of the total number of samples analyzed. Median relative amounts of the specific volatile compounds are given, and compounds are arranged according to chemical class and sorted by the Kovats retention index (RI; ZB-5 column). Minimum/maximum values are given in parentheses. Total median amount of scent released per flower per hour (ng) is given in the final row. For *M. furvanthera*, only diurnal scent samples contained compounds, while zero of the two analyzed night scent samples contained compounds. Pollinator groups: FR = flowerpiercer/rodent; HB = hummingbird/bat; HR = hummingbird/rodent.

[a] Identified based on the retention time and mass spectra of synthetic standard compounds.

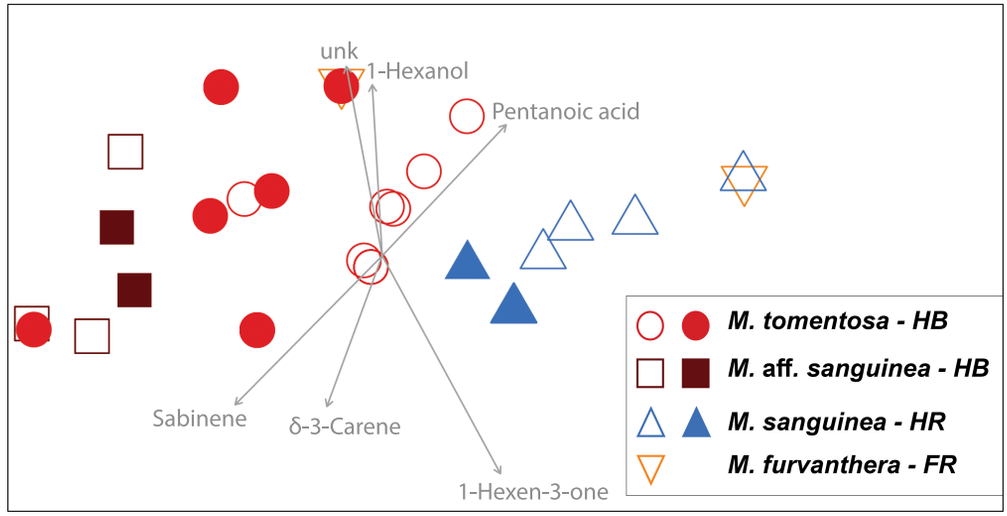

Figure 4: Nonmetric multidimensional scaling based on a Bray-Curtis dissimilarity matrix to display semiquantitative differences in day and night scent profiles of the four *Meriania* species. The stress value of 0.018 indicates a good representation of the observed similarities among scent samples. The six compounds correlating best with the coordinates are given. Pollinator groups: FR = flowerpiercer/rodent; HB = hummingbird/bat; HR = hummingbird/rodent.

apparent: nectar sugar composition follows typical diurnal bird pollinator preferences (Johnson and Nicolson 2008), and scent profiles partially show adaptations to the different nocturnal pollinators.

