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Pollinator Shifts and Floral Evolution in Merianieae (Melastomataceae)

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TABLE OF CONTENTS

ABSTRACT

Pollinator-mediated selection is a major driver of flower diversity in angiosperms. Recurring floral trait combinations (pollination syndromes) have been associated with convergent evolution in response to parallel selection pressures imposed by pollinators of the same functional group (e.g. hummingbirds). Shifts between functional groups (e.g. bees to hummingbirds) change selection regimes on flowers and ultimately lead to flower diversification and distinct pollination syndromes. Pollinator-mediated selection is not necessarily homogeneous across the flower. Traits involved in attracting pollinators (e.g. scent, colour, display size) may underlie different selection pressures than 'efficiency traits' mediating efficient pollen transfer (e.g. anther-stigma distance). The direct comparison of closely related plant taxa that have shifted functional pollinator groups has great power in elucidating how selection affects floral diversification, trait functioning and angiosperm evolution in general. Nevertheless, relatively few study groups exist where pollinator shifts and floral trait change have been explored across macroevolutionary scales.

It was the aim of my PhD project to work towards establishing the Melastomataceae tribe Merianieae as a new model system for the study of floral evolution and pollinator-mediated selection. Merianieae occur in the Neotropics with their centre of diversity in the tropical Andes. At the level of Melastomataceae as a whole, the vast majority (ca. 98%) of species is pollinated by buzzing bees but literature suggested occasional shifts to vertebrate pollination. I combined extensive fieldwork in Ecuador and Costa Rica with detailed morphological and structural assessments of flowers of 61 Merianieae species and multivariate statistics to characterize pollination syndromes in the tribe. Together with my collaborators Darin Penneys and Fabián Michelangeli, I produced a Merianieae phylogeny, which allowed tracing pollinator shifts and floral evolution in the group.

I found that buzz-pollination by bees, with a pollen reward and vibratile pollen release, is the ancestral and most widespread pollination syndrome in Merianieae. The 'buzz-bee' syndrome has been destabilized repeatedly and multiple independent shifts into two novel vertebrate pollination syndromes have occurred. The new 'mixed-vertebrate' syndrome is characterized by nectar rewards, a 'salt-shaker' like pollen release mechanism and combinations of different diurnal (hummingbirds, flowerpiercers) and nocturnal (bats, rodents) functional pollinator groups. The 'passerine' syndrome is characterized by an

elaborate ‘bellows’ mechanism for pollen expulsion and associated food-body rewards. I could further demonstrate that floral evolution happened via coordinated changes of functionally related traits (modules), even across floral organ categories (whorls). Fit with the different functional pollinator groups was apparently optimized in each syndrome independently. Given the prevalence of buzz-pollination in Merianieae and Melastomataceae, I hypothesize that the high levels of floral modularity detected in the ‘buzz-bee’ syndrome were key to their evolutionary success. The relative independence of distinct functional floral modules possibly enhanced their flexibility to respond to slightly different selection pressures imposed by the large diversity of bees capable of buzz-pollination. This may have allowed for extensive ‘adaptive wandering’ on the ‘buzz-bee’ pollination plateau.

My results exemplify the great need of basic fieldwork, particularly in the notoriously understudied biodiversity hotspots of our planet such as the tropical Andes, to understand the natural history of its organisms. Among other things, I have documented rodent pollination in Merianieae, which is extremely rare outside of South Africa. Rodent pollinated Merianieae release a scent compound otherwise only known from mammal pollinated South African plants from another order (Malvales, as opposed to Myrtales where Melastomataceae belong to), and hence opens up new questions on convergent evolution of a compound apparently involved in the communication between plants and mammals. Exploratory fieldwork, which forms the basis of this project, may hence help to generate the potential for new ideas, hypotheses and concepts for future research and conservation.

ZUSAMMENFASSUNG

Die enorme Blütendiversität der Angiospermen ist vor allem durch Selektion durch unterschiedliche Bestäuber entstanden. Gewisse Merkmalskombinationen (Bestäubungssyndrome) treten häufig in Verbindung mit bestimmten funktionellen Bestäubergruppen auf und repräsentieren vermutlich konvergente Anpassungen an diese Bestäubergruppen. Wechsel zwischen Bestäubergruppen (z.B. von Bienen zu Kolibris) gehen Hand in Hand mit einer Veränderung der Selektionsdrücke auf Blüten und führen zur Entstehung unterschiedlicher Blüten. Selektion durch Bestäuber wirkt aber nicht zwangsläufig auf alle Blütenmerkmale gleich. Merkmale wie Duft, Farbe oder Blütengröße, die vor allem der Bestäuberanlockung dienen, unterliegen aller Wahrscheinlichkeit nach anderen Selektionsdrücken als Merkmale, die den optimalen Pollentransfer (Passform mit dem Bestäuber) gewährleisten. Um zu verstehen, wie Selektion Blütenvielfalt, die Funktion von Blütenorganen und die Evolution der Blütenpflanzen im Allgemeinen beeinflusst, ist der Vergleich nah verwandter Pflanzenarten, die Bestäuber gewechselt haben, besonders aufschlussreich. Nichtsdestotrotz gibt es nur relativ wenige Pflanzengruppen, wo der Einfluss von Bestäuberwechseln und Blütenevolution auf makroevolutiver Ebene getestet wurde.

Ziel meines Dissertationsprojekts war eine grundlegende blüten- und bestäubungsbiologische Charakterisierung der Tribus Merianieae (Melastomataceae), die im Weiteren als Modellsystem für Forschung zu Bestäuberwechseln dienen kann. Merianieae kommen in den Neotropen vor und sind im tropischen Teil der Anden am häufigsten. Etwa 98% der Arten dieser Familie werden durch Bienen vibrationsbestäubt (buzz-pollinated), doch in der Literatur finden sich auch einzelne Hinweise auf Bestäuberwechsel zu Vertebraten. Um Bestäubungssyndrome in Merianieae zu charakterisieren, verband ich umfassende Feldarbeiten in Ekuador und Costa Rica mit einer detaillierten morphologischen und strukturellen Aufarbeitung von Blütenmaterial von 61 Arten sowie multivariaten statistischen Methoden. Gemeinsam mit meinen Kooperationspartnern Darin Penneys und Fabián Michelangeli erarbeitete ich eine Phylogenie für Merianieae, die es mir erlaubte, Bestäuberwechsel und die Veränderung von Blütenmerkmalen in einen evolutionären Kontext zu setzen.

Vibrationsbestäubung durch Bienen ist das ursprüngliche und am weitesten verbreitete Bestäubungssystem in den Merianieae und ist durch Pollen als Bestäuberbelohnung sowie Pollenfreisetzung durch Vibrationen gekennzeichnet. Mehrere unabhängige Wechsel von Bienen zu zwei Vertebratenbestäubungssyndromen haben bei Merianieae stattgefunden. Ich habe ein neues gemischtes Vertebratenbestäubungssyndrom beschrieben, in dem jede Art von

je einer tag- und einer nachtaktiven funktionellen Bestäubergruppe besucht wird (z.B. Kolibris und Fledermäuse oder Kolibris und Mäuse). Als Bestäuberbelohnung ist Nektar entstanden und einer neuer Mechanismus der Pollenfreisetzung hat sich entwickelt, den ich „Salzstreuermechanismus“ genannt habe. Das zweite neue Bestäubungs-syndrom ist ein Sperlingsvogelsyndrom mit einem komplexen Blasebalg-mechanismus zur Pollenfreisetzung und einer damit zusammenhängenden Bestäuber-belohnung, Futterkörperchen, die von den Staubblättern gebildet werden. Des Weiteren konnte ich zeigen, dass Blütenevolution in Merianieae durch die koordinierte Veränderung von Blütenmerkmalen passiert, die eine gemeinsame Funktion im Bestäubungs-prozess übernehmen (Module). Dies hat auch über verschiedene Organkategorien hinweg Bestand. Die optimale Passform zwischen Blüte und Bestäuber wurde offensichtlich in jedem Syndrom durch eine Veränderung dieser funktionellen Module erreicht. Ich stelle die Hypothese auf, dass die hohe Unabhängigkeit dieser funktionellen Module bei den bienenbestäubten Merianieae ihr Schlüssel zum evolutionären Erfolg war. Die Möglichkeit zur relativ unabhängigen Veränderung funktioneller Module untereinander könnte die Anpassungsfähigkeit an leicht unterschiedliche Selektionsdrücke deutlich erhöhen. Entsprechend unterschiedliche Selektionsdrücke sind aufgrund der hohen Diversität an Bienen, die Vibrationsbestäubung durchführen können, zu erwarten, und könnten zu dem sehr diversen „Anpassungsplateau“ (adaptive plateau) der Bienenbestäubten beigetragen haben.

Meine Ergebnisse zeigen die Notwendigkeit und den wissenschaftlichen Wert von grundlegender, beschreibender Feldarbeit, vor allem in den nach wie vor unzureichend untersuchten Biodiversitätshotspots der Welt wie beispielsweise den tropischen Anden. Ich habe unteranderem Mäusebestäubung in Merianieae entdeckt, die außerhalb von Südafrika bislang nur sehr selten dokumentiert wurde. Mäusebestäubte Merianieae produzieren spezielle Duftstoffe, die sonst nur von einer südafrikanischen elefanten-spitzmausbestäubten Pflanze aus einer andern Ordnung (Malvales, im Gegensatz zu Myrtales, zu denen Melastomataceae gehören) bekannt sind. Diese Entdeckung eröffnet neue Fragen über konvergente Evolution von Duftstoffen, die in der Kommunikation zwischen Pflanzen und Säugetieren bedeutend sind. Dokumentarische Feldarbeit wie in diesem Projekt schafft die Grundlage zur Entwicklung neuer Ideen, Hypothesen und Konzepte für zukünftige Forschungsfragen und den Naturschutz.

1. INTRODUCTION

SETTING THE FRAME – BACKGROUND

This doctoral thesis is part of a larger project on pollinator shifts and floral evolution in the Merianieae (Melastomataceae) initiated by Jürg Schönenberger (PhD supervisor), Darin Penneys (international collaborator) and myself. The work we have accomplished so far provides a solid baseline of knowledge on the natural history, in particular the pollination biology, of the group. Merianieae belong to the world's seventh largest plant family (dos Santos et al. 2012). As in many other tropical lineages, however, the group's biology is poorly understood and broad generalizations for hundreds of species are based on a handful of studied taxa only. As of the year 2012, for example, pollinators had only been observed in seven of the approximately 300 species of the tribe (ca. 2%; Renner 1989, Vogel 1988). It was our collaborator Darin Penneys who suggested investigating the pollination biology of the Merianieae genus *Axinaea*. Our discovery of a novel passerine pollination system in *Axinaea* (my master thesis' work and resulting publication: Dellinger et al. 2014), together with bee, hummingbird and bat pollination documented in the literature (Renner 1989), rendered Merianieae an excellent system for studying drivers and consequences of pollinator shifts. We take a broad, comparative approach with the aim of integrating microevolutionary (ecological) patterns into a macroevolutionary (phylogenetic) framework to better understand the role of pollinator-mediated selection on angiosperm diversification (Smith 2010, van der Niet et al. 2014). In the following chapters, I will first introduce the concepts and theories which underlie my PhD thesis. I will then spend some words on the field- and lab work I did, which consumed a large part of my project's time and involves/d the collaboration with a number of invaluable people. The introductory sections are followed by three papers/manuscripts (one published, one under review, and one ready for submission), which make up the core of my dissertation work. I conclude the thesis by a general discussion.

FLORAL EVOLUTION AND POLLINATION BIOLOGY

Flowers are the defining structure of all angiosperms and represent an astounding diversity in structure, colour, reward and scent (Endress 1996, Kay et al. 2005, Specht &

Bartlett 2009, Sauquet et al. 2017). This diversity has largely been attributed to selection imposed by pollinating agents, and, to a lesser extent, by floral antagonists and abiotic factors (reviewed in Strauss & Whitall 2006, Harder & Johnson 2009, van der Niet et al. 2014, Gervasi & Schiestl 2017, Campbell et al. 2018). Pollinator mediated floral diversification may arise through obligate co-evolutionary processes between flowers and pollinators, impressively exemplified by the tight relationships between figs and fig-wasps (Thompson 2005). Alternatively, the same pollinator could be 'used divergently' by different plant species, e.g. by pollen placement on different parts of the pollinator's body (e.g. *Pedicularis*, Huang & Shi 2013, Stewart & Dudash 2017). A third mechanism, common in many angiosperm radiations, are pollinator shifts (e.g. Kay et al. 2005, Whittall & Hodges 2007, Smith et al. 2008, Thomson & Wilson 2008, Lagomarsino et al. 2016).

The pollinator-shift model, conceptualized by Grant & Grant (1965) and Stebbins (1970), and formalized by Johnson (2006), proposes convergent floral adaptation (pollination syndromes, Delpino 1890, Faegri & van der Pijl 1979) to specific functional pollinator groups. Functional pollinator groups are defined as groups of pollinators selecting for the same floral phenotype, while different functional groups will select for different phenotypes (Fenster et al. 2004). Thus, per definition, shifts between functional pollinator groups (e.g. from bee to hummingbird) can ultimately be related to floral morphological diversification as they entail major changes in phenotypic selection regimes (Harder & Johnson 2009, van der Niet et al. 2014, Smith & Kriebel 2018).

The pollination syndrome concept as a framework for structuring floral diversity in angiosperms has received considerable attention and stimulated controversial debate in recent years (e.g. Waser et al. 1996, Ollerton et al. 2009, Armbruster et al. 2011, Rosas-Guerrero et al. 2014, Lagomarsino et al. 2017). Over-simplification of complex plant-animal interactions and the lack of a unified terminology have been identified as major shortfalls of the concept (e.g. pollination biology pioneer Stefan Vogel refused to recognize a 'beetle syndrome', which other authors consider as valid; Johnson & Wester 2017). At this point, it is essential to note that early authors like Stebbins were extremely cautious in formulating 'character syndromes' and their applicability. In his much-cited 1970 paper, Stebbins phrases the 'most effective pollinator principle', which assumes floral adaptation to a plant's most frequent and most efficient (in removing and depositing pollen) pollinator. Stebbins stresses that 'character syndromes' do not ultimately preclude the existence of secondary, less efficient pollinators, which are actually common in many

systems (Rosas-Guerrero et al. 2014). Most importantly, “at least in our present state of knowledge”, Stebbins calls for “direct studies of the functional relationships of particular kinds of flowers to clearly identify pollinators” before making broad generalizations (Stebbins 1970). Although almost 50 years have gone by and the field of pollination biology has matured, generalizations of pollination syndromes still need to be treated with care (Rosas-Guerrero et al. 2014). A classic and much investigated generalization is found in the traditional ‘hummingbird syndrome’: red, unscented flowers (Faegri & van der Pijl 1979). Already Grant (1966) demonstrated that hummingbirds do not select for red colour per se. More recent studies have shown that ‘red’ might rather be an avoidance strategy against (less efficient) bee pollinators, which cannot see red (Lunau et al. 2011, Camargo et al. 2018). Also, colouration of co-occurring hummingbird pollinated flowers may be driven by interspecific competition rather than colour preferences (Muchhala et al. 2014). Still, a large number of hummingbird flowers are indeed red, but the evolutionary mechanisms leading to this hummingbird-red association are apparently manifold and not straightforward (Cronk & Ojeda 2008). On the one hand, these examples show the necessity of carefully re-reading the work of the founders of the field of pollination biology. On the other hand, they give an idea of the complexity of interactions and evolutionary processes which may generate floral diversity.

At least two other factors have to be considered when thinking about floral evolution and pollinator mediated selection. The first is the study system’s own evolutionary history and possible developmental and genetic constraints, which may limit the number of trait combinations that could possibly evolve (Campbell et al. 1994, Campbell 1996, Smith & Rausher 2008, O’Meara et al. 2016, Smith 2016). Darwin recognized the importance of the “evolutionary starting point” and several authors have discussed how similar selective pressures, acting on different starting points, will ultimately generate different adaptive responses and outcomes (Stebbins 1950, Armbruster 2005). These ideas could be applied directly to flowers that have been ancestrally bee pollinated (starting point), and which have shifted to hummingbird pollination (a shift-directionality reported in many systems; Thomson & Wilson 2008), such as, for instance, *Aquilegia, Costus, Ipomoea, Mimulus, Salvia* and *Silene*. The overall resemblance of the hummingbird flowers across these systems is striking (hummingbird syndrome), but certain lineage-specific ‘starting points’, e.g. five separate nectar spurs in *Aquilegia*, or radial symmetry in *Ipomoea* and *Silene* (as opposed to zygomorphy in many hummingbird pollinated flowers, Cronk & Ojeda 2008), were conserved.

The second factor which needs attention is that not all floral traits are affected equally by pollinator mediated selection (Pérez et al. 2008, Pérez-Barrales et al. 2014, Ordano et al. 2008). Flowers are integrated structures where traits function in a coordinated manner to achieve pollinator attraction, deterrence of herbivores, transfer of pollen, and, to some extent, protection of fruits and seeds from predation (Murren 2002, Armbruster et al. 2004, Ordano et al. 2008, Endress 2016). To further complicate matters, flowers are made up by distinct ontogenetic organ categories, generally the perianth (produced by one or more whorls/sets of organs), the androecium and the gynoecium (Endress 1994). In her pioneering work on correlation pleiades, Berg (1960) demonstrated the relative independence between vegetative and reproductive (floral) plant traits. Since then, a limited number of studies attempted to test whether flowers are integrated throughout, or whether they can be divided into modules, which can evolve relatively independently from each other (Armbruster et al. 2004, Armbruster et al. 2014, Pérez-Barrales et al. 2014, Diggle 2014). While hypotheses on modularity and form evolution are well established in anthropology and zoology, patterns in plants remain unclear and have rarely been tested at a macroevolutionary scale (Herrera et al. 2002, Benítez-Vieyra et al. 2006, Fenster et al. 2009, Diggle 2014, Esteve-Altava 2016). Some authors indeed found support for 'developmental modules' in flowers, which correspond to ontogenetic organ categories (e.g. *Helleborus*, Herrera et al. 2002). The majority of studies, however, has detected 'functional modules', which span different organ categories and are united by shared function, most likely driven by pollinator mediated selection (Benítez-Vieyra et al. 2006, Rosas-Guerrero et al. 2011, Esteve-Altava 2016). For example, flowers could be partitioned into two functional modules, one module comprising traits involved in pollinator attraction ('advertisement module') and the second module comprising traits involved in mediating fit with the pollinator ('efficiency module'). Both the type and strength of selection on these modules are likely different, however (Armbruster et al. 2005, Benítez-Vieyra et al. 2006, Strauss & Whitall 2006, Rosas-Guerrero et al. 2011). 'Advertisement traits/modules' are generally the first, coarse filters that determine which animals are attracted to a given flower (Thomson & Wilson 2008). These traits may hence underlie conflicting selection regimes, including attraction of pollinators and deterrence of herbivores or less efficient pollinators (Armbruster et al. 2005, Camargo et al. 2018). 'Efficiency traits/modules', on the other hand, mediate the fine-tuning between flower and pollinator and are thought to evolve under strong directional or stabilizing

selection (Benítez-Vieyra et al. 2006, Thomson & Wilson 2008, Rosas-Guerrero et al. 2011).

POLLINATOR SHIFTS AND THE NEOTROPICS

Pollinator efficiency is defined as the product of pollinator quantity (visitation rate) and quality (the capability of pollen transfer (Ne'eman et al. 2010)) and has been proposed as the main trigger of pollinator shifts (Thomson & Wilson 2008). Generally, stabilizing selection will act in the present pollination system, balancing a plant species on the 'optimal' trait combination to guarantee high fitness (siring success and seed set, Armbruster et al. 2009). For a pollinator shift to occur, this stabilizing selection has to be overcome. Traditional concepts envision a scenario where different populations of a plant species experience slightly different selection regimes due to differences in the geographic distribution mosaic of pollinators ('Grant-Stebbins model', Johnson 2006). This will create 'pollination ecotypes', which, over time, may result in taxonomically noteworthy differentiation among populations. They are, however, not comparable to the pronounced flower trait changes occurring with shifts between distinct functional pollinator groups (Thomson & Wilson 2008). Shifts in functional pollinator groups will most likely result in highly differentiated floral phenotypes which are optimized to attract and fit to the new functional pollinator group. More recently, attempts have been made to identify the mechanisms of the pollinator shift process. Thomson & Wilson (2008) proposed a scenario where an extrinsic environmental factor first decreases the visitation frequency of the ancestral (most efficient) pollinator. Pollination services by a second (possibly previously less efficient) pollinator may hence become more important. If this situation persists long enough, floral traits may gradually respond to selection by the second pollinator with increased attractiveness and optimization of conspecific pollen delivery (Toon et al. 2014). As a side note, the appearance of an alien (e.g. invasive) pollinator in the modern, human-altered world, could also trigger such a pollinator shift (Medel et al. 2018).

Pollinator shifts have occurred across the globe. Some relatively well studied systems are found in the South African flora (Vogel 1954, Johnson 2010) and the Neotropics (e.g. *Costus* (Costaceae), Kay et al. 2005; *Iochroma* (Solanaceae), Smith et al. 2008; Lobelioideae (Campanulaceae), Lagomarsino et al. 2016; Gesneriaceae, Serrano-Serrano et al. 2017; *Salvia* (Lamiaceae), Fragoso-Martínez et al. 2018). In the above-mentioned

Neotropical lineages, a strong association between shifts from bee to vertebrate (predominantly hummingbird) pollination and growth at medium to high elevations is apparent (less clear in *Costus*). Reduced efficiency of bee pollinators under adverse weather conditions at high elevations has been hypothesized as a major cause of this pattern (Cruden 1972). While bees are sensitive to lower temperatures, strong winds and rain common in tropical montane ecosystems (e.g. cloud forests), vertebrates are less impeded by these conditions. Most of the studies which have tried to trace pollinator shifts on dated phylogenies found relatively recent origins of these shifts, coinciding with the Andean uplift and (in some cases) with hummingbird diversification (Kay et al. 2005, Tripp and McDade 2013; Givnish et al. 2014; Roalson and Roberts, 2016; Serrano-Serrano et al. 2017; Tripp and Tsai 2017, Lagomarsino et al. 2016). Thus, the pollinator shift scenario envisioned by Thomson & Wilson (2008), where an extrinsic environmental factor changes visitation frequencies, seems plausible. If plant lineages tracked and colonized the new habitats gradually developing with the Andean uplift, they potentially did not only move from their ancestral abiotic niches, but also moved away from habitats where bees are most efficient pollinators (e.g. tropical lowland rainforests). Migration into the new montane habitats may have destabilized the bee pollination systems in some instances and triggered shifts to vertebrate pollination.

Pollination Biology and Floral Morphology in Melastomataceae

With approximately 5000 species, Melastomataceae are the seventh largest plant family (dos Santos et al. 2012) and pantropically distributed, with their major centre of diversity in the Neotropics (70% of species, Veranso-Libalah et al. 2017). Acrodromal leaf venation and the loss of an endothecium functional in the anther dehiscence are two characters shared by the New World Melastomataceae (Clausing & Renner 2001). A hypothesis proposed by Clausing & Renner (2001) states that the loss of a functional endothecium preceded another character found in the majority of Melastomataceae: poricidal anther dehiscence. Poricidal anther dehiscence is the crucial morphological character related to the pollination system prevalent in the family: buzz-pollination by bees. Buzz-pollination is a functionally highly specialized pollination system, in which bees apply high-frequency vibrations to stamens in order to extract pollen from the tubular anthers (Buchmann 1983). Buzz-pollination has evolved repeatedly across

angiosperms and is found in approximately 8-10% of species representing at least 72 families (Cardinal et al. 2018). The diversity of bees exhibiting the buzzing behaviour is equally scattered across the bee phylogenetic tree and found in an at least 74 bee genera (de Luca & Vallejo-Marín 2013).

In Melastomataceae, buzz-pollination is the most common pollination system and has been estimated to occur in about 98% of species (Renner 1989). The prevalence of this pollination system has been related to exceptional evolutionary success and described as an 'adaptive plateau' the family is wandering upon (Macior 1971, Berger et al. 2016, Reginato & Michelangeli 2016). In the remaining 2% of species, however, the adaptive plateau has apparently been de-stabilized and pollinator shifts have occurred. These shifts include shifts to other insect pollinators (e.g. flies, wasps) or vertebrates (hummingbirds, passerine birds, bats, rodents; Lumer 1980; Renner 1989; Vogel 1997; Dellinger et al. 2014; Brito et al. 2017). Up to now, pollinator shifts have only been documented in four tribes of New World melastomes and not in the Old World (Renner 1989). In three of these tribes (Blakeae, Merianieae, Tibouchineae), pollinator shifts show a strict association to growth at higher elevations, and all of these shifts are shifts to vertebrate pollinators (Renner 1989, Varassin 2008). Also in the fourth tribe (Miconieae), shifts to vertebrate pollinators are related to higher elevations while some lowland species have apparently shifted towards more generalized insect pollination systems (Renner 1989, Brito et al. 2017). In all species which have undergone pollinator shifts, new reward types have evolved (Varassin et al. 2008, Dellinger et al. 2014). While pollen is the only reward in the buzz-bee pollinated species, most shifted species offer nectar as a reward and a small group of species in the Merianieae has evolved a food-body reward (Dellinger et al. 2014).

With the clear dominance of bee pollination, the repeated shifts to different pollination strategies, and the diversity in species numbers and colonized habitats, Melastomataceae offer an ideal system for studying longstanding questions of angiosperm evolution and floral diversification.

AIMS AND RESEARCH OUTLINE

It is the aim of my PhD thesis to establish a broad and thorough understanding of the pollination biology and floral evolution in the Melastomataceae tribe Merianieae in order to develop a new model system for the study of pollinator shifts in the tropical Andes.

The tropical Andes are the world's most species rich biodiversity hotspot, yet very little is known about the natural history of the species making up this diversity (Myers et al. 2000). Thus, my project focused on increasing the number of Merianieae species with documented pollinators and understanding the pollination process by extensive fieldwork and comparative experiments. In addition, detailed structural studies on flowers across a wide taxonomic sampling of the tribe are aimed at providing insights into patterns of floral evolution. Finally, a modern model system does not make 'sense except in the light of evolution' (Dobzhansky 1973), and thanks to the efforts made by my collaborators Darin Penneys and Fabián Michelangeli, we managed to put together a first phylogeny of 150 Merianieae species (ca. 50% of the tribe). This phylogeny allows to trace pollinator shifts and floral evolution through evolutionary time and provides, next to my work on pollination systems, the second important pillar for exciting future research in the group.

Chapter I presents the essential results from my attempts of organizing and understanding flower morphological diversity in Merianieae. I employed the pollination syndrome concept to test whether it is possible to define distinct syndromes in Merainieae and whether they are useful in predicting pollinators for species where pollinators are yet unknown. Indeed, I detected three distinct pollination syndromes ('buzz-bee', 'mixed-vertebrate' and 'passerine') in the tribe. They are, however, not characterized by traditional syndrome traits but rather by two specific character complexes, the mechanism of pollen expulsion and the reward type. Extensive fieldwork to increase pollinator observations and structural work on flowers of 61 species form the data basis for this study.

In Chapter II, I focus on one of the three detected pollination syndromes, namely the 'mixed-vertebrate' syndrome. This syndrome is peculiar in that all species investigated in the field are visited both by a diurnal (hummingbirds or flowerpiercers) and by a nocturnal (bats or rodents) functional pollinator group. I explore whether both diurnal and nocturnal visitors actually are efficient pollinators and whether nectar and scent traits show adaptations to either functional group. I find relative equality in terms of effectiveness of both pollinator groups in each species. Nectar traits mostly follow documented bird pollinator preferences while scent profiles indicate some adaptation to nocturnal pollinators. I hence conclude that the 'mixed-vertebrate' syndrome of Merianieae is apparently made up of different 'bimodal' pollination systems.

Finally, in Chapter III, I explore floral evolution by testing competing hypotheses on floral modularity, including hypotheses on floral ontogenetic modules (organ categories) and pollination-related functional modules. All hypotheses are tested both within syndromes and across the phylogeny and are based on geometric morphometric assessments of 3D-models of flowers produced by High-Resolution X-ray Computed Tomography of flowers. I find significant shifts in floral modularity with pollinator shifts and strong support for function driving floral modularity patterns rather than ontogeny. Very high degrees of modularity within the 'buzz-bee' syndrome potentially explain how these species could remain on their 'adaptive plateau' as they were flexible to respond to small changes in selection regimes. High levels of modularity can also explain how Merianieae could shift from the functionally highly specialized 'buzz-bee' syndrome to the vertebrate pollination syndromes.

2. Fieldwork and Lab Experience

Fieldwork in Ecuador and Costa Rica

In order to increase the number of species with documented pollinators in Merianieae, to carry out pollination experiments, and to collect more flower material, I conducted five fieldtrips to Latin America between 2015 and 2018 (Table 1). These trips consisted of longer stays at different research stations to carry out pollination experiments and observations, and shorter stays in various nature reserves to collect additional species (Table 2). For pollinator observations, I used video cameras both during day- and night time or directly observed flowers myself. In seven species (two bee pollinated, three pollinated by mixed assemblages of diurnal and nocturnal vertebrates, two passerine pollinated), I conducted pollination experiments and mostly focused on understanding pollen transfer efficiency in the different pollination systems. I presented flowers to pollinators for standardized time intervals (e.g. 6 hours, 12 hours, 24 hours, …), and collected styles and stamens separately for later pollen counting. Thus, I could assess whether the different functional pollinator groups differed in their efficiency in transferring pollen. In addition, I checked nectar availability and measured nectar concentration in nectar producing species using refractometers. In the 2017 fieldtrip, I collected nectar in capillary tubes for later analyses of sugar composition and my field assistant Lisa Scheer collected scent using dynamic headspace techniques (Dötterl & Jürgens 2005). Two master students, Silvia Artuso and Lisa Scheer, assisted during fieldwork in Ecuador in 2016 and 2017, respectively. Also, my local collaborator Diana Fernández-Fernández joined on several expeditions during the two Ecuador fieldtrips and shared her invaluable knowledge on Melastomataceae with me.

Table 1: Fieldtrips undertaken to Latin America.

Country	Period	Type of work
Costa Rica	February & March 2015	Pollination experiments with *Axinaea costaricensis* Cogn. and collections of floral material, leaf samples in sliqua gel and herbarium vouchers
Costa Rica	November & December 2015	Pollinator observations with *Meriania phlomoides* (Triana) Alemda and collections of floral material, leaf samples in sliqua gel and herbarium vouchers
Costa Rica	February 2018	Pollination experiments with *Adelobotrys adscendens* (Sw.) Triana and collection of floral material

Ecuador	September, November, December 2016	Pollination experiments with *A. confusa* E. Cotton & Borchs., *M. hernandoi* L. Uribe, *M. sanguinea* Wurdack, *M. tomentosa* (Cogn.) Wurdack and collections of floral material, leaf samples in sliqua gel and herbarium vouchers
Ecuador	October & November 2017	Pollination experiments with *M.* aff. *sanguinea, M. sanguinea* Wurdack, *M. tomentosa* (Cogn.) Wurdack and collections of floral material, leaf samples in sliqua gel and herbarium vouchers

Table 2: Research stations, National Parks, and nature reserves visited during fieldtrips.

Country	**Station**
Costa Rica	Tropical Field Station La Gamba, Monteverde Biological Station, Cerro Dantas Biological Station, private housing at 'Truchas Selva Madre'; visits to various National Parks (e.g. Braulio-Carillo, Volcán Tenorio, La Amistad, Chirripó, Los Quetzales), Cerros de Escazú, Montverde Cloudforest Reserve
Ecuador	San Francisco Biological Station, Yanayacu Biological Station, Bellavista Cloudforest Reserve, Guanderas Biological Station (Jatun Sacha Foundation), Rio Zunac Reserve (EcoMinga Foundation), Pococarpus National Park (Cajanuma), Cerro Toledo, El Tiro (Loja), Tapichalaca Reserve (Jocotoco Foundation), Yacuri National Park, Pahuma Reserve

LAB WORK

Besides the flower material I collected in the field myself, the majority of material came from three collaborators, Darin Penneys (University of North Carolina-Wilmington), Frank Almeda (California Academy of Sciences) and Fabián Michelangeli (New York Botanical Garden). This material had been collected on various sampling trips they had undertaken in previous years. Without their contributions, I would never have reached the broad sampling across the major Merianieae clades and throughout their geographical distribution.

In order to compile the large comparative dataset for 61 species presented in Chapter I, I mostly employed scanning electron microscopy and light microscopy on petals, stamens and gynoecia of all species. In addition, and most important for Chapter III, I also scanned flowers of these species using the High-Resolution X-ray Computed Tomography Scanner available at our department at the University of Vienna.

I produced results presented in Chapter II by counting pollen grains on stigmas using fluorescence microscopy. My collaborator Stefan Dötterl (University of Salzburg) and our master student Lisa Scheer used Gas-Chromatography-Light-Spectrometry (GC-MS) to analyse scents collected in the field by Lisa and myself. Raimund Tenhaken (University of Salzburg) processed nectar collections using High-Performance Liquid Chromatography.

My collaborator Darin Penneys has produced a large seven-marker phylogeny of Melastomataceae over the past years and shared sequence data for Merianieae with me. In order to complete the sampling for all species which I had included in the floral morphological studies, I followed the invitation of my collaborator Fabián Michelangeli to travel to the New York Botanical Garden to do the sequencing for the missing species in October 2017.

In addition, I have gained training in Next Generation Sequencing methods with my collaborator Ovidiu Paun (University of Vienna), working on the population genomics of seven selected Merianieae species. I am currently analysing the data from these studies and will present the results elsewhere.

Details on field experiments and lab work are given in Chapter I to III.

3. Chapter I: Beyond Buzz-Pollination – Departures from an Adaptive Plateau lead to new Pollination Syndromes

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Research

Beyond buzz-pollination – departures from an adaptive plateau lead to new pollination syndromes

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Summary

• Pollination syndromes describe recurring adaptation to selection imposed by distinct pollinators. We tested for pollination syndromes in Merianieae (Melastomataceae), which contain bee- (buzz-), hummingbird-, flowerpiercer-, passerine-, bat- and rodent-pollinated species. Further, we explored trait changes correlated with the repeated shifts away from buzz-pollination, which represents an 'adaptive plateau' in Melastomataceae.

• We used random forest analyses to identify key traits associated with the different pollinators of 19 Merianieae species and estimated the pollination syndromes of 42 more species. We employed morphospace analyses to compare the morphological diversity (disparity) among syndromes.

• We identified three pollination syndromes ('buzz-bee', 'mixed-vertebrate' and 'passerine'), characterized by different pollen expulsion mechanisms and reward types, but not by traditional syndrome characters. Further, we found that 'efficiency' rather than 'attraction' traits were important for syndrome circumscription. Contrary to syndrome theory, our study supports the pooling of different pollinators (hummingbirds, bats, rodents and flowerpiercers) into the 'mixed-vertebrate' syndrome, and we found that disparity was highest in the 'buzz-bee' syndrome.

• We conclude that the highly adaptive buzz-pollination system may have prevented shifts towards classical pollination syndromes, but provided the starting point for the evolution of a novel set of distinct syndromes, all having retained multifunctional stamens that provide pollen expulsion, reward and attraction.

Introduction

The observation of recurring floral phenotypes associated with distinct pollinator groups has given rise to the concept of pollination syndromes (Delpino, 1890; Vogel, 1954; Stebbins, 1970; Faegri & van der Pijl, 1979; Endress, 1996). Pollinators are grouped into functional categories, i.e. groups of animals probably exerting similar selective pressures on flowers as a result of shared morphology, foraging behaviour/preferences and sensory abilities (Fenster *et al.*, 2004). Thus, flowers pollinated by the same functional group of pollinators are expected to converge onto similar phenotypes in response to selection imposed by the most effective pollinators (defined as the product of visitation frequency and pollen transfer efficiency; e.g. Armbruster, 1988; Ne'eman *et al.*, 2010; Ashworth *et al.*, 2015; Fenster *et al.*, 2015). Although a large body of literature reports pollination syndromes for certain plant lineages (Lázaro *et al.*, 2008; Armbruster *et al.*, 2011; Lagomarsino *et al.*, 2017), and a recent quantitative evaluation of the concept found strong support even across angiosperms (Rosas-Guerrero *et al.*, 2014), other studies have raised concerns about the utility of the concept (e.g. Waser *et al.*, 1996; Kingston & McQuillan, 2000; Ollerton *et al.*, 2009). Major points of criticism include an over-simplification of complex plant–animal interactions, a lack of clear terminology and difficulties in making comparisons across different taxonomic levels (summarized by Ollerton *et al.*, 2009). Not all 'classical' traits (e.g. red coloration in bird syndrome, musty odour in bat syndrome) are necessarily equally selected for in all systems or geographical regions (Rosas-Guerrero *et al.*, 2014). Besides selection generated by pollinator effectiveness, the evolution of floral traits may also be mediated by antagonistic interactions (e.g. red coloration as bee avoidance in hummingbird flowers; Papiorek *et al.*, 2014), competition for

pollinators (e.g. colour variation in hummingbird-pollinated Iochrominae; Muchhala *et al.*, 2014) or the evolutionary history of the clade, and the developmental constraints embedded therein (e.g. constraints of possible floral trait combinations; Smith & Rausher, 2008; O'Meara *et al.*, 2016). These interactions may lead to narrower, clade-specific syndromes (e.g. Pérez *et al.*, 2006; Johnson, 2013; Serrano-Serrano *et al.*, 2017).

Classical pollination syndromes are conceptually interpreted as systems specialized on only one ('most effective') functional group of pollinators, although it has long been recognized that additional secondary (less effective) pollinators are common (e.g. Rosas-Guerrero *et al.*, 2014; Ashworth *et al.*, 2015). Indeed, Rosas-Guerrero *et al.* (2014) showed that there is a non-random association of pollination syndromes (e.g. bee–hummingbird, hummingbird–bat) and that ancestral pollinators are often retained as secondary pollinators as long as they do not incur a fitness cost (see also Aigner, 2006).

Finally, syndromes should capture adaptations for how to 'attract and utilize' (Fenster *et al.*, 2004) pollinators. Many existing studies focus on a reduced set of traits primarily from the 'attraction' component (e.g. colour, reward and scent). This is particularly troublesome as the literature suggests stronger selection on the 'utilization' component (fitted with the pollinator to ensure pollen transfer, 'efficiency function traits'; Ordano *et al.*, 2008; Rosas-Guerrero *et al.*, 2011). Thus, it is timely to take a novel approach to pollination syndrome studies. Here, we integrate pollinator observations and floral trait data on both 'classical' syndrome traits and any trait that may be relevant for our study system ('bottom up' approach outlined by Ollerton *et al.*, 2009), and use multivariate analyses to detect convergent associations between flower traits and pollinators ('top down' approach; Ollerton *et al.*, 2009).

Buzz-pollination by bees has evolved independently in many angiosperm lineages (found in at least 72 families) and is present in *c.* 22 000 species (Cardinal *et al.*, 2018). A typical buzz-pollinated flower is characterized by poricidal anthers, lack of nectar and pollen being the sole reward offered to pollinating bees (Buchmann, 1983). The functional group of 'buzzing bees' is taxonomically and morphologically highly diverse, as bees from at least 74 genera (seven families) are capable of producing distinct high-frequency vibrations ('buzz') (de Luca & Vallejo-Marín, 2013; Cardinal *et al.*, 2018). The buzz-pollination syndrome is not evenly distributed across angiosperms, however; whilst some lineages contain only a few species adapted for buzz-pollination, some genera, such as *Solanum*, and families, such as Melastomataceae, show a conspicuous predominance of buzz-pollination. In the latter, an estimated 98% of the *c.* 5000 species are buzz-pollinated (Renner, 1989; Berger *et al.*, 2016). Evolutionary success has been proposed as an explanation for the prevalence of buzz-pollination in Melastomataceae, balancing the majority of species on an 'adaptive peak' (Macior, 1971). Given the considerable floral disparity (morphological diversity) amongst buzz-bee-pollinated Melastomataceae (e.g. genus *Leandra*; Reginato & Michelangeli, 2016), it is probably more appropriate to speak of an 'adaptive plateau' on which the family is wandering. Interestingly, recent studies have reported various departures from the buzz-pollination syndrome to alternative pollinators (flies, wasps, hummingbirds, bats, passerines and rodents) in Melastomataceae (Lumer, 1980; Renner, 1989; Vogel, 1997; Dellinger *et al.*, 2014; Brito *et al.*, 2017). Although not yet formally tested, these shifts seem to be associated with complex changes in reward type (from pollen to nectar; Varassin *et al.*, 2008 or to food bodies; Dellinger *et al.*, 2014) or pollen expulsion mechanisms (e.g. from buzzing to a bellows mechanism; Dellinger *et al.*, 2014). As buzz-pollinated flowers represent a functionally highly complex, specialized pollination system very distinct from the majority of bee pollination systems, an understanding of trait combinations and associated new syndromes derived therefrom is particularly interesting.

Here, we analyse the floral morphology and pollination ecology of members of the Neotropical Melastomataceae tribe Merianieae (*c.* 300 species), which offers an ideal model system to investigate floral adaptations to different functional pollinator groups. Buzz-pollination is clearly ancestral in Merianieae and independent shifts to different vertebrate pollination systems (including mixed hummingbird/bat and passerine pollination) have occurred repeatedly (Dellinger *et al.*, 2014; see the Results section). We use state-of-the-art statistical tools (random forests, Johnson, 2013; morphospaces, Chartier *et al.*, 2017) to (1) describe the pollination syndromes (based on 61 floral traits) of 19 Merianieae species with known pollinators, (2) determine the respective roles of 'classical' pollination syndrome traits and Merianieae-specific traits, and (3) predict pollinators for 42 species, for which pollinators have never been observed. This enables us to provide a broad understanding of the floral morphologies that characterize the 'buzz'-morphology as the evolutionary starting point in Merianieae, and to understand the floral trait changes that have occurred along the evolutionary paths away from the 'buzz-pollination plateau' to different vertebrate pollination systems. Furthermore, by mapping pollination syndromes onto a phylogeny, we provide evidence that floral adaptations in Merianieae indeed represent convergences to different functional pollinator groups, as postulated under the pollination syndrome concept.