Our finding of effective rodent pollination in *Meriania sanguinea* and *Meriania furvanthera* is particularly interesting given the rarity of documented cases of rodent pollination in general and especially in the New World (e.g., Melastomataceae [Lumer 1980]; Loasaceae [Cocucci and Sérsic 1998]; Proteaceae [Cárdenas et al. 2017]). Both species with rodent pollination show modifications in their inflorescence architecture (short-pediceled flowers in leaf axils in *M. furvanthera*; fig. 1*B*) or growth form (procumbent habit of *M. sanguinea*; fig. 1*C*), which facilitate access to flowers by perching pollinators. This is in contrast to flowers protruding on long inflorescence stalks in *Meriania tomentosa* and *Meriania* aff. *sanguinea* (fig. 1*A*), which are visited only by pollinators capable of hovering while drinking nectar (HB). Although rodent visitation rates were 10 times lower than hummingbird visitation rates in *M. sanguinea* (table 1), rodents contributed substantially to pollen deposition on stigmas and hence must be considered as legitimate pollinators (fig. 2). Likewise, hummingbirds were more frequent visitors than bats in *M. tomentosa* and *M.* aff. *sanguinea* but deposited the same or lower amounts of pollen. It is possible that the relatively small experimental sample sizes have reduced the power of detecting significant differences between the diurnal and nocturnal pollinators in *M. sanguinea* and *M. tomentosa*. Interestingly, excluding either pollinator group did not significantly reduce stigma pollen loads as compared to open controls in these two species. This merits further investigation, as it could indicate that each plant species could successfully reproduce if visited by one pollinator group only. In *M.* aff. *sanguinea*, bats were more effective pollinators than hummingbirds. However, there are clearly more aspects to pollinator quality than only pollen deposition (but see Muchhala et al. 2009 for a similar approach to ours). Quality differences between pollinators also involve differences in the efficiency of removing pollen, which then gets deposited (and not lost), and the "purity" of deposited pollen (e.g., amount of heterospecific pollen; see Morales and Traveset 2008; Queiroz et al. 2015), as well as the genetic compatibility/viability of deposited pollen (e.g., self-/outcross pollen and consequently fitness of offspring; Minnaar et al. 2018). Manual pollination experiments in *M. sanguinea* and *M. tomentosa* showed self-compatibility (A. S. Dellinger, unpublished data). Thus, more fine-grained assessments of stigmatic pollen loads could bring out subtle quality differences between the different pollinator groups in the future.

We found nectar secretion only from stamens, which contradicts findings on hypanthial nectar secretion in Merianieae (Varassin et al. 2008; but see also Stein and Tobe 1989). Although the exact location of nectar secretion is variable, the systems are overall similar in having unspecialized staminal nectaries with direct connection to the phloem. Possibly, the pronounced stamen movement in early stages of anthesis (fig. 1*H*–1*J*) leads to high pressure in the tissue,

which causes tissue rupture and phloem sap leakage (Vogel 1997; De la Barrera and Nobel 2004). This idea is supported by our finding that ruptures form only within the first hours of anthesis but are not present in the bud stage. Generally, invertases can change sucrose-rich phloem composition in the nectary (Nicolson et al. 2007), and plants have been found to even be capable of changing their nectar composition between day and night (e.g., in *Inga sessilis*; Amorim et al. 2013). In the *Meriania* species studied here, nectar sugar composition did not change between day and night and sugar compositions corresponded to preferences described for bird pollinators (Johnson and Nicolson 2008). We found a clear differentiation between specialized nectar feeders (hummingbirds, sucrose rich: *M. tomentosa*, *M.* aff. *sanguinea*, *M. sanguinea*) and more generalist nectar feeders (flowerpiercers, hexose rich: *M. furvanthera*; fig. 3). The hexose-rich nectar of *M. furvanthera*, however, indicates the presence of nectary invertases despite the unspecialized nectar leakage (De la Barrera and Nobel 2004; for hexose-rich food bodies in closely related passerine-pollinated *Axinaea*, see also Dellinger et al. 2014). The origin of the large variability in nectar sugar composition in *M. tomentosa* remains unknown but could be interpreted as a means of meeting both hummingbird and bat preferences (Abrahamczyk et al. 2017).