Materials and Methods

Taxon sampling and floral traits

We aimed to capture both the morphological and taxonomic diversity in Merianieae by selecting 61 species (*c.* 20% of Merianieae) from five of the eight currently recognized genera for our study. Flower material was collected throughout the distribution range of Merianieae (north to south from Costa Rica to Brazil, east to west from Antilles to Ecuador) and stored in 70% ethanol; details on sampling localities can be found in Supporting Information Table S1.

Based on earlier studies of pollination syndromes (e.g. Ollerton *et al.*, 2009) and on floral morphology in Melastomataceae (e.g. Varassin *et al.*, 2008; Mendoza-Cifuentes & Fernández-Alonso, 2010; Cotton *et al.*, 2014; Dellinger *et al.*, 2014), we have compiled a list of 61 floral characters potentially important for pollination (for the justification of character choice, see

Notes S1). Our floral dataset is based on direct field observations, photographs, descriptions on herbarium sheet labels, scanning electron microscopy (SEM), light microscopy and high-resolution X-ray computed tomography (HR-XCT). For SEM, flowers were dissected and, for each species, the hypanthium, one petal, two stamens and one style were prepared (for details on preparation, see Dellinger *et al.*, 2014). For HR-XCT, entire flowers or stamens of 57 species were placed into a contrasting agent (1% phosphotungstic acid–70% ethanol) for 4 wk and mounted for scanning by placing them into plastic cups (Semadeni Plastics Group, Ostermundigen, Switzerland) with acrylic pillow foam arranged around the samples to prevent them from moving during the scanning procedure (for details on the HR-XCT methodology, see Staedler *et al.*, 2013, 2018). Three-dimensional models of flowers and stamens were reconstructed (XML-Reconstructor) and visualized in the software AMIRA; raw scan data have been deposited on the open source platform PHAIDRA (https://phaidra.univie.ac.at/).

Pollinator observations

Pollinator information from the literature was available for eight species. In addition, we monitored pollinators using video cameras (Sony Camcorder, Tokyo, Japan) and direct observations at field sites in Ecuador (2016/2017) and Costa Rica (2015/2018) for 11 more species (Tables 1, S2). We filmed single inflorescences during daytime (06:00–18:00 h) and night monitored (18:00–00:00 h) five species. For each video, we replayed a minimum of three random 30-min intervals using the software PLAYMEMORIESHOME (total average of 11.3 h of daytime and 8.2 h of night-time observation per species). We scored visitors as pollinators if they caused pollen release from stamens and came into contact with stigmas. Floral visitors were classified as 'buzz-bee', 'hummingbird', 'bat', 'flowerpiercer' (nectar-consuming passerine birds), 'passerine' (in this study, including Thraupidae visiting flowers for non-nectar rewards) and 'rodent' (Table 1). Bat and rodent visits to *Meriania* were recorded only during the night. This resulted in a total of 19 species with known pollinators in Merianieae. Of these species, six (*M.* aff. *sanguinea*, *M. furvanthera*, *M. phlomoides*, *M. pichinchensis*, *M. sanguinea* and *M. tomentosa*) are pollinated by two types of pollinators (e.g. diurnal hummingbirds and nocturnal bats, see Table 1) and would usually be classified into two different functional groups (e.g. Faegri & van der Pijl, 1979). In *Meriania*, these pollinators actually all visit flowers looking for the same reward (nectar). For the two other nectar-producing species, *M. costata* and *M. quintuplinervis*, no nocturnal observations were made, but additional nocturnal pollinators (bats and/or rodents) cannot be ruled out. This lack of information must be treated with care in pollinator classification analyses (see next paragraph).

Identification of floral characters differentiating pollinator groups

We used the statistical classification method of random forests (RF) to identify the most important floral characters differentiating functional pollinator groups in Merianieae with known pollinators (Breiman, 2001; for application in the same context, see Johnson, 2013). In RF analyses, a large number of decision trees are built on subsets of data by trying different variables at each node and assessing the quality of the specific variable in reducing the tree's entropy (i.e. power of character in splitting data into known classes). As only 63% of input data are used in each tree, the remaining out-of-bag (OOB) observations are used to estimate classification error and reduction in model accuracy when one character is removed (reduction in Gini index; Cutler *et al.*, 2007). We ran two different models: (1) a 'six-syndrome model' separating pollinators into six functional pollinator groups ('buzz-bee', 'hummingbird/?', 'hummingbird/bat', 'hummingbird/rodent', 'flowerpiercer/rodent', 'passerine'); and (2) a 'three-syndrome model' separating pollinators into three functional groups ('buzz-bee', 'mixed-vertebrate' and 'passerine'). The 'mixed-vertebrate' group encompasses all nectar-secreting Merianieae species where pollinators foraging for nectar cause pollen release when inserting tongues/bills/heads into flowers, and hence possibly selected for a common pollen expulsion mechanism (to compare the flower morphology of these species, see Fig. S1). We calculated 100 RFs of 500 trees each and seven variables tried at each split (mtry). The importance of each variable (floral character) in separating the pollinator groups was ranked by the mean decrease in Gini index over all 100 RFs. All analyses were run using the RANDOMFOREST package 4.6-12 (Liaw & Wiener, 2002) in R 3.3.0 (R Core Team, 2017).

Estimation of pollinators

To estimate the pollinators of species for which no observations were available, we ran the function *predict* (STATS) on the RFs previously trained with data from the 19 species with known pollinators (Table S2). As RFs cannot handle missing data, the variables 'reward type' (69.1% of data missing) and 'pollen expulsion mechanism' (95.2% of data missing) were removed from the dataset despite their importance (see Results). As the removal of characters with high predictive power may reduce model accuracy, we first ascertained that the error rates remained low by re-running predictions of species with known pollinators on the reduced trait dataset (see Table S3). In 19 of the 42 species for which we predicted pollinators, additional characters included missing data. For these, we ran separate predictions excluding the characters with missing data (Table S4). Predictions from these separate runs were collated with the results obtained from the other runs. We ran predictions for the 'three-syndrome model' only because the 'six-syndrome model' failed to predict species with two pollinator types into separate syndromes (see Results). All predictions were run 100 times to account for possible inconsistencies in group assignment.

Morphospace analyses and disparity

To understand the variation in morphological diversity (disparity), we constructed a morphospace from the full set of 61 floral characters. We grouped species into the three pollination syndromes ('buzz-bee', 'mixed-vertebrate' and 'passerine') estimated

Table 1 Merianieae species with known pollinators, source of pollinator observation and syndrome estimation using random forest (RF) analyses for the 'six-syndrome model' ('buzz-bee', 'hummingbird/?', 'hummingbird/bat', 'hummingbird/rodent', 'flowerpiercer/rodent', 'passerine') and 'three-syndrome model' ('buzz-bee', 'mixed-vertebrate', 'passerine').

Species	Confirmed pollinator group	Source of pollinator observation	Estimation 'six-syndrome model'	Estimation 'three-syndrome model'
Adelobotrys adscendens (Sw.) Triana	**Buzz-bee**	A. S. Dellinger (pers. obs.)	**Buzz-bee (0.94)**/ passerine (0.06)	**Buzz-bee (0.9)**/ passerine (0.1)
Graffenrieda cucullata (Triana) L.O. Williams	**Buzz-bee**	A. S. Dellinger (pers. obs.)	**Buzz-bee (0.59)**/ passerine (0.41)	Passerine (0.55)/ **buzz-bee (0.45)**
Meriania drakei (Cogn.) Wurdack	**Buzz-bee**	A. S. Dellinger (pers. obs.)	**Buzz-bee (1)**	**Buzz-bee (1)**
Meriania hernandoi L. Uribe	**Buzz-bee**	A. S. Dellinger (pers. obs.)	**Buzz-bee (1)**	**Buzz-bee (1)**
Meriania longifolia (Naudin) Cogn.	**Buzz-bee**	Renner (1989)	**Buzz-bee (1)**	**Buzz-bee (1)**
Meriania maguirei Wurdack	**Buzz-bee**	A. S. Dellinger (pers. obs.)	**Buzz-bee (1)**	**Buzz-bee (1)**
Meriania maxima Markgr.	**Buzz-bee**	A. S. Dellinger (pers. obs.)	**Buzz-bee (1)**	**Buzz-bee (1)**
Meriania furvanthera Wurdack	**Flowerpiercer/rodent**	A. S. Dellinger (pers. obs.)	HB (0.67)/**FR (0)**	**MV (1)**
Meriania costata Wurdack	**Hummingbird/?**	A. S. Dellinger (pers. obs.)	HB (0.89)/**H/? (0)**	**MV (1)**
Meriania quintuplinervis Naudin	**Hummingbird/?**	E. Calderón-Sáenz (unpublished)	HB (1)/**H/? (0)**	**MV (1)**
Meriania pichinchensis Wurdack	**Hummingbird/bat**	Muchhala & Jarrin-V (2002); A. S. Dellinger (pers. obs.)	**HB (0.8)**/H (0.2)	**MV (1)**
Meriania aff. *sanguinea*	**Hummingbird/bat**	A. S. Dellinger (pers. obs.)	HR (1)/**HB (0)**	**MV (1)**
Meriania phlomoides (Triana) Almeda	**Hummingbird/bat**	Vogel (1997); A. S. Dellinger (pers. obs.)	**HB (0.84)**/H (0.16)	**MV (1)**
Meriania tomentosa (Cogn.) Wurdack	**Hummingbird/bat**	A. S. Dellinger (pers. obs.)	**HB (1)**	**MV (1)**
Meriania sanguinea Wurdack	**Hummingbird/rodent**	A. S. Dellinger (pers. obs.)	HB (1)/**HR (0)**	**MV (1)**
Axinaea confusa E. Cotton & Borchs.	**Passerine**	Dellinger *et al.* (2014)	**Passerine (1)**	**Passerine (1)**
Axinaea costaricensis Cogn.	**Passerine**	Dellinger *et al.* (2014)	**Passerine (1)**	**Passerine (1)**
Axinaea macrophylla (Naudin) Triana	**Passerine**	Rojas-Nossa (2007)	**Passerine (1)**	**Passerine (1)**
Axinaea sclerophylla Triana	**Passerine**	Dellinger *et al.*, 2014	**Passerine (1)**	**Passerine (1)**

The first and second most probable group assignments and estimation probabilities (0 (0%)–1 (100%)) are given for each species. The variable group assignment in buzz-bee-pollinated *A. adscendens* and *G. cucullata* is due to these flowers presenting highly distinct morphologies from all other buzz-bee-pollinated species with known pollinators, underpinning the diversity of the 'buzz-bee' syndrome; misclassification is alleviated once more species with similar morphologies are included in syndrome estimation. '?' indicates a lack of nocturnal pollinator observations. Abbreviations: H/?, 'hummingbird/?'; HB, 'hummingbird/bat'; HR, 'hummingbird/rodent'; FR, 'flowerpiercer/rodent'; MV, mixed-vertebrate. Bold type indicates the correct pollination syndrome.

from RF analyses (Table S4). A dissimilarity matrix (mean character difference D between each pair of taxa; Foote, 1999) was calculated following Chartier *et al.* (2017), whose approach allows the accommodation of all types of data (binary, categorical and continuous). Principal coordinates analyses (PCoAs) were calculated on the dissimilarity matrix to visualize morphospace occupation. A PERMANOVA was run on the dissimilarity matrix to test for morphological differences between pollination syndromes using the function *adonis* (VEGAN) (Oksanen *et al.*, 2018) in R, with 10 000 permutations to calculate a pseudo F-ratio. We estimated the disparity from the distance matrix as the mean pairwise dissimilarity ($\bar{D}$) for each pollination syndrome and compared among groups with a non-parametric Kruskal–Wallis test. Partial disparity (partial contribution of each pollination system to total disparity) was calculated from the coordinates of each species in the morphospace following Foote (1993).

Phylogeny and ancestral character estimation

To ascertain whether pollinator shifts in Merianieae have occurred repeatedly, and hence similar floral phenotypes indeed represent convergences to different pollinator groups as assumed under the concept of pollination syndromes, we used a trimmed phylogeny for the 61 Merianieae species included in this study. The presented phylogeny stems from larger phylogenetic analyses for the entire Merianieae, which will be discussed in detail elsewhere (F.A. Michelangeli *et al.*, unpublished; for details, see Table S5). The expanded Merianiae phylogeny has 190 terminals representing 150 taxa of Merianieae and eight outgroups (four species of Miconieae, three species of *Physeterostemon* and one species of *Eriocnema*). Some species for which species boundaries are problematic are represented by more than one accession. Total genomic DNA was isolated from silica-dried or herbarium material using the DNAeasy plant mini kit from Qiagen (Qiagen, Valencia, CA, USA) following the modifications suggested by Alexander *et al.* (2007) and Martin *et al.* (2008). Some samples were isolated using the cetyltrimethylammonium bromide (CTAB) method as modified by Doyle & Doyle (1987), scaled down for 600 µl of extraction buffer. The molecular dataset includes six loci markers, including two nuclear ribosomal loci (internal and external transcribed spacers, nrITS and nrETS) and four plastid loci (portions of the *ndhF* and *rbcL* genes and the intergenic spacers *accD-psaI* and *psbK-psbL*). All of these regions have been widely used in Melastomataceae systematics, and PCR primers and conditions follow Clausing & Renner (2001), Fritsch *et al.* (2004), Michelangeli *et al.* (2004, 2008, 2013),

Martin *et al.* (2008), Reginato *et al.* (2010) and Kriebel *et al.* (2015). Cycle sequencing was performed with the same forward and reverse primers as used for amplification through the high-throughput sequencing service of the University of Washington or Macrogen (Rockville, MD, USA). Sequence contigs were built with SEQUENCHER 4.9 (GeneCode Corp., Ann Arbor, MI, USA) or GENEIOUS v7.1.9. (Biomatters Ltd., Auckland, New Zealand). Sequence alignment was performed with MUSCLE (Edgar, 2004) as implemented through the GENEIOUS plugin. Sequence evolution models for each locus were set to GTR. Separate phylogenetic analyses were conducted for each dataset using maximum likelihood (ML) in RAxML v. 8.2.10 (Stamatakis, 2014) and run through the CIPRES Science Gateway (http://www.phylo.org/; Miller *et al.*, 2010). Rapid bootstrapping (BS) was performed on the ML tree using RAxML at 1000 replicates to determine branch support. Once we had ensured that there was no topological conflict among loci (BS threshold > 70), all loci were combined into a single matrix. ML was run on the combined matrix with six partitions maintaining the same parameters as above.

Ancestral states of pollination syndromes and three of the most important floral characters with data present for all species (Table 2: 'appendage shape' (as a proxy of 'pollen expulsion mechanism' and 'reward type'), 'filament ruptures' (as a proxy of 'reward type'), 'relative position of stigma vs corolla opening') were estimated using ML methods. For all four characters, models with 'equal rates' and 'all rates different' were run using the function *ace* (APE; Paradis *et al.*, 2004) and a likelihood ratio test was subsequently performed to select the best-fit model for each character. Stochastic character mapping (1000 iterations) with the empirical Bayes method on the optimal model was performed with the function *make.simmap* (PHYTOOLS; Revell, 2009) to validate ML estimation.

Results

Differentiation of functional pollinator groups

Classification of the 19 species with known pollinators (Table 1) into six syndromes ('buzz-bee', 'hummingbird', 'hummingbird/bat', 'hummingbird/rodent', 'flowerpiercer/rodent' and 'passerine; 'six-syndrome model') using OOB data led to an overall median error rate of 31% over all 100 RFs. RFs were unable to separate nectar-rewarding species correctly into separate syndromes as reflected by high levels of misclassification ('hummingbird', 100%; 'hummingbird/bat', 25%; 'hummingbird/rodent', 100%; 'flowerpiercer/rodent', 100%); classification was correct in the 'buzz-bee' and 'passerine' (both 0% misclassification) pollinated species. However, classification of the 19 species into three syndromes ('buzz-bee', 'mixed-vertebrate' and 'passerine') noticeably reduced the overall median error rate to 5.2%. All nectar-secreting species were correctly classified as 'mixed-vertebrate' (0% misclassification). Accordingly, the 'three-syndrome model' was chosen for further analyses.

Floral characters differentiating pollination syndromes

The 20 most important floral characters differentiating the 19 Merianieae species with known pollinators into 'buzz-bee', 'mixed-vertebrate' or 'passerine' pollination syndromes are listed in Table 2 (for a complete list of all 61 characters over 100 RFs, see Fig. S2). Four characters (mode of pollen expulsion, reward type, relative position of stigma vs corolla opening, presence of filament ruptures) were particularly informative, as the removal of any of these characters reduced the mean model accuracy (and hence the accuracy of pollination syndrome classification)

Table 2 Twenty floral characters of Merianieae ranked by importance (mean decrease in model accuracy and Gini index) in separating the three pollination syndromes and mean decrease in accuracy per syndrome averaged for the 100 RFs; * indicates classical pollination syndrome characters; detailed information on the floral characters can be found in Supporting Information Notes S1 and S2.

Floral characters ranked by importance	Mean decrease in model accuracy	Mean decrease in Gini index	'Buzz-bee'	'Mixed-vertebrate'	'Passerine'
Mode of pollen expulsion	0.087	1.533	0.127	0.074	0.071
Reward type*	0.051	1.1	0.065	0.03	0.095
Relative position of stigma vs corolla opening*	0.056	0.942	0.043	0.084	0.041
Filament ruptures	0.055	0.881	0.041	0.078	0.045
Petal gloss*	0.047	0.753	0.037	0.068	0.033
Orientation of flower*	0.041	0.648	0.021	0.051	0.045
Corolla height*	0.022	0.604	0.042	0.022	0.005
Stigma shape	0.029	0.572	0.038	0.022	0.029
Pollen grain diameter	0.023	0.534	0.049	0	0.029
Relation corolla diameter : height	0.011	0.491	0.016	0.004	0.007
Corolla shape*	0.025	0.478	0.016	0.007	0.061
Structure of stamen appendage	0.018	0.468	0.002	0.028	0.029
Corolla shape change during anthesis	0.022	0.454	0.044	0.018	−0.002
Change of androecial arrangement during anthesis	0.021	0.397	0.008	0.034	0.026
Structure of adaxial thecal wall*	0.011	0.385	0.01	0.012	0.013
Level of anther pore*	0.017	0.372	0.01	0.022	0.019
Stamen appendage shape	0.009	0.283	0.002	0.008	0.027
Dimorphism in appendage volume	0.007	0.259	−0.003	0.005	0.025
Stigma diameter	0.007	0.225	−0.004	0.012	0.014
Style curvature	0.01	0.176	0.02	0	0.013

by > 5% (Table 2). Floral characters vary in their predictive power among syndromes: certain characters were more predictive for one syndrome than for the other two, reflected by differences in reduction in syndrome-specific model accuracy (Table 2). For instance, all flowers in the 'mixed-vertebrate' syndrome are pendant, whereas flower orientation varies in the other two syndromes. When comparing the relative importance of 'classical' pollination syndrome traits, eight of 14 fell within the 20 most important characters, whereas the remaining six were of less importance (Table 2). The latter include colour, scent, symmetry, corolla diameter and inflorescence position (Table S6).

Pollination syndromes in Merianieae

Pollination syndromes and pollinator behaviour (observed by ASD, Table 1) are described on the basis of species with known pollinators; a syndrome summary is provided in Table 3 and a more detailed description is given in Notes S2.

Within the 'buzz-bee' syndrome, three major groups have been distinguished (*Graffenrieda* species, group 1; *Adelobotrys adscendens*, group 2; *Meriania* species, group 3), and syndrome description is organized accordingly. Features shared by all 'buzz-bee' syndrome species in Merianieae are the pollen reward and buzz-pollination (Table 3). Corollas are wide bowl-shaped to reflexed with papillate petal epidermis, providing a landing platform for pollinating bees (Figs 1a–g, S3a). Flower colours range from white to orange, fuchsia and lilac, with stamens forming a strong colour contrast against the petals (Fig. 1a,c,e,f). Stamens are either distributed more or less regularly in the flower (Fig. 1a, b, group 1) or arranged on one side of the flower (thus monosymmetric appearance, Fig. 1c–g, groups 2 and 3); heteranthery is found in some species in groups 2 and 3. Anthers can be erect (group 1), bringing pores close to the stigma (Fig. 1b), or remain geniculate (the condition found in bud stage in all species) with pores remaining close to the base of the style in the floral centre (Fig. 1d,g; groups 2 and 3). Stamen appendages in Merianieae are always dorsal; in groups 2 and 3 conspicuous and large (Figs 1e,f, 2b), in group 1 small and acuminate (Fig. 1c). Thecae are located on the ventral side of the connective and usually have strongly corrugated and rigid walls (Figs 1b,d,g, S3d,g); pollen can only be released by applying strong vibrations (buzzes). Pores may be located on the ventral (group 1) or dorsal (groups 2 and 3) side of the anther. Styles are usually exserted and often strongly curved right beneath the stigma. In many species, stigmas are small and punctiform. In species of groups 1 and 2 (flower diameter < 2 cm), visiting bees were seen to crouch above the entire androecium, head pointing towards the flower centre, and buzzing the entire androecium. In large-flowered species of group 3 (flower diameter > 2 cm), pollinating bees oriented their bodies in parallel to individual stamens, with their head at the appendage and their abdomen pointing towards the pores. They bit into the appendage and buzzed individual stamens at a time. Thus, the 'buzz-bee' syndrome encompasses three distinct flower morphologies and two different types of interaction between flowers and buzzing bees.

Flowers belonging to the 'mixed-vertebrate' syndrome are recognized by nectar rewards secreted from stamens and pseudo-campanulate, pendant flowers (Fig. 1h,i), with a flat petal epidermis and glossy appearance (Fig. S3b). Colours range from white, pinkish, salmon to scarlet red, and flowers are often scented. All species have androecia arranged on one side of the flower and stamens undergoing a strong deflexion movement in the early phase of anthesis, bringing pores close to stigmas (anthers erect, Fig. 1i). Stamen appendages are mostly smaller than in bee-pollinated group 3 species (Fig. 2b), and relatively inconspicuous coloration (same colour as anther) in some species (e.g. *M. tomentosa* (hummingbird/bat)), but larger and contrasting in colour to thecae in others (e.g. *M. sanguinea* (hummingbird/rodent)). Heteranthery is absent in all species with known pollinators. In many species, thecae are attached laterally to the connective and have a soft, easily deformable (e.g. by a hummingbird's bill) wall (Fig. S3e, h). Apical anther pores are usually directed towards the stigma.

Table 3 Summary of floral characters characterizing the three pollination syndromes ('bee', 'mixed-vertebrate' and 'passerine') in Merianieae and traditional pollination syndrome characters; three groups can be distinguished in the 'buzz-bee' syndrome (see Fig. 3).

Floral trait	'Buzz-bee'	'Mixed-vertebrate'	'Passerine'
Orientation of flower	Upright, horizontal	Pendant	Upright, horizontal, pendant
Corolla shape	Flat to reflexed (groups 1, 3); urceolate (group 2)	Pseudo-campanulate	Urceolate
Corolla colour	White (groups 1, 2); lilac, orange, fuchsia	White, salmon, light pink, red	Red, light pink
Petal epidermis and gloss	Conical, matt	Flat, glossy	Flat to conical, matt
Scent	Scentless, flowery	Scentless, flowery, solvent-like	Scentless
Reward type	Pollen	Nectar	Food bodies
Pollen expulsion mechanism	Buzzing	Salt-shaker	Bellows
Stamen appendage shape	Small acuminate (group 1), acuminate bifid (group 2), large pyramidal (group 3)	Reduced in size, crown-like	Bulbous
Anther reflexion	Yes (group 1), no (groups 2, 3)	Yes	No
Thecal attachment	Ventral	Lateral or ventral	Ventral
Structure of adaxial thecal wall	Corrugated, sturdy	Crumpled, soft	Smooth, sturdy
Location of pore	Ventral (group 1), dorsal (groups 2, 3)	Mostly apical	Dorsal
Relative position of stigma vs corolla opening	Far exserted	At level of corolla opening	Slightly exserted

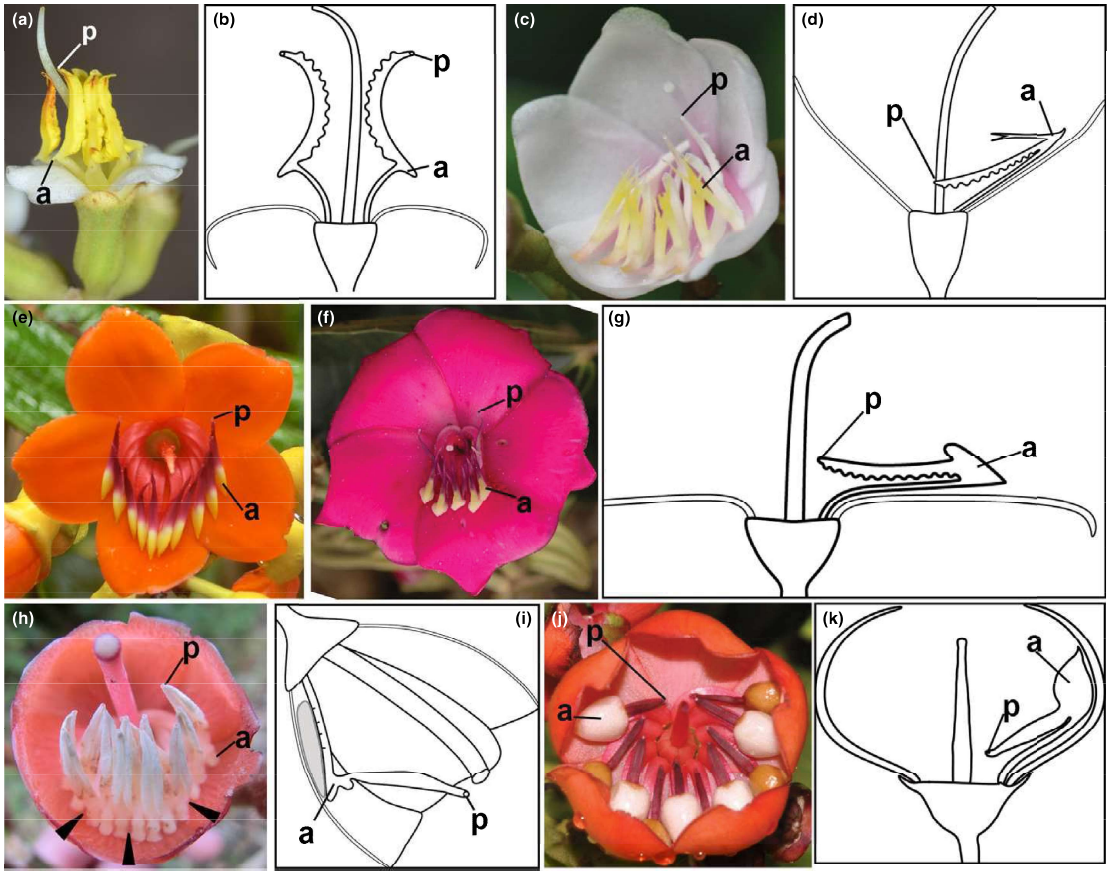

Fig. 1 Flowers of Merianieae species. (a) Buzz-bee-pollinated *Graffenrieda maklenkensis*. (b) Schematic drawing of buzz-bee-pollinated *Graffenrieda* with reflexed corolla and radially symmetric androecium with erect stamens; note corrugated thecal wall. (c) Buzz-bee-pollinated *Adelobotrys adscendens*. (d) Schematic drawing of buzz-bee-pollinated *Adelobotrys* with urceolate corolla and heterantherous, monosymmetric androecium with geniculate stamens; note corrugated thecal wall. (e) Buzz-bee-pollinated *Meriania hernandoi* with reflexed corolla and isomorphic geniculate stamens. (f) Buzz-bee-pollinated *M. maxima* with reflexed corolla and heteranthery. (g) Schematic drawing of 'buzz-bee' syndrome *Meriania* flower with reflexed corolla and monosymmetric androecium with geniculate stamens; note corrugated thecal wall. (h) Hummingbird/bat-pollinated *M. tomentosa* with pseudo-campanulate corolla and reflexed stamens; arrowheads indicate site of nectar aggregation. (i) Schematic drawing of 'mixed-vertebrate' flower with pseudo-campanulate corolla and monosymmetric androecium with erect stamens; grey-shaded area indicates nectar aggregation between stamens and corolla. (j) Passerine-pollinated *Axinaea costaricensis*. (k) Schematic drawing of 'passerine' syndrome flower with urceolate corolla and monosymmetric androecium with bulbous stamen appendages serving as food bodies for passerines. a, Appendage of one stamen; p, pore of one stamen.

Styles are often straight, not exceeding the corolla length, and often bear enlarged, slightly flattened stigmas. Vertebrate pollinators insert their bills, tongues or heads into the pseudo-campanulate corollas to lick nectar aggregated on petals beneath the stamens. To reach the nectar, they have to push through the densely arranged anthers and thereby touch the soft, laterally attached thecae and cause pollen release from the apical pores. As all stamens are arranged with pores pointing downwards, out of the pendant flower, we term this mechanism 'salt-shaker'-like pollen release. Table 3 summarizes the most important features differentiating the 'mixed-vertebrate' from the 'buzz-bee' syndrome: pendant, pseudo-campanulate flowers in combination with erect stamens, nectar rewards, and soft, easily deformable thecae from which pollen can be released by applying external pressure.

The 'passerine' pollination syndrome is characterized by food body rewards provided by bulbous stamen appendages and urceolate corollas (Figs 1j,k, 2b) with a flat petal epidermis (Fig. S3c). Colours range from white, light pink to red. In all species, the brightly coloured stamen appendages form a strong colour contrast with the corolla. Stamens are arranged on one side of the flower (monosymmetric) and, in contrast with the 'mixed-

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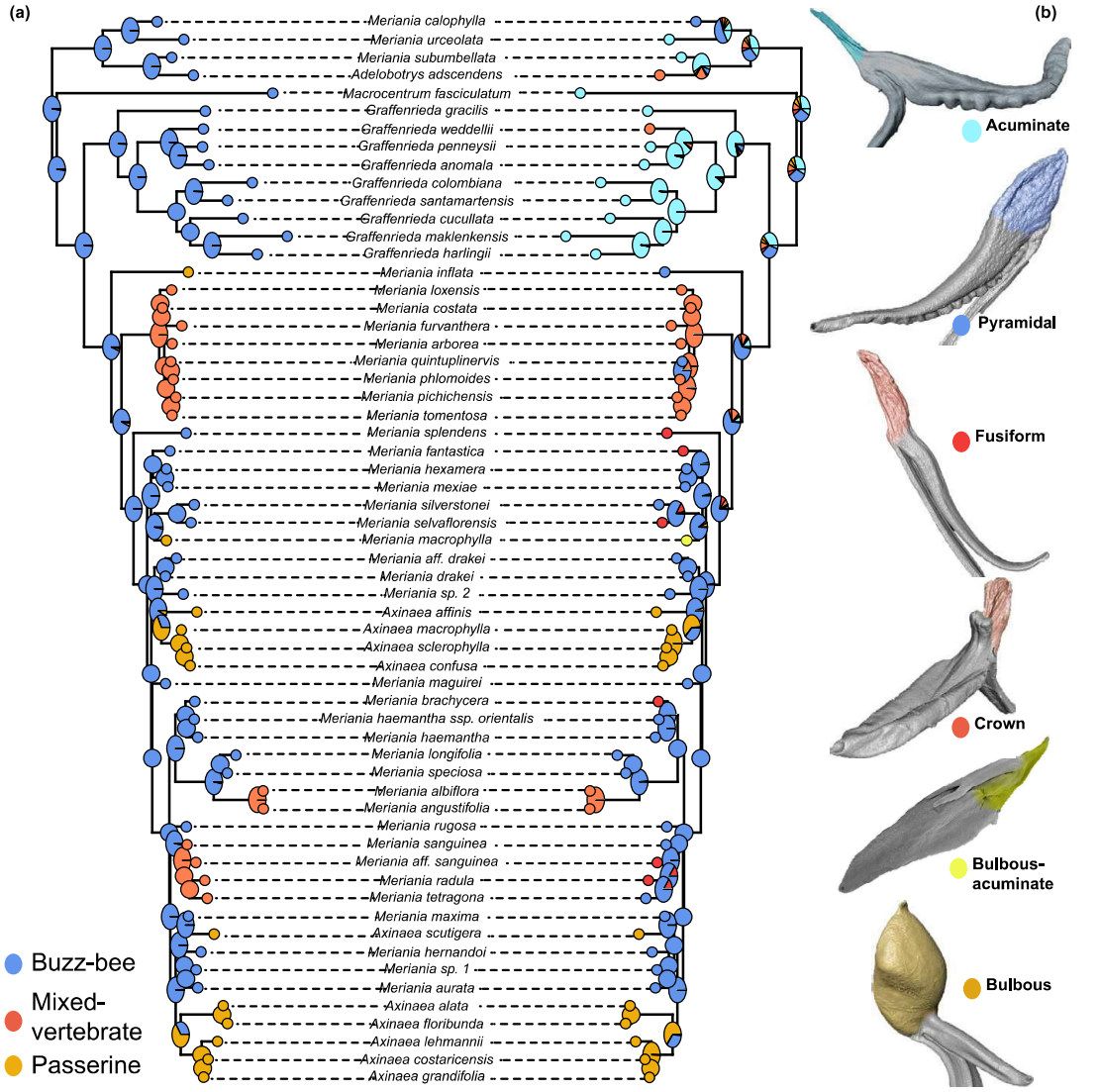

Fig. 2 Stochastic character mapping of the three pollination syndromes ('buzz-bee', 'mixed-vertebrate' and 'passerine') and stamen appendage evolution in Merianieae. Circles at the nodes represent ancestral states estimated from 1000 mapping runs using the 'equal rates' ('ER') model. (a) The 'buzz-bee' syndrome represents the ancestral pollination system in Merianieae and repeated independent shifts occurred to the 'mixed-vertebrate' and the 'passerine' syndrome. (b) Evolution of the primary stamen appendage, with the largest diversity of primary appendage types (acuminate, pyramidal, fusiform) found within the 'buzz-bee' syndrome, two types (crown and fusiform) found within the 'mixed-vertebrate' syndrome and bulbous appendages (bellows organs) restricted to the 'passerine' syndrome. Single stamens from computed tomography (CT) scans and scanning electron microscopy (SEM) are shown; primary appendages are coloured, secondary appendages (if present) were not considered (*Graffenrieda weddellii*, acuminate; *Meriania hernandoi*, pyramidal; *M. fantastica*, fusiform; *M. phlomoides*, crown; *M. macrophylla,* bulbous-acuminate; *Axinaea costaricensis*, bulbous).

vertebrate' syndrome, they do not deflex during anthesis, so that pores remain more or less around the mid-length of the style (Fig. 1k). Most species show moderate heteranthery (appendage volume and colour). Thecae are located on the ventral side of the connective and have a smooth, sturdy wall (Fig. S3f,i). Pores are located on the dorsal side of the anther. Styles are usually partially

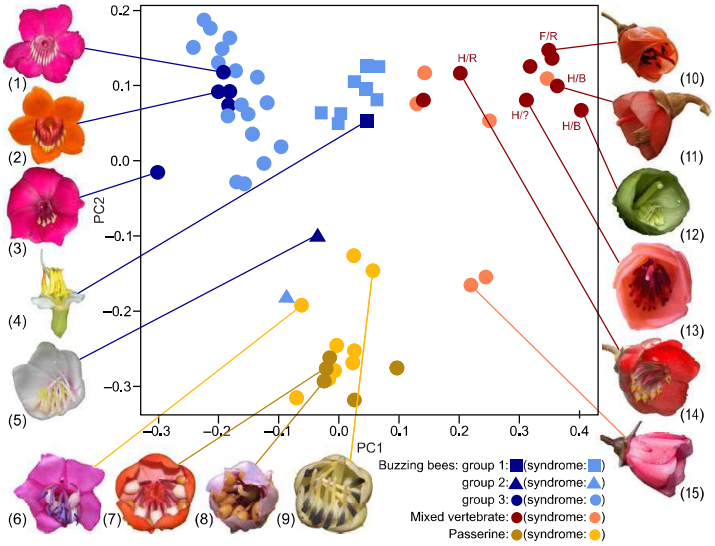

Fig. 3 Morphospace of the three Merianieae pollination syndromes: 'buzz-bee', 'mixed-vertebrate' and 'passerine'. Colours indicate known pollinators and pollination syndromes; functional pollinator groups of the 'mixed-vertebrate' syndrome (H/B, hummingbird/bat; H/R, hummingbird/rodent, F/R, flowerpiercers/rodent; H/?, hummingbird/unknown) are given to underpin convergence despite pollination by different functional groups. The 'buzz-bee' syndrome is scattered in three clusters (group 1 (flower 4), group 2 (flower 5), group 3). Single species were selected to exemplify the morphological diversity of the group: (1) *Meriania maguirei*, (2) *M. hernandoi*, (3) *M. maxima*, (4) *Graffenrieda maklenkensis*, (5) *Adelobotrys adscendens*, (6) *M. macrophylla*, (7) *Axinaea costaricensis*, (8) *A. sclerophylla*, (9) *M. inflata*, (10) *M. furvanthera*, (11) *M. tomentosa*, (12) *M. phlomoides*, (13) *M. costata*, (14) *M. sanguinea* and (15) *M. angustifolia*. [Correction added after online publication 12 October 2018: the figure and associated legend have been updated.]

exserted from the urceolate corollas, with relatively small, conical stigmas. Pollen release is ultimately connected to the ubiquitous bulbous appendages: besides functioning as sugary food body reward, the bulbous appendages work as 'bellows' organs (Dellinger *et al.*, 2014). When passerines grab the appendages for consumption, the compression forces contained air into and through the thecae, dusting the birds with pollen grains that are ejected out of the apical thecal pores. Thus, the bulbous stamen appendages are the most important character differentiating the 'passerine' syndrome from both 'buzz-bee' and 'mixed-vertebrate' syndromes (Table 3).

Estimation of pollination syndromes and ancestral character estimation

All 42 species, for which pollinators were unknown, could be classified into one of the three pollination syndromes using RF. Group assignment over 100 RFs was 100% consistent in 41 species and 97% consistent in one species (Table S4). Estimation yielded 27 'buzz-bee' syndrome flowers in the genera *Meriania*, *Graffenrieda*, *Macrocentrum* and *Adelobotrys*, six 'mixed-vertebrate' syndrome flowers in the genus *Meriania*, and nine 'passerine' syndrome flowers in the genera *Meriania* and *Axinaea*. Buzz-bee pollination was resolved as the ancestral pollination system at the root with the equal rates model performing best (Akaike information criterion (AIC), 70.4; log-likelihood, −34.2; scaled likelihood: 'buzz-bee', 97.7%; 'mixed-vertebrate', 1.1%; 'passerine', 1.1%; AIC of 'all rates different' model, 76.6; log-likelihood, −32.3; see Table S7 for syndrome transition rates; likelihood ratio-test, $P = 0.57$). The mapping of three crucial traits ('appendage shape' (Fig. 2b), 'relation between stigma and corolla opening' and 'filament ruptures' (Figs S4, S5)) confirmed the trait change patterns found in RF analyses.

Disparity of different syndromes

PCoA on the 61 species showed clear grouping according to pollination syndromes and occupation of different areas of morphospace (Fig. 3); 59.2% of the variation was explained by the first three axes. Significant differences in morphospace occupation were detected between syndromes ($F = 21.785$, df = 2, $P < 0.0001$; for details on *post-hoc* group differences, see Table S8). Also, syndromes differed significantly in disparity (Kruskal–Wallis: $Chi^2 = 65.7$, df = 2, $P < 0.0001$; for details on *post-hoc* group differences, see Table S9). The 'buzz-bee' pollination syndrome was morphologically most diverse (mean pairwise dissimilarity $\overline{D} = 0.364 \pm 0.131$ (SD)), i.e. occupied the largest area in the morphospace. Three 'buzz-bee' syndrome clusters could be distinguished, encompassing very different floral morphologies: small-flowered species with reflexed petals and erect stamens (Fig. 1a,b; group 1, differentiated mostly by PCO3, Fig. S6); large-flowered species with reflexed petals and geniculate stamens (Fig. 1e–g; group 3), which occupied a large and distinct area of the space (negative PCO1, positive PCO2); and bee-

pollinated species with urceolate corollas and slightly erect stamens (Fig. 1c,d, group 2), which occupied an area close to the 'passerine' syndrome. The second largest disparity was found in the 'mixed-vertebrate' syndrome ($\bar{D} = 0.318 \pm 0.130$), which is clearly differentiated from the 'bee' syndrome by PCO1 and from the passerine syndrome by PCO2. The different functional pollinator groups ('hummingbird', 'hummingbird/bat', 'hummingbird/rodent', 'flowerpiercer/rodent') could not be distinguished in the morphospace (Fig. 3). The 'passerine' syndrome occupied the smallest area ($\bar{D} = 0.242 \pm 0.087$) of the space, differentiated by PCO2. When assessing the contribution to total disparity, the 'buzz-bee' syndrome alone contributed 51.3%, whereas the 'mixed-vertebrate' and 'passerine' syndromes only contributed 28.8% and 20.0%, respectively.