Contrary to our expectation of increased floral scent release during nighttime as an adaptation to bat and rodent attraction (Dobson 2006), flowers did not smell more strongly at night. At the level of scent classes, *M. tomentosa* and *M.* aff. *sanguinea* (both HB) released higher amounts of terpenoids, known to be important in bat pollination, while aliphatic compounds were dominant in rodent-pollinated *M. sanguinea* and *M. furvanthera* (fig. A4; Knudsen et al. 1995; Pettersson et al. 2004; Dobson et al. 2006). *Meriania sanguinea* is particularly interesting in this context: 1-hexen-3-one (mostly confined to nocturnal scent samples) is known as flower scent only from *Cytinus visseri* (Cytinaceae, Malvales), a parasitic South African plant pollinated by rodents and shrews (Johnson et al. 2010). Curiously, 1-hexen-3-one worked as a repellent when tested alone in a pollinator behavioral assay but had no negative effects when tested in combination with the strong attractant 3-hexanone, also released by *C. visseri* (Johnson et al. 2010). In *M. sanguinea*, however, 3-hexanone was detected only during daytime when rodents are not active. Thus, the role of 1-hexen-3-one as pollinator attractant in *M. sanguinea* remains equivocal. At the larger scale, however, the simultaneous occurrence of 1-hexen-3-one in plants of different orders (Myrtales, Malvales) and continents (South America, Africa) points toward convergence in the evolution of this compound to communicate with ground-dwelling mammals. Given the lack of detectable scent compounds at night in *M. furvanthera*, it remains unclear how this species attracts its mammal pollinators. Interestingly, these results are in line with a study reporting lack of floral scent in other Melastomataceae species (genus *Blakea*) for which rodent visitation has been reported (Lumer 1980; Wester et al. 2016). Furthermore, it is notable that all four *Meriania* taxa released scents during daytime (table 3). In traditional pollination syndrome theory, "bird" flowers usually attract pollinators by visual cues (showy red flowers) rather than scent (Dobson et al. 2006). More recent studies, however, indicate that birds use olfactory cues in addition to vision when foraging (Kessler and Baldwin 2007).

Taken together, our results support the view that nectar-producing *Meriania* species, summarized into a mixed vertebrate pollination syndrome, comprise different bimodal pollination systems. While studies on nectar-secreting Melastomataceae from other tribes (e.g., Miconieae) report an increased "generalization" (e.g., Kriebel and Zumbado 2014; Brito et al. 2017), our mixed vertebrate syndrome is better described as "specialized bimodal" (cf. Manning and Goldblatt 2005). Such bimodal systems have been considered as labile, possibly representing evolutionary transitions between distinct pollination syndromes (Manning and Goldblatt 2005). Given the ancestral bee-buzz pollination syndrome in Merianieae, one could expect such transitions between (ancestral) bee pollinators and a (derived) vertebrate pollinator or further transitions between two functional vertebrate pollinators (e.g., hummingbird to bat; Rosas-Guerrero et al. 2014). Alternatively, bimodal pollination systems in *Meriania* could have arisen without prior specialization on one new functional group but actually represent stable systems adapted to exploit two complementary groups of pollinators. This scenario seems plausible in *Meriania* given the lack of (ancestral) bee pollinators in the mixed vertebrate syndrome and the fact that there is, to date, no nectar-secreting *Meriania* species known to be pollinated by only one type of vertebrate pollinator (either hummingbirds, flowerpiercers, bats, or rodents). The repeated independent origin of different bimodal systems (shift 1: *M. tomentosa* [HB], *M. furvanthera* [FR]; shift 2: *M.* aff. *sanguinea* [HB], *M. sanguinea* [HR]) further supports the idea of a stable pollination strategy. This is particularly interesting given that much work on pollinator shifts has found strong adaptive trade-offs in species visited by two functional pollinator groups (Castellanos et al. 2004; Muchhala 2007). While clear "pro-bird, anti-bee" changes in floral traits have been documented in *Penstemon* (Castellanos et al. 2004), our results indicate that *Meriania* flowers can successfully exploit two different functional pollinator groups. Taking this thought further, our results suggest that certain combinations of pollinators, such as hummingbirds and bats, may potentially come without (or with very small) fitness trade-offs, at least in some systems. More detailed experiments are needed, however, to clarify these ideas.

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Black-thighed puffleg (*Eriocnemis derbyi*) sitting on infructescence of *Meriania tetragona* after visiting its flowers in the Tapichalaca Reserve (Jocotoco Foundation). Photo © Francisco Sornoza.