Discussion

Our results corroborate the general concept of pollination syndromes and allow the detection and description of convergence of multiple floral traits into three distinct pollination syndromes in Merianieae: the ancestral 'buzz-bee', the 'mixed-vertebrate' and the 'passerine' syndromes (Fig. 2a). These syndromes are best described by a series of traits specific to Merianieae, rather than by 'classical' pollination syndrome characters, as indicated by the relatively low contribution to the differentiation model of the latter type of character (Tables 2, S6; Faegri & van der Pijl, 1979; Ollerton *et al.*, 2009; Serrano-Serrano *et al.*, 2017). Our results generally support the hypothesis that 'attraction' traits (e.g. exposure of flower, display size, scent, colour, flower symmetry and timing of anthesis) are less important in differentiating syndromes than 'efficiency' traits involved in the direct physical interaction between flower and pollinators (e.g. flower shape and orientation, position of reproductive organs). This is particularly important for two reasons. First, most studies on phenotypic selection detected selection only on attraction traits (e.g. Armbruster *et al.*, 2005). Attraction traits, however, can be subject to opposing selection in the presence of floral antagonists or trade-offs in pollen delivery, and hence selection will be less consistent and weaker than on traits involved in accurate pollen transfer (e.g. Armbruster *et al.*, 2005; Strauss & Whittall, 2006; Rosas-Guerrero *et al.*, 2011). Second, 'classical' syndrome characters, such as the 'attraction' traits colour and display size, are regularly included in studies on pollination syndromes (Lagomarsino *et al.*, 2017; Wilson *et al.*, 2017), whereas 'efficiency' traits, such as anther–stigma distance, have generally received less attention. At least in Merianieae, certain 'classical' syndrome characters either did not vary consistently between syndromes (e.g. timing of anthesis (most flowers are open during day- and night-time); flower size (both smallest and largest flowers are found in the 'buzz-bee' syndrome)), or they contradicted traditional syndrome expectations (e.g. floral colour (many pale pink and white bird-pollinated flowers instead of the 'red–bird' association)). We wish to point out, however, that one 'classical' syndrome trait (reward type) involved in pollinator attraction was the second-most important character in differentiating syndromes (see discussion on association of reward and androecium).

The difficulty in delimiting 'classical' pollination syndromes in Merianieae is further illustrated by the 'mixed-vertebrate' syndrome. Pollination syndrome theory (e.g. Faegri & van der Pijl, 1979) would split the various combinations of different vertebrate pollinators that we observed visiting Merianieae species ('hummingbird/?', 'hummingbird/bat', 'hummingbird/rodent' and 'flowerpiercer/rodent') into separate functional groups (hummingbirds, flowerpiercers, bats and rodents) based on differences in timing of activity (diurnal/nocturnal), means of localizing flowers (visual/scent/echolocation), foraging behaviour (hovering/perching), morphological fit with flowers (tubular/bowl-shaped flowers) and nectar preferences (sucroses/hexoses). However, our RF and disparity analyses did not support syndromes related to any individual pollinator group or did not separate syndromes related to the different mixed pollinator assemblages. On the contrary, our results underscore that these pollinator groups are part of the same 'functional group' based on their shared interest in the nectar reward and their ability to cause pollen release via the 'salt-shaker' mechanism. Indeed, the 'mixed-vertebrate' syndrome in Merianieae could encompass different cases of specialized bimodal pollination systems, which are systems representing intermediate adaptations to two different (equally effective) functional pollinator groups (Manning & Goldblatt, 2005). Mixed pollinator assemblages can also be the result of retaining ancestral pollinators whilst being specialized on a more effective primary pollinator (Rosas-Guerrero *et al.*, 2014). In bird syndromes, ancestral bee pollinators are disproportionately common, as well as ancestral bird pollinators in bat syndromes (e.g. Buzato *et al.*, 1994; Wilson *et al.*, 2007; Tripp & Manos, 2008). In Merianieae, bees have not been observed as pollinators in either the 'mixed-vertebrate' or the 'passerine' syndrome. The 'mixed-vertebrate' syndrome, however, could potentially represent a transition stage between ancestral bird and novel bat/rodent pollination, or vice versa. Alternatively, pollinator shifts in Merianieae could have passed directly from a buzz-bee system to the different combinations of vertebrate pollinators. A salient feature of all Merianieae with a 'mixed-vertebrate' syndrome is that they all combine a diurnal with a nocturnal pollinator. We hypothesize that such a '24/7 access' to pollinators may be an important adaptive advantage that could have driven these pollinator shifts in Merianieae with Andean distribution (Varassin *et al.*, 2008). A few other systems employing hummingbirds and bats as pollinators are known from Neotropical cloud forests (e.g. *Aphelandra* (Acanthaceae), Muchhala *et al.*, 2009; *Encholirium* (Bromeliaceae), Queiroz *et al.*, 2016), and the combination of these pollinators has been interpreted as a pollination assurance mechanism under harsh montane weather conditions. However, the diversity of combinations of different functional groups in Merianieae is unparalleled in other families. More detailed studies on the population level of species belonging to the 'mixed-vertebrate' syndrome may allow the testing of the hypotheses outlined above and may shed light on the evolutionary history of pollinator shifts in Merianieae.

Experimental studies show that selection, and hence pollination syndrome evolution, operates on complex trait combinations, which do not always match 'classical' syndromes in all traits. Instead, they may represent clade-specific syndromes, which are possibly phylogenetically constrained (Smith & Rausher, 2008; Fenster *et al.*, 2015; O'Meara *et al.*, 2016; Wilson *et al.*, 2017). Buzz-bee pollination in Merianieae represents a highly specialized pollination system in itself (Buchmann, 1983). It is possible that the ancestral 'buzz' morphology in Merianieae, with relatively open corollas and poricidal anthers, partly prevented the evolution of the group towards derived 'classical' syndromes, which have not originated from buzz-pollinated flowers. Compared with other systems, access to flowers is not physically restricted by the corolla in Merianieae (e.g. no narrow corolla tubes typical of the classical hummingbird syndrome), and nectar rewards can be retrieved by a variety of pollinators. In pollen-rewarding Merianieae, however, poricidal anthers strictly confine access to the reward to bees capable of buzzing. Poricidal anthers were retained in all Merianieae species, which could be due to an anatomical constraint (lack of endothecium) hindering the evolution of longitudinal anther dehiscence (Keijzer, 1987). Interestingly, in the Melastomataceae genus *Miconia*, this constraint was apparently overcome as longitudinal anther dehiscence has evolved at least three times (Goldenberg *et al.*, 2003) and has resulted in pollination by non-buzzing insects (Brito *et al.*, 2017). Conserving the poricidal anther morphology whilst shifting to non-buzzing pollinators in Merianieae, however, made the evolution of alternative pollen expulsion mechanisms a necessity. It is thus not surprising that the pollen expulsion mechanism was the most important floral trait separating the three pollination syndromes in Merianieae, with buzzing in the 'buzz-bee' syndrome, the 'salt-shaker' mechanism in the 'mixed-vertebrate' syndrome and the 'bellows' mechanism in the 'passerine' syndrome. The complex functioning of these two new mechanisms was achieved by considerable morphological modifications in the androecium (Fig. 2b). In the 'mixed-vertebrate' syndrome, stamens have deflexed so that pores point towards the opening of the pendant corolla, the location of the thecae has changed from dorsal to lateral, and thecal walls have softened in most species so that pollen is easily released when external pressure is applied (e.g. by a hummingbird's bill). Together, these changes promote the 'salt-shaker'-like release of pollen. In the 'passerine' syndrome, stamen appendages have been modified into inflated bulbous 'bellows' organs which cause pollen ejection from thecae when seized by the foraging passerines (Dellinger *et al.*, 2014).

In addition to promoting pollen dispersal, the androecium provides the reward in all three syndromes: pollen in the 'buzz-bee' syndrome, nectar in the 'mixed-vertebrate' syndrome, which is secreted from staminal filament ruptures, and sucrose-rich food bodies in the 'passerine' syndrome, which are formed by the bulbous stamen appendages (Dellinger *et al.*, 2014). This androecium–reward association in Merianieae is particularly important when compared with rewarding structures across angiosperms: both staminal food bodies and nectar release by stamens are otherwise rare. Staminal food bodies are mainly associated with beetle pollination (e.g. Cyclanthaceae, Bernhard, 1996; Calycanthaceae, Gottsberger, 2015) and staminal nectar release usually occurs by specialized nectaries at the filament base, but not by ruptures along filaments as in Merianieae (staminal nectar release has been reported in Laurales, Magnoliales, Caryophyllales and Geraniales; Bernardello, 2007). In addition to the pollen transfer and rewarding function of the androecium, stamen appendages in buzz-bee-pollinated species form strong colour contrasts with the corolla and therefore also carry an advertisement function. This function has been retained in the 'passerine' syndrome, where bulbous appendages also contrast against petals, and partially in the 'mixed-vertebrate' syndrome (in some species (Fig. 3, flower 14), appendages form the contrast; in others, entire stamens (Fig. 3, flowers 10, 11 and 13) or there is no contrast (Fig. 3, flower 12)). Thus, the androecial multifunctionality of the buzz syndrome has been almost completely retained throughout pollinator shifts in Merianieae and both the complex pollen expulsion mechanisms and unusual rewarding structures are the result of the evolutionary starting point (buzz-pollination syndrome). The strong effect of such evolutionary starting points (genetic context) on adaptation (evolutionary outcome) as a source of trait diversity was recognized by Darwin (Darwin, 1859; Armbruster, 2002).

Merianieae pollination syndromes differed markedly in their levels of floral disparity, with the 'buzz-bee' syndrome clearly being most variable, occupying three distinct areas of morphospace. This is in line with previous studies describing buzz-pollinated Melastomataceae as 'wandering on an adaptive peak' (Macior, 1971; Reginato & Michelangeli, 2016). Apparently, the evolutionarily successful buzz-pollination system does not strictly constrain the floral phenotype, but can be achieved by a variety of floral constructions, united by a common reward type (pollen) and pollen expulsion mechanism (buzzing). This, in turn, broadens the exploitable buzz-bee pollinator niche. A typical buzz syndrome flower is often associated with the architecture of the '*Solanum*-type' flower (Buchmann, 1983; de Luca & Vallejo-Marín, 2013), a small, polysymmetric, pendant flower with reflexed petals and anthers forming a cone on which the bees crouch for buzzing. In the Merianieae species studied here, this phenotype is only realized by a part of the species (buzz-bee group 1, Fig. 3, flower 4). All other buzz-pollinated Merianieae have relatively large flowers with a polysymmetric perianth, but a distinctly monosymmetric androecium. Similar buzz-pollinated flowers are present in the genus *Senna* (Fabaceae, Marazzi & Endress, 2008; Amorim *et al.*, 2017). Although *Senna* flowers are usually urceolate with pronounced heteranthery (Buchmann, 1983; Marazzi *et al.*, 2007), this character combination is found only in buzz-bee group 2 (Fig. 3, flower 5). In comparison with the 'buzz-bee' syndrome, the 'mixed-vertebrate' and 'passerine' syndromes show much lower levels of disparity. Apparently, migration from the 'buzz-bee plateau' happened along two relatively narrow ridges in combination with a change in reward type, pollen expulsion mechanism, corolla shape and androecial arrangement. Although not yet formally tested, this seems to be in line with pollinator shifts reported for the three other Neotropical Melastomataceae tribes (Blakeeae, Melastomateae, Miconieae, e.g. Goldenberg *et al.*, 2008; Varassin *et al.*, 2008; Penneys & Judd, 2011). As in Merianieae, the vast

majority of species in the rest of Melastomataceae are buzz-bee-pollinated (*c.* 89%, Renner, 1989) and show a tremendous diversity of floral morphologies. Shifts to alternative specialized and more generalized pollination systems always involve changes in reward type and pollen release (Renner, 1989; Varassin *et al.*, 2008; Brito *et al.*, 2016).

In conclusion, our results provide an important step forward in the study of floral morphological and functional adaptations to different pollinator groups. We demonstrate that the highly specialized buzz-pollination syndrome largely channelled the evolution of alternative pollination systems, and that the multifunctionality of the androecium (pollen expulsion, reward, attraction) was retained throughout pollinator shifts. Our results further emphasize the value and validity of the pollination syndrome concept, but, at the same time, point out that pollination syndromes need to be evaluated carefully in each study group.

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Author contributions

ASD and JS conceived the idea and designed the study, ASD, DF-F, DSP, MA, FA and FAM carried out fieldwork and flower sampling, YS assisted in HR-XCT scanning, MC gave support in statistical analyses and WSA in discussions on pollination concepts. All authors contributed to writing and revising the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Nectar-producing *Meriania* species with known pollinators grouped into the 'mixed-vertebrate' pollination syndrome.

Fig. S2 Ranking of all 61 floral traits by decrease in Gini index using random forest (RF) analyses.

Fig. S3 Structural properties of petals and stamens in Merianieae.

Fig. S4 Stochastic character mapping of pollination syndromes and the 'filament structure'.

Fig. S5 Stochastic character mapping of pollination syndromes and the character 'relation style to corolla'.

Fig. S6 Merianieae morphospace PC1–3.

Notes S1 Sixty-one floral characters and character states recorded for Merianieae.

Notes S2 Detailed description of Merianieae pollination syndromes.

Table S1 Merianieae species included in the morphospace and information on sampling localities.

Table S2 Pollinator information for the 19 Merianieae species used for the delimitation of pollination syndromes.

Table S3 Misclassification percentage of 19 Merianieae species with known pollinators.

Table S4 Probability of pollinator classification by random forest (RF) analyses.

Table S5 Merianieae species included in the full phylogeny, sampling localities, collector and voucher information and GenBank accession numbers for genes used for construction of the phylogeny.

Table S6 Predictive value of floral characters used in traditional pollination syndromes.

Table S7 Estimated average number of pollination syndrome shifts across 1000 stochastic character mappings.

Table S8 Results from *post-hoc* test on morphological differences between pollination syndromes.

Table S9 Results from *post-hoc* test on significant differences in disparity between pollination syndromes.

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***New Phytologist* Supporting Information**

Article title: **Beyond buzz-pollination – departures from an adaptive plateau lead to new pollination syndromes**

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The following Supporting Information is available for this article:

Table S1. Merianieae species included in morphospace and information on sampling localities.
Table S2. Pollinator information for the 19 Merianieae species used for delimiting pollination syndromes.
Table S3. Misclassification percentage of 19 Merianieae species with known pollinators.
Table S4. Probability of pollinator classification by Random Forest Analyses (RF).
Table S5. Merianieae species included in the full phylogeny, sampling localities, collector and voucher information and GenBank accession numbers for genes used for constructing the phylogeny.
Table S6. Predictive value of floral characters used in traditional pollination syndromes.
Table S7. Estimated average number of pollination syndrome shifts across 1000 stochastic character mappings.
Table S8. Results from post-hoc test on morphological differences between pollination syndromes.
Table S9. Results from post-hoc test on significant differences in disparity between pollination syndromes.
Figure S1. Nectar producing *Meriania* species with known pollinators grouped into the 'mixed vertebrate' pollination syndrome.
Figure S2. Ranking of all 61 floral traits by decrease in Gini Index using RF analyses.
Figure S3. Structural properties of petals and stamens in Merianieae.
Figure S4. Stochastic character mapping of pollination syndromes (left) and the 'filament structure' (right).
Figure S5. Stochastic character mapping of pollination syndromes (left) and the character 'relation style to corolla' (right).
Figure S6. Merianieae morphospace PC1-3.
Notes S1. 61 floral characters and character states recorded for Merianieae.
Notes S2. Detailed description of Merianieae pollination syndromes.

Table S1. Merianieae species included in morphospace and information on sampling localities.

species	collectin no	collector	country	state/province	elevation	collection date	voucher
Adelobotrys adscendens	FA10230	Frank Alemda	Colombia	Valle del Cauca	593	04.02.2011	CAS 1120080
Axinaea affinis	AD41	Agnes Dellinger	Ecuador	Azuay	3200	29.11.2012	-
Axinaea alata	NM55309	M Nee	Bolivia	Cochabamba	2845	03.05.2007	NY02424039
Axinaea floribunda	FM1981	Fabián Michelangeli	Peru	Cusco	2558	21.06.2012	NY02540381
Axinaea confusa	AD127	Agnes Dellinger	Ecuador	Loja	1800	13.09.2016	WU 0092828
Axinaea costaricensis	AD75	Agnes Dellinger	Costa Rica	San José	2600	03.02.2016	WU
Axinaea grandifolia 1	MA1697	Marcela Alvear	Colombia	Narino	2922	25.01.2013	CAS 1156779
Axinaea grandifolia 2	FM650	Fabián Michelangeli	Venezuela	Merida	2500-2700	13.01.2001	BH
Axinaea lehmannii	FA10322	Frank Alemda	Colombia	Valle del Cauca	2080	13.02.2011	CAS
Axinaea macrophylla	DSP1598	Darin S. Penneys	Ecuador	Morona-Santiago	2400	28.09.2003	NY02450495
Axinaea sclerophylla	AD24	Agnes Dellinger	Ecuador	Loja	2750	20.10.2012	WU 0072429
Axinaea scutigera	AD129	Agnes Dellinger	Ecuador	Napo	2715	14.10.2016	WU 0092827
Graffenrieda anomala	FA10434	Frank Alemda	Colombia	Chocó	99	31.01.2012	CAS 1127619
Graffenrieda colombiana	MA1862	Marcela Alvear	Colombia	Putumayo	699	18.02.2013	CAS 1156711
Graffenrieda cucullata	MA1735	Marcela Alvear	Colombia	Narino	1362	02.02.2013	CAS 1156955
Graffenrieda gracilis	FM1763	Fabián Michelangeli	Peru	Amazonas	764	18.03.2012	NY02540393
Graffenrieda harlingii	CU1843	Carmen Ulloa	Ecuador	Loja	2465-3230	04.06.2010	NY1596631
Graffenrieda maklekensis	FA10643	Frank Alemda	Colombia	Santander	1900	09.03.2012	CAS 1127617
Graffenrieda penneysii	AD184	Agnes Dellinger	Ecuador	Zamora-Chinchipe	2539	13.11.2017	QCNE
Graffenrieda santamartensis	FA10636	Frank Alemda	Colombia	Santander	1715	07.03.2012	CAS 1127621
Graffenrieda weddellii	MA1503	Marcela Alvear	Colombia	Risaralda	1374	05.01.2013	CAS 1155724
Macrocentrum fasciculatum	FM2144	Fabián Michelangeli	Suriname	Sipaliwini	720	20.08.2013	NYBG1637020

Meriania aff sanguinea	AD176	Agnes Dellinger	Ecuador	Carchi	3100	01.11.2017	QCNE
Meriania aff. drakei	AD141/DF2278	Agnes Dellinger	Ecuador	Pastaza	1843	13.11.2016	QCNE
Meriania albiflora	FM2211	Fabián Michelangeli	Cuba	Granma	885	08.11.2013	NYBG2361494
Meriania angustifolia	FM2241	Fabián Michelangeli	Cuba	Holguín	250	13.11.2013	NYBG02499331
Meriania arborea	FA10564	Frank Alemda	Colombia	Norte de Santander	2300	28.02.2012	CAS 1128115
Meriania aurata	AD145/DF2282	Agnes Dellinger	Ecuador	Pastaza	2208	13.11.2018	QCNE
Meriania brachycera	FA10547	Frank Alemda	Colombia	Norte de Santander	2600	26.02.2012	CAS 1127903
Meriania calophylla	FA1609	Fabián Michelangeli	Brazil	Espírito Santo	837	08.02.2011	NY1654154
Meriania cf costata	AD106/DF2214	Agnes Dellinger	Ecuador	Loja	2900	10.09.2016	WU 0092833
Meriania drakei	AD132	Agnes Dellinger	Ecuador	Napo	2052	14.10.2016	WU 0092805
Meriania sp. nov2	AD146/DF2285	Agnes Dellinger	Ecuador	Pastaza	1568	14.11.2016	WU 0092844
Meriania fantastica	MA1951	Marcela Alvear	Colombia	Putumayo	2314	16.02.2013	CAS 1156637
Meriania furvanthera	AD113/DF2236	Agnes Dellinger	Ecuador	Loja	2800	13.09.2016	WU 0092838
Meriania haemantha ssp. haemantha	FA10569	Frank Alemda	Colombia	Norte de Santander	2550	28.02.2012	CAS 1127905
Meriania haemantha ssp. orientalis	FA10651	Frank Alemda	Colombia	Santander	1700	11.03.2012	CAS 1128063
Meriania hernandoi	MA1856	Marcela Alvear	Colombia	Putumayo	2151	16.02.2013	CAS 1156636
Meriania hexamera	MA1854	Marcela Alvear	Colombia	Putumayo	2314	16.02.2013	CAS 1156638
Meriania inflata	RG2078	Renato Goldenberg	Brazil	Bahia	675	13.10.2014	NY02286571
Meriania longifolia	FA10536	Frank Alemda	Colombia	Norte de Santander	1259	25.02.2012	CAS 1127902
Meriania loxensis	AD115/DF2226	Agnes Dellinger	Ecuador	Loja	2700	12.09.2016	WU 0092836
Meriania macrophylla	MA1496	Marcela Alvear	Colombia	Risaralda	1338	05.01.2013	CAS 1156342
Meriania maguirei	AD110/DF	Agnes Dellinger	Ecuador	Loja	2850	11.09.2016	QCNE
Meriania maxima	MA1768	Marcela Alvear	Colombia	Narino	1888	06.02.2013	CAS 1155921

Meriania mexiae	MA1853	Marcela Alvear	Colombia	Putumayo	2314	16.02.2013	CAS 1156500
Meriania phlomoides	MA1733	Marcela Alvear	Colombia	Narino	1414	02.02.2013	CAS 1156124
Meriania pichinchensis	DSP1905	Darin Penneys	Ecuador	Pichincha	1930	06/12/2005	NY02500177
Meriania quintuplinervis	FA10306	Frank Alemda	Colombia	Valle del Cauca	2140	11.02.2011	CAS 1120552
Meriania radula	AD201/DF	Agnes Dellinger	Ecuador	Zamora-Chinchipe	3180	15.11.2017	QCNE
Meriania rugosa	FM1725	Fabián Michelangeli	Peru	Amazonas	2400	12.03.2012	NY02540643
Meriania sanguinea	AD108/DF2215	Agnes Dellinger	Ecuador	Loja	2850	10.09.2016	WU 0092832
Meriania selvaflorensis	MA1465	Marcela Alvear	Colombia	Caldas	1732	02.03.2011	CAS 1119760
Meriania silverstonei	FA10210	Frank Alemda	Colombia	Valle del Cauca	1960	01.02.2011	CAS 1120063
Meriania sp. nov1	AD158/DF2304	Agnes Dellinger	Ecuador	Pastaza	2533	15.11.2016	WU 0092856
Meriania speciosa	FA10219	Frank Alemda	Colombia	Valle del Cauca	1875	02.02.2011	CAS 1119942
Meriania splendens	MA1690	Marcela Alvear	Colombia	Narino	2922	25.01.2013	CAS 1156411
Meriania subumbellata	FM819	Fabián Michelangeli	Venezuela	Aragua	1550	03.01.2002	NYBG01101015
Meriania tetragona	AD187/DF	Agnes Dellinger	Ecuador	Zamora-Chinchipe	1859	14.11.2017	QCNE
Meriania tomentosa	AD105	Agnes Dellinger	Ecuador	Pichincha	1700	08.09.2016	WU 0092814
Meriania urceolata	KR1446	Karen Redden	Guyana	Cuyuni-Mazaruni	490	8.12.2002	NY02513392

Table S2. Pollinator information for the 19 Merianieae species used for delimiting pollination syndromes and as training set for Random Forest classification for pollinator estimation. The total number of days/nights when pollinator monitoring was made is given as well as the total number of hours of reviewed video material; a minimum of three 30 minute intervals was reviewed from every observation day.

species	pollinator group	source	study site	number of days filmed	number of nights filmed	hours reviewed daytime	hours reviewed nighttime
Adelobotrys adscendens	buzz-bee	A. S.Dellinger, pers. obs.	Costa Rica, Field Station La Gamba	7	-	13	-
Graffenrieda cucullata	buzz-bee	A. S.Dellinger, pers. obs.	Ecuador, Field Station Reserva Drákula	-	-	2h direct observation	-
Meriania drakei	buzz-bee	A. S.Dellinger, pers. obs.	Ecuador, Orchid Garden in Cosanga	-	-	2h direct observation	-
Meriania hernandoi	buzz-bee	A. S.Dellinger, pers. obs.	Ecuador, Orchid Garden in Cosanga	5	-	22	-
Meriania longifolia	buzz-bee	Renner 1989	-	-	-	-	-
Meriania maguirei	buzz-bee	A. S.Dellinger, pers. obs.	Ecuador, Podocarpus National Park	8	-	20	-
Meriania maxima	buzz-bee	A. S.Dellinger, pers. obs.	Ecuador, Bellavista Reserve	4	-	12	-
Meriania furvanthera	flowerpiercer/rodent	A. S.Dellinger, pers. obs.	Ecuador, Podocarpus National Park	2	3	8	7
Meriania costata	hummingbird/?bat	A. S.Dellinger, pers. obs.	Ecuador, Podocarpus National Park	2	-	5	-
Meriania quintuplinervis	hummingbird/?bat	Calderón-Sáenz 2012	-	-	-	-	-
Meriania pichichensis	hummingbird/bat	Muchhala & V.-Jarrín 2004, A. S.Dellinger, pers. obs.	Ecuador, Bellavista Cloudforest Reserve	-	-	-	-
Meriania aff. sanguinea	hummingbird/bat	A. S.Dellinger, pers. obs.	Ecuador, Guanderas Reserve	5	4	13	9.4

Meriania phlomoides	hummingbird/bat	Vogel 1997, A. S.Dellinger, pers. obs.	Costa Rica, Field Station Monteverde	5	3	10	12
Meriania tomentosa	hummingbird/bat	A. S.Dellinger, pers. obs.	Ecuador, Bellavista Cloudforest Reserve	8	7	15	6
Meriania sanguinea	hummingbird/rodent	A. S.Dellinger, pers. obs.	Ecuador, Podocarpus National Park	7	4	10	36
Axinaea confusa	passerine	Dellinger et al. 2014	-	-	-	-	-
Axinaea costaricensis	passerine	Dellinger et al. 2014	-	-	-	-	-
Axinaea macrophylla	passerine	Rojas-Nossa 2007	-	-	-	-	-
Axinaea sclerophylla	passerine	Dellinger et al. 2014	-	-	-	-	-

Table S3. Misclassification percentage of 19 Merianieae species with known pollinators when running models without the two most important predictive traits "pollen expulsion mechanism" and "reward type" (median error rate: 10.5%, 'buzz-bee': 28.6%, 'mixed-vertebrate' (MV): 0%, 'passerine': 0%). Misclassification only occurred in the two known buzz-bee pollinated species (*Adelobotrys adscendens*, *Graffenrieda cucullata*) with morphologies very distinct from the majority of buzz-bee pollinated Merianieae, which also displayed slight classification uncertainty in the full trait dataset. Classification errors disappeared when including all 61 species which encompass additional taxa sharing these distinct morphologies. Thus, models were considered accurate enough for pollination syndrome predictions.

species	known pollinator	% correct prediction
Adelobotrys adscendens	buzz-bee	0.07
Axinaea confusa	passerine	1
Axinaea costaricensis	passerine	1
Axinaea macrophylla	passerine	1
Axinaea sclerophylla	passerine	1
Graffenrieda cucullata	buzz-bee	0.01
Meriania costata	MV	1
Meriania drakei	buzz-bee	1
Meriania furvanthera	MV	1
Meriania hernandoi	buzz-bee	1
Meriania longifolia	buzz-bee	1
Meriania maguirei	buzz-bee	1
Meriania maxima	buzz-bee	1
Meriania phlomoides	MV	1
Meriania pichinchensis	MV	1
Meriania quintuplinervis	MV	1
Meriania sanguinea	MV	1
Meriania tomentosa	MV	1
Meriania aff. sanguinea	MV	1

Table S4. Probability of pollinator classification by Random Forest Analyses (RF) using 100 RFs with 500 trees each. For all species, the characters "reward type" and "pollen expulsion mechanism" were removed prior to estimation; additional characters which had to be removed due to missing data are listed in the column 'characters removed'.

species	buzz-bee	hb	pass	characters removed
Axinaea affinis	0	0	1	-
Axinaea alata	0	0	1	7, 9, 20
Axinaea cf floribunda	0	0	1	6
Axinaea grandifolia	0	0	1	22, 23
Axinaea grandifolia	0	0	1	7, 8, 9, 11, 13, 21, 46, 48, 49, 50, 51
Axinaea lehmannii	0	0	1	-
Axinaea scutigera	0	0	1	22, 23

Graffenrieda anomala	1	0	0	2, 3, 12, 13, 42, 43, 44, 45, 48, 49, 50, 51, 52
Graffenrieda colombiana	1	0	0	-
Graffenrieda gracilis	1	0	0	6, 15, 32
Graffenrieda harlingii	1	0	0	-
Graffenrieda maklekensis	1	0	0	6, 15, 32
Graffenrieda penneysii	1	0	0	32, 34, 35, 36
Graffenrieda santamartensis	1	0	0	6, 15, 32
Graffenrieda weddellii	1	0	0	-
Macrocentrum frutucosum	1	0	0	11
Meriania aff. drakei	1	0	0	-
Meriania albiflora	0	1	0	6
Meriania angustifolia	0	1	0	-
Meriania arborea	0	1	0	-
Meriania aurata	1	0	0	-
Meriania brachycera	1	0	0	-
Meriania calophylla	1	0	0	37
Meriania faldas	1	0	0	8, 9, 32
Meriania fantastica	1	0	0	-
Meriania haemantha ssp. haemantha	1	0	0	-
Meriania haemantha ssp. orientalis	1	0	0	-
Meriania hexamera	1	0	0	-
Meriania inflata	0	0	1	-
Meriania loxensis	0	1	0	-
Meriania macrophylla	0	0	1	35, 36
Meriania mexiae	1	0	0	-
Meriania radula	0	1	0	-
Meriania rugosa	1	0	0	-
Meriania selvaflorensis	1	0	0	21, 42, 43, 44, 45, 46, 48, 49, 50, 51, 52
Meriania silverstonei	1	0	0	-
Meriania sp. nov	0.97	0	0.03	-
Meriania speciosa	1	0	0	-
Meriania splendens	1	0	0	-
Meriania subumbellata	1	0	0	47
Meriania urceolata	1	0	0	6
Meriania tetragona	0	1	0	35, 36, 37

8

Table S5. Merianieae species included in the full phylogeny, sampling localities, collector and voucher information and Genbank accession numbers for genes used for constructing the phylogeny. "no" indicate genes where no data was obtained, 'xxxxxxxx' indicate sequences submitted to Genbank but no accession numbers received by the date of submission (28072018).

sequence_ID	ACCD	ETS	ITS	ndhf	psbk	rbcl	collected_by	Country	specimen_voucher
Adelobotrys_adscendens_FA10230_T185	MG198218	MF029158	KY991642	MF105310	MF104724	MF069642	Almeda F. 10230	Colombia	COL, CAS
Adelobotrys_barbata_T1641	KF819861	KF820580	AY460446	no	KF821781	no	Caddah M.K. 528	Brazil	UPCB
Adelobotrys_boissieriana_AF215530	no	no	no	no	no	AF215530	GENBANK ONLY	GENEBANK ONLY	GENEBANK ONLY
Adelobotrys_klugii_R&T11820	no	no	KF821398	no	no	no	GENBANK ONLY	GENEBANK ONLY	GENEBANK ONLY
Adelobotrys_macrantha_KR11159	no	no	AY966413	no	no	AF215531	Ruokolainen 11159	GENEBANK ONLY	NY
Adelobotrys_permixta_KMR1515	KF819862	MF029601	EU055643	MF105723	KF821782	MF070022	Redden K.M. 1515	Guyana	NY, US
Adelobotrys_praetexta_Schulman195	no	no	KF821399	no	no	no	GENBANK ONLY	GENEBANK ONLY	GENEBANK ONLY
Adelobotrys_ruokolainenii_Schulman219	no	no	AY966410	no	no	no	GENBANK ONLY	GENEBANK ONLY	GENEBANK ONLY
Adelobotrys_scandens_Schulman133	no	no	AY966406	AY966414	no	no	GENBANK ONLY	GENEBANK ONLY	GENEBANK ONLY
Adelobotrys_spruceana_CMK587	KF819863	KF820581	KF821400	MH760282	KF821783	MH747566	Caddah M.K. 587	Brazil	UPCB
Adelobotrys_subsessilis_T2963	no	MH781591	AY966407	MH760283	MH781651	MH747567	Michelangeli F.A. 493	Peru	BH, USM
Adelobotrys_tessmannii_KR11834	no	no	no	AY966415	no	no	GENBANK ONLY	GENEBANK ONLY	GENEBANK ONLY
Axinaea_affinis_NA	no	no	AY460447	no	no	no	Luteyn J. 14130	Ecuador	NY
Axinaea_alata_T646	KF819865	KF820583	KF821401	MH760284	KF821785	MH747568	Nee M.H. 55301	Bolivia	NY
Axinaea_confusa_AD127	no	MH781592	MH819864	MH760285	MH781652	MH747569	Dellinger A. 127	Ecuador	QCNE, W
Axinaea_costaricensis_FA10183	MG198210	MF029147	KY991632	MF105300	MF104713	MF069633	Almeda F. 10183	Colombia	COL, CAS
Axinaea_costaricensis_T365	KF819866	KF820584	KF821402	no	KF821786	no	Michelangeli F.A. 1223	Costa Rica	NY
Axinaea_fallax_T2659	MH781548	MH781593	MH819865	MH760286	MH781653	MH747570	Gonzalez M. F. 927	Colombia	COL, NY
Axinaea_floribunda_T2766	MH781549	MH781594	MH819866	no	MH781654	no	Michelangeli F.A. 1981	Peru	NY, USM
Axinaea_floribunda_T2914	MH781550	MH781595	MH819867	MH760287	MH781655	MH747571	Michelangeli F.A. 1957	Peru	NY, USM
Axinaea_grandifolia_FAM650	KF819867	KF820585	KF821404	MF105579	KF821787	MH747572	Michelangeli F.A. 650	Venezuela	BH, VEN
Axinaea_lehmannii_FA10322	MG198244	MF029184	KY991668	MF105347	MF104768	MF069679	Almeda F. 10322	Colombia	COL, CAS
Axinaea_macrophylla_cf_AD117	MH781551	no	MH819868	MH760288	MH781656	MH747573	Dellinger A. 117	Ecuador	QCNE, W
Axinaea_macrophylla_DSP1598	MG198483	no	KY991536	no	no	MF069943	Penneys D. S. 1598	Ecuador	NY

Axinaea_macrophylla_T1180	KF819870	KF820588	KF821405	no	KF821790	no	Michelangeli F.A. 1265	Venezuela	NY, VEN
Axinaea_minutiflora_T2752	no	MH781596	MH819869	no	MH781657	no	Pedraza P.P. 2203	Colombia	NY
Axinaea_nitida_T3049	MH781552	MH781597	MH819870	MH760289	MH781658	MH747574	Michelangeli F.A. 2616	Peru	NY, USM
Axinaea_pauciflora_cf_DSP1590	MG198482	no	KY991535	no	no	MF069941	Penneys D. S. 1590	Ecuador	NY
Axinaea_sclerophylla_DSP1878_T1670	MG198501	KF820586	KF821403	no	KF821788	MF069977	Ulloa C. U. 1769	Ecuador	MO
Axinaea_scutigera_MEM1758	no	no	KY991968	no	no	no	Morales, M. E. 1758	Colombia	UPTC
Axinaea_scutigera_T3337	no	no	MH819871	no	MH781659	no	Dellinger A. 129	Ecuador	QCNE, W
Axinaea_sp_T3114	no	MH781598	MH819872	no	MH781660	no	Michelangeli F.A. 2737	Peru	NY, USM
Axinaea_tomentosa_T2004	MH781553	MH781599	MH819873	MH760290	MH781661	MH747575	Michelangeli F. A. 1688	Peru	NY, USM
Axinaea_wurdackii_T3065	MH781554	MH781600	MH819874	MH760291	MH781662	MH747576	Michelangeli F.A. 2668	Peru	NY, USM
Centronia_laurifolia_DN14973	no	no	KY991530	no	MF105116	MF069925	Neill D. 14973	Ecuador	MO
Centronia_laurifolia_T3323	KF819890	no	KF821419	no	MH781663	no	Ulloa C. 1780	Ecuador	MO
Clidemia rubra	KF819953	KF820692	AY460481	AF215579	KF821892	AF215535	Michelangeli, F. A., 825 (NY)	Venezuela	NY
Eriocnema_fulva_T366_CVM222_T366_T366	KF819990	KF820735	AY460481	AY553781	KF821935	AY553777	Almeda F. 8414	Brazil	CAS
Graffenrieda_anomala_FA10434	no	MF029205	KY991689	MF105356	MF104786	MH747577	Almeda F. 10434	Colombia	COL, CAS
Graffenrieda_bella_DSP1657	MG198488	no	KY991541	MF105629	MF105149	MF069953	Penneys D. S. 1657	Panama	FLAS
Graffenrieda_colombiana_MA1862_MA2608	no	MH781601	MH819875	MH760292	MH781664	no	Alvear M. 1862	Colombia	COL, CAS
Graffenrieda_cucullata_DSP1873_T1673	MG198500	MF029543	KY991556	MF105675	KF821936	MF069976	Penneys D. S. 1873	Ecuador	NY
Graffenrieda_emarginata_cf_T3072_T3115	MH781555	MH781602	MH819876	no	MH781665	no	Michelangeli F.A. 2687	Peru	NY, USM
Graffenrieda_emarginata_DSP1890	no	MF029547	KY991559	MF105573	MF105100	MF069902	Penneys D. S. 1890	Ecuador	NY
Graffenrieda_emarginata_T1676	KF819992	KF820737	KF821476	no	KF821937	no	Ulloa C.U. 1803	Ecuador	MO
Graffenrieda_galeottii_T1936	KF819993	KF820738	AY460449	MH760293	KF821938	MH747578	David H. 3242	Colombia	HUA
Graffenrieda_glandulosa_T977	KF819994	KF820739	KF821477	MH760294	KF821939	no	Goldenberg R. 938	Brazil	UPCB
Graffenrieda_goldenbergii_T983	KF820004	MH781603	KF821485	MH760295	MH781666	MH747579	Goldenberg R. 962	Brazil	UPCB
Graffenrieda_gracilis_T975	KF819995	KF820740	KF821478	MH760296	KF821940	MH747580	Goldenberg R. 935	Brazil	UPCB
Graffenrieda_harlingii_T1671	KF819996	KF820741	KF821479	MH760297	KF821941	MH747581	Ulloa C.U. 1774	Ecuador	MO
Graffenrieda_hitchcockii_T1242	KF819997	KF820742	KF821480	no	KF821942	no	Michelangeli F.A. 359	Venezuela	BH, VEN
Graffenrieda_intermedia_T579	KF819998	KF820743	EU055684	MF105536	KF821943	MF069866	Goldenberg R. 855	Brazil	UPCB

Graffenrieda_irwinii_T2696	no	MH781604	no	MH760298	MH781667		Michelangeli F. A. 2696	Guyana	NY
Graffenrieda_jefensis_DSP1687	no	no	no	MF105633	no	MF069956	Penneys D. S. 1687	Panama	FLAS
Graffenrieda_laevicarpa_T2937	MH781556	MH781605	AY460450	MH760299	MH781668	MH747582	Goldenberg R. 1940	Brazil	UPCB
Graffenrieda_latifolia_DSP1303	KY821079	MF029485	EF683143	EU055943	MF105119	MF069928	Penneys D. S. 1303	Dominica	FLAS
Graffenrieda_latifolia_FAM794	JQ730297	KF820744	no	no	JQ730503	no	Michelangeli F.A. 794	Venezuela	BH, VEN
Graffenrieda_limbata_T786	KF819999	KF820745	KF821481	MH760300	KF821944	MH747583	Goldenberg R. 998	Brazil	UPCB
Graffenrieda_maklenkensis_T2080	no	MF029227	KY991711	MH760301	MF104805	no	Almeda F. 10643	Colombia	COL, CAS
Graffenrieda_miconioides_T773	KF820000	KF820746	KF821482	MH760302	KF821945	MH747584	Goldenberg R. 929	Brazil	UPCB
Graffenrieda_micrantha_aff_DSP1511	MG198479	MF029492	KY991532	MF105600	MF105125	MF069935	Penneys D. S. 1511	Costa Rica	FLAS
Graffenrieda_micrantha_T1373	KF820001	KF820747	KF821483	MH760303	KF821946	MH747585	Kriebel R. 5503	Costa Rica	NY
Graffenrieda_moaensis_T774	KF820002	KF820748	KF821484	MH760304	KF821947	MH747586	Goldenberg R. 931	Brazil	UPCB
Graffenrieda_moritziana_FAM832	JQ730298	KF820749	AY460451	EU055944	JQ730504	EU711390	Michelangeli F.A. 832	Venezuela	BH, VEN
Graffenrieda_penneysii_DSP1891_T2903	MH781557	MH781606	MH819877	MH760305	MH781669	MH747587	Ulloa C. 1804	Ecuador	MO
Graffenrieda_reticulata_T3028	no	MH781607	MH819878	MH760306	MH781670	MH747588	Forzza R. 7150	Brazil	RB
Graffenrieda_rotundifolia_C&R	no	no	AF215532	AF215576	no	AF215532	Genebank only	GENEBANK ONLY	GENEBANK ONLY
Graffenrieda_rufescens_T2668	MH781558	MH781608	MH819879	no?	MH781671	MH747589	Michelangeli F. A. 2214	Cuba	HAJB, NY
Graffenrieda_santamartensis_aff_FA10650	no	MF029229	KY991713	MF105371	MF104807	no	Almeda F. 10650	Colombia	COL, CAS
Graffenrieda_santamartensis_FA10193	no	MF029150	KY991634	MF105303	MF104716	MF069613	Almeda F. 10193	Colombia	COL, CAS
Graffenrieda_sessilifolia_FAM510	KF820003	KF820750	AY460452	MH760307	KF821948	MH747590	Michelangeli F.A. 510	Venezuela	BH, VEN
Graffenrieda_sp_T1028	MH781559	MH781609	no	MH760308	MH781672	MH747591	Nee M. 55646	Bolivia	NY
Graffenrieda_spnov_T3026	MH781560	no	MH819880	no	MH781673	no	Forzza R. 6590	Brazil	RB
Graffenrieda_tamana_T2286	MH781561	MF029214	KY991698	MF105359	MF104792	MH747592	Almeda F. 10540	Colombia	COL, CAS
Graffenrieda_uribei_aff_FA10222	MG198217	MF029157	KY991641	MF105309	MF104723	MF069641	Almeda F. 10222	Colombia	COL, CAS
Graffenrieda_uribei_HM17594	KF820005	KF820752	KF821486	MH760309	KF821950	MH747593	Mendoza H. 17594	Colombia	FMB
Graffenrieda_weddellii_KMR4548	MG198197	KF820753	KF821487	no	KF821951	no	Redden K.M. 4548	Guyana	NY, US
Leandra mexicana	no	KF820811	GU968799	AF215580	KF822003	AF215536	Genebank only	GENEBANK ONLY	GENEBANK ONLY
Macrocentrum_anfractum_T797	KF820085	KF820851	KF821521	MH760310	KF822037	MF070024	Redden K.M. 5676	Guyana	NY, US
Macrocentrum_brevipedicellatum_T2941	MH781562	MH781610	MH819881	MH760311	MH781674	MH747594	Radosavljevic A. 183	Guyana	NY, US

Macrocentrum_cristatum_microphyllum_T807	KF820086	no	KF821522	no?	KF822038	???????	Wurdack K.J. 4218	Guyana	NY, US
Macrocentrum_cristatum_T2943	MG198564	MH781611	KY991908	no	MF105286	MF070050	Radosavljevic A. 251	Guyana	NY, US
Macrocentrum_droseroides_T805	KF820087	KF820852	KY991906	MF105745	MF070049	MF070049	Wurdack K.J. 4188	Guyana	NY, US
Macrocentrum_fasciculatum_T969	KF820088	no	KY991909	no	KF822040	MF070051	Wurdack K.J. 4342	Guyana	NY, US
Macrocentrum_gesneriaceum_T1105	KF820089	no	KF821525	MH760312	KF822041	MH747595	Redden K.M. 5001	Guyana	NY, US
Macrocentrum_minus_T1104	KF820090	KF820854	KF821526	MH760313	KF822042	MF069618	Redden K.M. 3813	Guyana	NY, US
Macrocentrum_neblinense_DD14049	KF820091	KF820855	KF821527	MH760314	MH781675	MH747596	Daly D. 14049	Colombia	NY
Macrocentrum_parvulum_T2556	no	MH781612	MH819882	MH760315	MH781676	MH747597	Michelangeli F. A. 2158	Suriname	NY
Macrocentrum_repens_T799	KF820092	KF820856	KF821528	MF105726	KF822043	MF070025	Redden K.M. 5821	Guyana	NY, US
Macrocentrum_vestitum_T2680_T2683	no	no	MH819883	MH760316	too short	MH747598	Michelangeli F. A. 2346	Guyana	NY
Maguireanthus_ayangannae_T2770_T2789A	no	MH781613	MH819884	MH760317	MH781677	MH747599	Radosavljevic A. 325	Guyana	NY, US
Meriania_acostae_T712	MG198470	KF820875	KF821537	MH760318	KF822061	MH747600	Moran R.C. 6838	Ecuador	NY
Meriania_albiflora_T2667	MH781563	MH781614	MH819885	MH760319	MH781678	MH747601	Michelangeli F. A. 2211	Cuba	HAJB, NY
Meriania_almedae_DF61	no	no	MH819886	no	no	no	Neill D. 16923	Ecuador	MO
Meriania_amplexicaulis_DF40	no	no	MH819887	no	no	no	Fernandez D. M. 1540	Ecuador	QCN
Meriania_angustifolia_T2670	MH781564	MH781615	MH819888	MH760320	MH781679	MH747602	Michelangeli F. A. 2241	Cuba	HAJB, NY
Meriania_aracaensis_T2936	MH781565	no	MH819889	MH760321	MH781680	MH747603	Goldenberg R. 1937	Brazil	UPCB
Meriania_arborea_FA10564	MH781566	MF029219	KY991703	MF105362	MF104797	MH747604	Almeda F. 10564	Colombia	COL, CAS
Meriania_aurata_AD145	no	MH781616	MH819890	MH760322	MH781681	MH747605	Dellinger A. 145	Ecuador	QCNE, W
Meriania_barbosae_MA1459	MH781567	MF029323	KY991929	MH760323	MF104900	MF069777	Alvear M. 1459	Colombia	COL, CAS
Meriania_brachycera_FA10593	no	MF029222	KY991706	MF105365	MF104800	no	Almeda F. 10593	Colombia	COL, CAS
Meriania_brachycera_T2916	MH781568	MH781617	MH819891	MH760324	MH781682	no	Almeda F. 10531	Colombia	COL, CAS
Meriania_brevipedunculata_T2663	KJ933883	KJ933924	KJ933971	MH760325	KJ934024	no	Majure L.C. 4279	Haiti	FLAS
Meriania_calophylla_T616	KF820112	KF820876	EU055707	MF105547	KF822062	MH747606	Kollmann L. 8843	Brazil	UPCB
Meriania_calyptrata_T811	KF820113	KF820877	KF821538	no	KF822063	no	Rochelle A. 351	Brazil	USP
Meriania_compressicaulis_DSP1759	KY821078	MF029531	KY782388	MF105568	MF105097	MF069900	Penneys D. S. 1759	Panama	NY
Meriania_costata_AD106	MH781569	MH781618	MH819892	MH760326	MH781683	MH747607	Dellinger A. 106	Ecuador	QCNE, W
Meriania_crassiramis_T2944	no	MH781619	MH819893	MH760327	MH781684	MH747608	Radosavljevic A. 258	Guyana	NY, US

Meriania_cuzcoana_T2658	MH781570	MH781620	MH819894	MH760328	MH781685	MH747609	Michelangeli F. A. 1908	Peru	NY, USM
Meriania_denticulata_DF64	no	no	MH819895	no	no	no	Homeier H. 2202	Ecuador	NY
Meriania_drakei_aff3_AD153	MH781571	MH781621	MH819896	MH760329	MH781686	MH747610	Dellinger A. 153	Ecuador	QCNE, W
Meriania_drakei_drakei_AD142	MH781572	MH781622	MH819897	MH760330	MH781687	MH747611	Dellinger A. 142	Ecuador	QCNE, W
Meriania_ekmanii_T2664	KJ933884	KJ933925	KJ933972	MH760331	KJ934025	MH747612	Majure L.C. 4299	Haiti	FLAS
Meriania_fantastica_MA1851	no	no	KY991960	MF105460	MF104929	no	Alvear M. 1851	Colombia	COL, CAS
Meriania_franciscana_CU1795	KF820114	KF820878	KF821539	MH760332	KF822064	MH747613	Ulloa C.U. 1795	Ecuador	MO
Meriania_furvanthera_AD23	MH781573	MH781623	MH819898	MH760333	MH781688	no	Dellinger A. 23	Ecuador	QCNE, W
Meriania_grandiflora_DSP1746	MG198496	MF029525	KY991550	MF105649	no	MF069964	Penneys D. S. 1746	Panama	FLAS
Meriania_haemantha_FA10546	no	MF029216	KY991700	MF105360	MF104794	no	Almeda F. 10546	Colombia	COL, CAS
Meriania_haemantha_v_orientalis_FA10651	KF819889	KF820609	KF821418	KY991658	MF104808	no	Almeda F. 10651	Colombia	COL, CAS
Meriania_hernandoi_FA10300	MG198234	MF029174	KY991658	MF105337	MF104758	MF069669	Almeda F. 10300	Colombia	COL, CAS
Meriania_hexamera_AD139	no	MH781624	MH819899	MH760334	MH781689	MH747614	Dellinger A. 139	Ecuador	QCNE, W
Meriania_hexamera_T1680	KF820115	KF820879	KF821540	MH760335	KF822065	MH747615	Ulloa C.U. 1825	Ecuador	MO
Meriania_inflata_T2786	MH781574	MH781625	MH819900	MH760336	MH781690	MH747616	Goldenberg R. 2078	Brazil	UPCB
Meriania_involucrata_T270	KF820116	KF820880	EF418874	MF105734	KF822066	MF070034	Skean D. 4097	Dom. Rep.	FLAS
Meriania_kirkbridei_DF48	no	no	MH819901	no	no	no	Fernandez D. M. 1541	Ecuador	QCN
Axinaea_lawsonnonii_cf_AD116	MH781575	MH781626	MH819902	MH760337	MH781691	MH747617	Dellinger A. 116	Ecuador	QCNE, W
Meriania_leucantha_T1695	KF820117	KF820881	KF821541	MH760338	KF822067	MH747618	Judd W.S. 8303	Jamaica	FLAS
Meriania_longifolia_FA10169	MG198207	MF029145	KY991629	MF105298	MF104711	MF069630	Almeda F. 10169	Colombia	COL, CAS
Meriania_longifolia_FAM610	JQ730316	KF820882	AY460454	no	KF822068	no	Michelangeli F.A. 610	Venezuela	BH, VEN
Meriania_loxensis_AD115	MH781576	MH781627	MH819903	MH760339	MH781692	MH747619	Dellinger A. 115	Ecuador	QCNE, W
Meriania_macrophylla_costanensis_FAM829	KF820118	KF820883	AY460455	no	KF822069	no	Michelangeli F.A. 829	Venezuela	BH, VEN
Meriania_macrophylla_macrophylla_DSP1741	MG198495	MF029524	KY991549	MF105647	MF105164	MF069962	Penneys D. S. 1741	Panama	NY
Meriania_maguirei_AD110	no	MH781628	MH819904	MH760340	MH781693	no	Dellinger A. 110	Ecuador	QCNE, W
Meriania_maxima_DSP1618	MG198486	MF029505	KY991539	MF105617	MF105138	MF069946	Penneys D. S. 1618	Ecuador	FLAS
Meriania_mexiae_DSP1848	no	no	KY991554	no	no	MF069974	Penneys D. S. 1848	Ecuador	NY
Meriania_nobilis_MEM1781	MG198468	MF029474	KY991969	no	no	no	Morales M. E. 1781	Colombia	UPTC

Meriania_nobilis_T2767	MH781577	MH781629	MH819905	no	MH781694	no	Clark J. L. 13051	Colombia	UNA
Meriania_panamensis_DSP1734	MG198493	MF029521	KY991546	MF105644	no	MF069960	Penneys D. S. 1734	Panama	FLAS
Meriania_paniculata_T3022	MH781578	MH781630	MH819906	no	MH781695	no	Reginato M. 1477	Brazil	NY, UPCB
Meriania_parvifolia_T2300	KJ933885	KJ933926	KJ933973	MH760341	KJ934026	MH747620	Skean D. 5048	Haiti	FLAS
Meriania_pastazana_AD143	no	no	MH819907	MH760342	MH781696	MH747621	Dellinger A. 143	Ecuador	QCNE, W
Meriania_peltata_AD148	no	MH781631	MH819908	MH760343	MH781697	MH747622	Dellinger A. 148	Ecuador	QCNE, W
Meriania_phlomoides_FA10354	MG198254	MF029196	KY991680	MF105353	MF104777	MF069687	Almeda F. 10354	Colombia	COL, CAS
Meriania_pichichensis_DSP1905	MG198506	MF029556	KY991563	MF105685	MF105197	MF069982	Penneys D. S. 1905	Ecuador	NY
Meriania_purpurea_T1696	KF820119	KF820885	KF821542	MH760344	KF822071	MH747623	Judd W.S. 8306	Jamaica	FLAS
Meriania_quintuplinervis_FA10306	MG198237	MF029177	KY991661	MF105340	MF104761	MF069672	Almeda F. 10306	Colombia	COL, CAS
Meriania_radula_cf_AD126	no	MH781632	MH819909	MH760345	MH781698	MH747624	Dellinger A. 126	Ecuador	QCNE, W
Meriania_radula_T2008	MH781579	MH781633	MH819910	MH760346	MH781699	MH747625	Michelangeli F. A. 1732	Peru	NY, USM
Meriania_rigida_aff_T3056	MH781580	no	MH819911	no	MH781700	no	Michelangeli F.A. 2635	Peru	NY, USM
Meriania_rigida_DSP1617	MG198485	MF029504	KY991538	MF105616	MF105137	MF069945	Penneys D. S. 1617	Ecuador	FLAS
Meriania_robusta_T1717	KF820120	KF820886	no	no	KF822072	no	Michelangeli F.A. 1623	Brazil	NY, UPCB
Meriania_rugosa_T2006	MH781581	MH781634	MH819912	MH760347	MH781701	MH747626	Michelangeli F. A. 1704	Peru	NY, USM
Meriania_sanguinea_aff_T3087	MH781582	MH781635	MH819913	MH760348	MH781702	MH747627	Michelangeli F.A. 2743	Peru	NY, USM
Meriania_sanguinea_DSP1588	MG198481	no	KY991534	MF105611	no	no	Penneys D. S. 1588	Ecuador	NY
Meriania_sanguinea_T3338	MH781583	no	no	MH760349	MH781703	MH747628	Fernandez D. M. 2215	Ecuador	QCN
Meriania_sclerophylla_T706	KF820121	KF820887	KY991910	no	KF822073	MF069919	Redden K.M. 1219	Guyana	NY, US
Meriania_selvaflorensis_MA1465	MG198326	MF029324	KY991930	no	MF104901	MF069778	Alvear M. 1465	Colombia	COL, CAS
Meriania_silverstonei_FA10348	MG198252	MF029193	KY991677	MF105352	MF104774	MF069684	Almeda F. 10348	Colombia	COL, CAS
Meriania_sp_AD149	no	MH781636	MH819914	MH760350	MH781704	MH747629	Dellinger A. 149	Ecuador	QCNE, W
Meriania_sp_AD155	no	MH781637	MH819915	MH760351	MH781705	MH747630	Dellinger A. 155	Ecuador	QCNE, W
Meriania_sp_FA10147	MG198203	MF029140	KY991623	no	MF104705	MF069624	Almeda F. 10147	Colombia	COL, CAS
Meriania_sp_FA10184	MG198211	MF029148	KY991633	MF105301	MF104714	MF069634	Almeda F. 10184	Colombia	COL, CAS
Meriania_sp_faldas_AD146	no	MH781638	MH819916	MH760352	MH781706	MH747631	Dellinger A. 146	Ecuador	QCNE, W
Meriania_sp_MA1475	MG198327	MF029325	KY991931	MF105437	MF104902	no	Alvear M. 1475	Colombia	COL, CAS

Meriania_sp_T2915	MH781584	MH781639	MH819917	no	MH781707	no	Michelangeli F.A. 1991	Peru	NY, USM
Meriania_speciosa_FA10219	MG198216	MF029156	KY991640	MF105308	MF104722	MF069640	Almeda F. 10219	Colombia	COL, CAS
Meriania_splendens_MA1690	no	MH781640	MH819918	MH760353	MH781708	MH747632	Alvear M. 1690	Colombia	COL, CAS
Meriania_spnov_AD157	no	MH781641	MH819919	MH760354	MH781709	MH747633	Dellinger A. 157	Ecuador	QCNE, W
Meriania_squamulosa_T2665	KJ933886	KJ933927	KJ933974	MH760355	KJ934027	no?	Skean D. 5053	Haiti	FLAS
Meriania_steyermarkii_FAM1266	MG198457	MF029462	KY991809	MF105577	MF105104	MF069903	Michelangeli F. A. 1266	Venezuela	NY, VEN
Meriania_subumbellata_FAM819	KF820122	KF820889	AY460457	MH760356	KF822075	MH747634	Michelangeli F.A. 819	Venezuela	BH, VEN
Meriania_tetragona_AD107	MH781585	MH781642	MH819920	MH760357	MH781710	MH747635	Dellinger A. 107	Ecuador	QCNE, W
Meriania_tetragona_T2009	no	MH781643	MH819921	MH760358	MH781711	MH747636	Michelangeli F. A. 1739	Peru	NY, USM
Meriania_tetramera_T972	no	MH781644	MH819922	MH760359	MH781712	MH747637	Goldenberg R. 911	Brazil	UPCB
Meriania_tomentosa_aff_AD144	MH781586	MH781645	MH819923	MH760360	MH781713	MH747638	Dellinger A. 144	Ecuador	QCNE, W
Meriania_tomentosa_DSP1899	MG198505	MF029553	KY991562	MF105682	no	MF069981	Penneys D. S. 1899	Ecuador	FLAS
Meriania_tomentosa_T3051	no	MH781646	MH819924	no	MH781714	no	Michelangeli F.A. 2623	Peru	NY, USM
Meriania_tuberculata_T2274	MH781587	MH781647	MH819925	MH760361	MH781715	MH747639	Pedraza P.P. 2142	Colombia	NY
Meriania_urceolata_FAM539	KF820124	KF820891	AY460458	no	KF822077	MH747640	Michelangeli F.A. 539	Venezuela	BH, VEN
Meriania_weberbaueri_T3078	MH781588	MH781648	MH819926	MH760362	MH781716	MH747641	Michelangeli F.A. 2714	Peru	NY, USM
Miconia calycina	KF820179	KF820956	EU055737	EU056001	KF822139	JF832003	Judd, W., 8210 (FLAS)	Puerto Rico	FLAS
Physeterostemon_fiaschii_T319	KF820526	KF821337	KF821756	EU711379	KF822520	EU711397	Amorim A.M. 4515	Brazil	CEPEC
Physeterostemon_jardimii_T742	KF820527	KF821338	KF821757	EU711382	KF822521	EU711399	Amorim A.M. 7064	Brazil	CEPEC
Physeterostemon_thomasii_T355	JQ730332	KF821339	KF821758	EU711383	JQ730542	EU711401	Amorim A.M. 5054	Brazil	CEPEC
Salpinga_glandulosa_T2938	MH781589	MH781649	MH819927	no?	MH781717	no	Goldenberg R. 1941	Brazil	UPCB
Salpinga_maranoensis_JLC6979	MG198373	MF029372	KY991873	JF831982	MF104983	JF832008	Clark J. L. 6979	Ecuador	NY
Salpinga_peruviana_T_3331_T3336	MH781590	MH781650	MH819928	MH760363	MH781718	MH747642	Clark J. L. 15100	Ecuador	UNA
Salpinga_secunda_FAM487	MG198459	no	KY991815	EU711384	MF105092	EU711402	Michelangeli F. A. 487	Peru	NY, USM
Tococa guianensis	KF820567	KF821385	AY460554	EU056136	KF822559	AM235650	Michelangeli, F. A., 703 (BH)	Venezuela	BH

Table S6. Predictive value of floral characters used in traditional pollination syndromes (e.g. Ollerton et al. 2009, Lagomarsino et al. 2016) in Merianieae (measured by reduction in Gini index), the floral traits belonging to the 20 most important floral characters identified are marked in bold.

Traditional pollination syndrome characters in Merianieae	Reduction in Gini index	Relative ranking
Reward type	0.802	**2**
Positioning of inflorescence	0.060	38
Flower orientation	0.624	**3**
Maximal corolla opening	0.141	23
Corolla height	0.490	**9**
Corolla shape	0.492	**7**
Corolla colour	0.122	25
Petal gloss	0.600	**5**
Scent	0.109	26
Arrangement of androecium relative to corolla	0.098	29
Level of anther pore relative to style	0.356	**15**
Adaxial thecal wall	0.368	**14**
Colour contrast appendage/thecae	0.059	44
Relation between stigma and corolla	0.622	**4**
Timing of anthesis	not included	not included

Table S7. Estimated average number of pollination syndrome shifts across 1000 stochastic character mappings, the total average number of pollination syndrome transitions is 10.675.

ancestral syndrome	shifted syndrome	average number of shifts
buzz-bee	mixed-vertebrate	3.402
buzz-bee	passerine	5.839
mixed-vertebrate	buzz-bee	0.468
mixed-vertebrate	passerine	0.277
passerine	buzz-bee	0.501
passerine	mixed-vertebrate	0.188

Table S8. Results from post-hoc test on morphological differences between pollination syndromes (Bonferroni corrected, PERMANOVA). F value is given in the upper part of each classification method, * indicates significant p-value 0.01667.

	buzz-bee	MV	pass
buzz-bee		34.389	25.717
MV	*		49.674
pass	*	*	

Table S9. Results from post-hoc test on significant differences in disparity (mean pairwise differences) between pollination syndromes. * indicates p-value < 0.001.

	buzz-bee	MV	pass
buzz-bee		2.985	7.862
MV	0.0085		3.971
pass	*	*	

17

Figure S1. Nectar producing *Meriania* species with known pollinators grouped into the 'mixed vertebrate' pollination syndrome. a-c: hummingbird/bat pollinated, (a) *M. tomentosa*, (b) *M. phlomoides*, (c) *M.* aff. *sanguinea*. d, e: flowerpiercer/rodent pollinated *M. furvanthera*. f: hummingbird/rodent pollinated *M. sanguinea*. g: hummingbird pollinated *M. quintuplinervis,* night observations have never been done. h: hummingbird pollinated *M. costata,* night observations have never been done. i: *M. tetragona*, hummingbirds observed close to flowers, night observations have never been done. Given the large similarity of g, h, i, to species where both day and night monitoring was conducted and both diurnal (hummingbirds, flowerpiercers) and nocturnal (bats, rodents) pollinators were observed, nocturnal pollinator visits in g, h, i are highly probable.

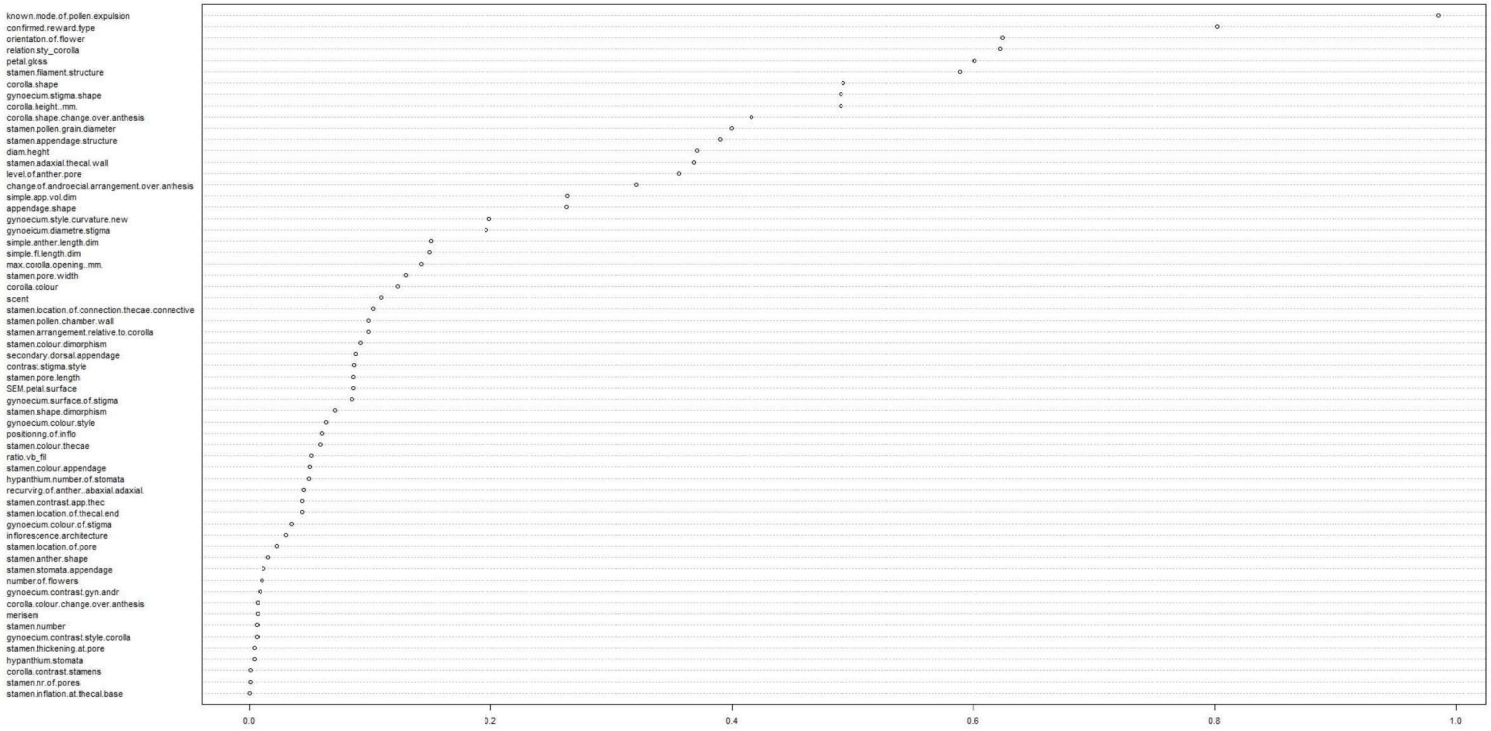
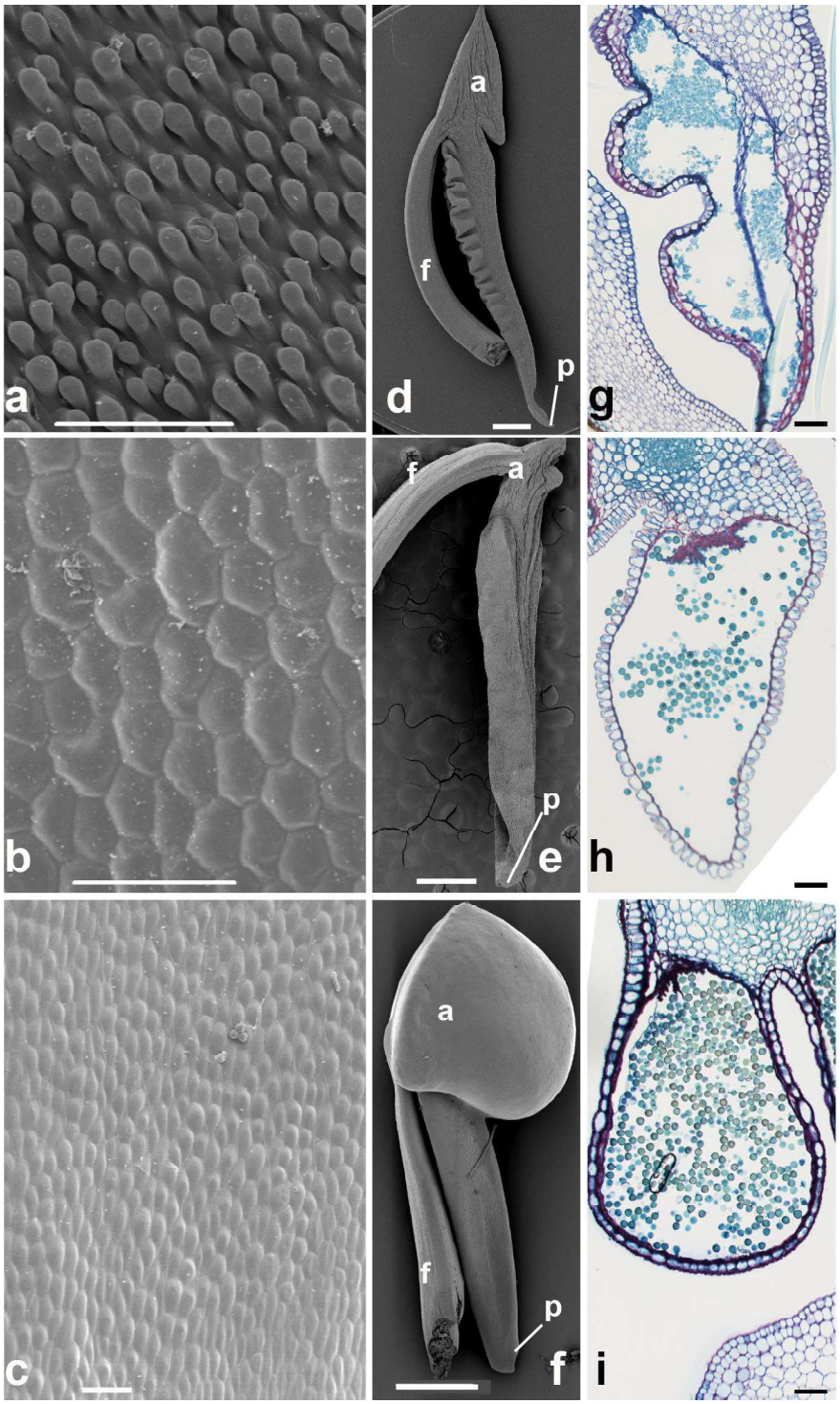


Figure S2. Ranking of all 61 assessed floral characters based on their importance in predicting pollination syndromes in Merianieae (based on Gini Index).



20

Figure S3. Structural properties of petals and stamens in Merianieae. (a) 'buzz-bee' syndrome petal surface with papillate epidermis of *Meriania brachycera*. (b) 'Mixed vertebrate' syndrome petal surface with almost smooth epidermis of *M. tomentosa*s (c) 'Passerine' syndrome petal surface with smooth epidermis of *Axinaea costaricensis*. (d) 'buzz-bee' syndrome stamen of *M. haemantha* ssp. *haemantha*, note ventral attachment of corrugated thecae to connective and sculptured appendage (e) 'Mixed vertebrate" syndrome stamen of *M. furvanthera*, note lateral attachment of pollen chambers to connective and small appendage. (f) 'Passerine' syndrome stamen of *Axinaea costaricensis* with bulbous appendage and ventral attachment of pollen chambers to connective. (g) Cross-section of theca of 'buzz-bee' syndrome *M. haemantha* ssp. *haemantha*, note epidermis and endothecium with thickened cell walls as well as corrugated structure of thecal wall and presence of septum separating the two pollen sacs of the theca. (h) Cross-section of theca of 'mixed vertebrate' syndrome *M. pichichensis* with flexible pollen chamber wall and collapsed septum (remnants indicated with arrowhead). (i) Cross-section of theca of 'passerine' syndrome *A. costaricensis* with smooth thecae with thickened cell walls in epidermis and collapsed septum (arrowhead). a – appendage, f – filament, p – pore, scale bars: (a), (b), (g), (h), (i), (j) 100 µm; (c), (l) 200 µm; (k) 500 µm; (d), (e), (f) 1 mm.

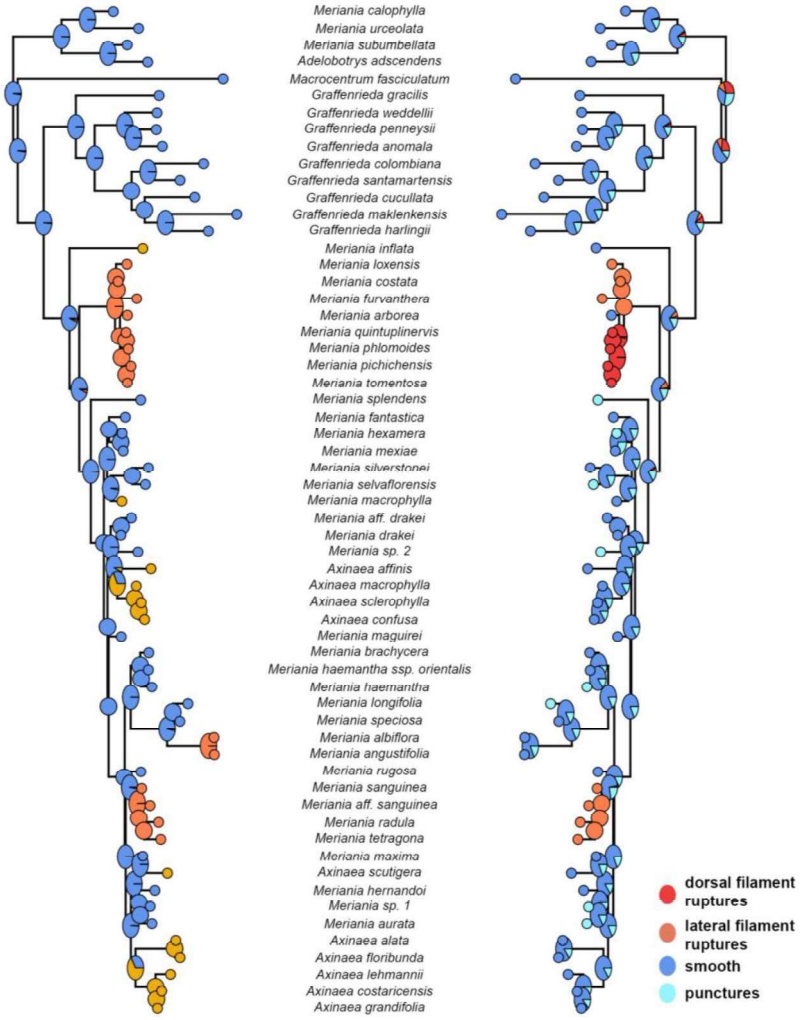


Figure S4. Stochastic character mapping of pollination syndromes (left) and the 'filament structure' (right). Note that filament ruptures are only found within the 'mixed-vertebrate' syndrome (in salmon on the left) while the ancestral 'buzz-bee' syndrome (blue on the left) and the 'passerine' syndrome (yellow on the left) do not show filament ruptures. The 'all rates different' model was chosen to estimate filament structure evolution as it performed significantly better than the 'equal rates' model (ER: log-likelihood: -53,5, AIC 109, ARD: log-likelihood: -36.6, AIC 97, ANOVA: $p < 0.001$).

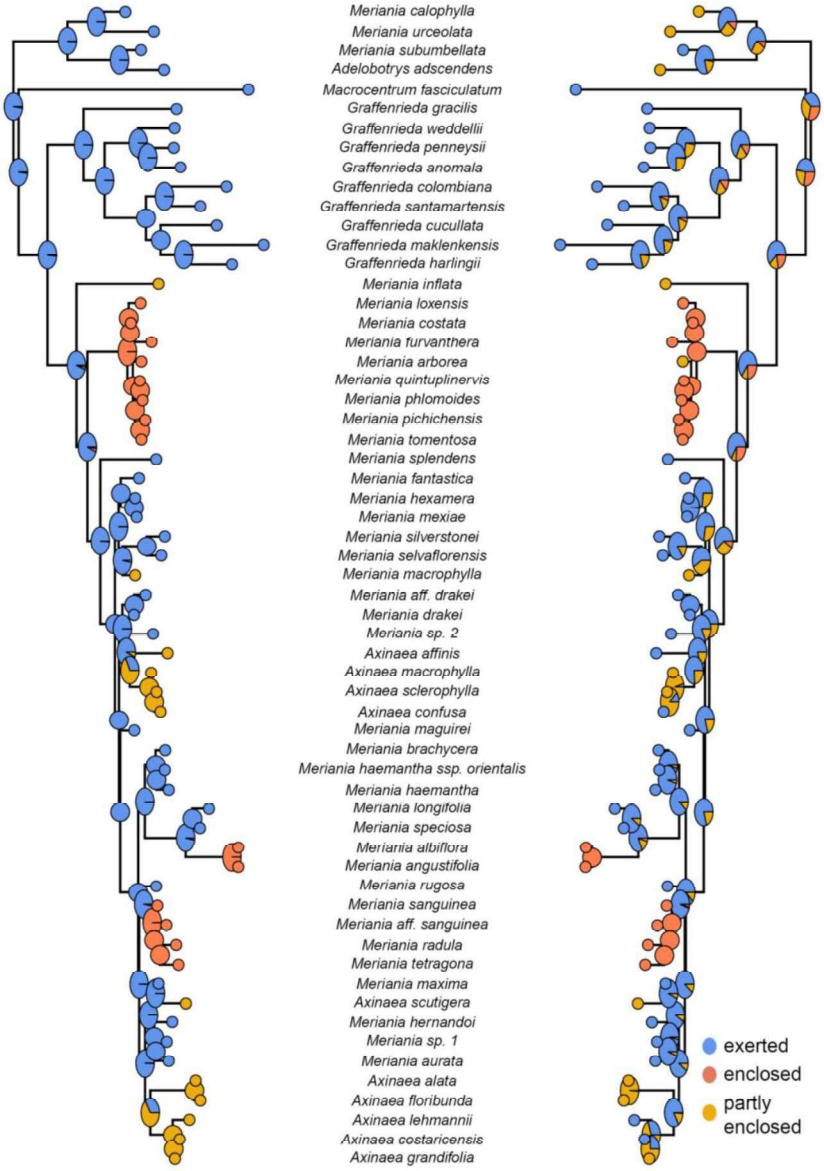


Figure S5. Stochastic character mapping of pollination syndromes (left) and the character 'relation style to corolla' (right). Note that in all 'mixed-vertebrate' species (in salmon on the left), styles are enclosed by the pseudo-campanulate corolla, while 'passerine' syndrome species (in yellow on the left) have more open corollas with only partly enclosed or exerted styles and most 'buzz-bee' syndrome flowers (in blue on the left) have fully exerted styles ('ER' model: log-likelihood -47.2, AIC96.4, 'ARD' model. Log-likelihood -40.1, AIC 92.3, ANOVA p 0.014).

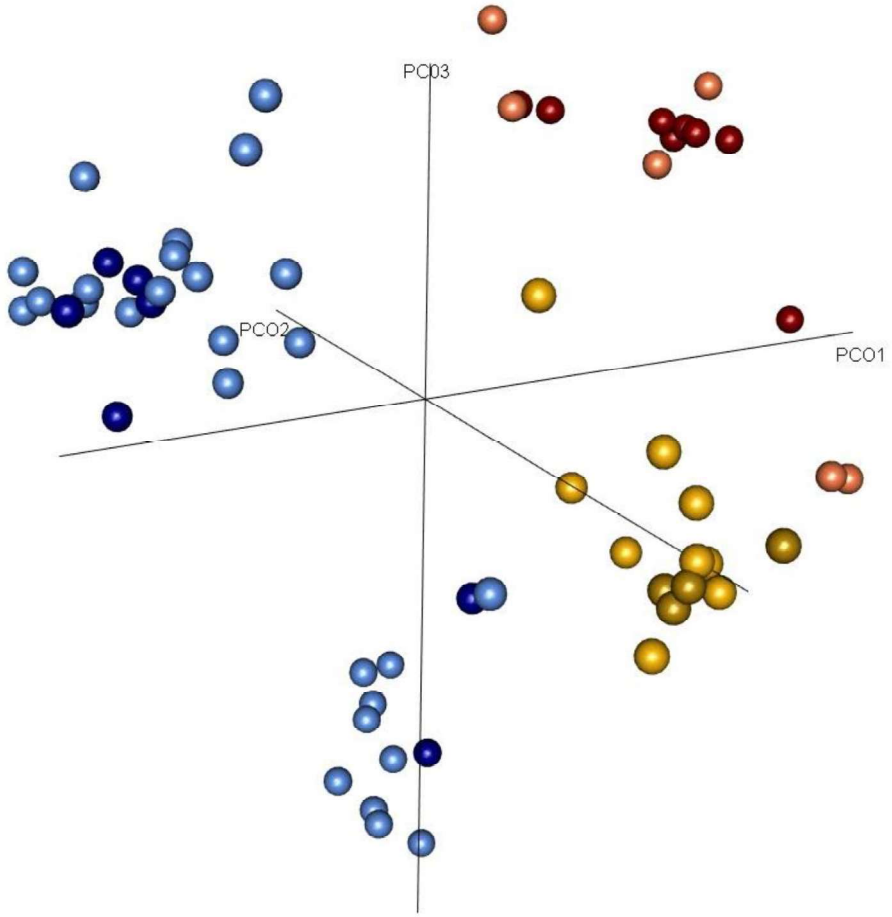


Figure S6. Merianieae morphospace PC1-3. The three pollination syndromes ('buzz-bee' – blue, 'mixed-vertebrate' – red, 'passerine' – yellow) are clearly differentiated; species with known pollinators are represented in darker colours while lighter colours represent species estimated into syndromes by RF analyses. Note the large disparity of buzz-bee pollinated species and the three distinct clusters found within the 'buzz-bee' syndrome.

Notes S1. 61 floral characters coded for Merianieae and used to evaluate pollination syndromes in the tribe

Descriptions of characters and decision criteria for character states are given. Characters relevant for understanding flower functioning and pollination biology in Merianieae were targeted while not focusing on characters only relevant for taxonomic treatments (justification of character choices are given in brackets). These floral characters could be used for the inclusion of further taxa within the tribe, but should mostly also be applicable to other Melastomataceae tribes.

1. **Reward type** (traditional pollination syndrome character)
   0) Pollen
   1) Nectar
   2) Food body
2. **Inflorescence architecture –** evaluated on photos, herbarium specimens and in the field, following description of inflorescences by Cotton et al. 2014 (possibly relevant for how pollinators can approach flowers; Harder & Prusinkiewicz, 2013)
   0) Compound or simple dichasium, subtended by a pair of leaf-like bracts, p. 14, Cotton et al. 2014, p.14, Figure 3C and D
   1) Elongate thyrse, elongated inflorescence with bracts absent or caduceus or occasional small leaf-like bracts, Cotton et al. 2014, p.14, Figure 3B
   2) Elongate whorls (whorls along an extended inflorescence stalk like e.g. *M. sanguinea*)
   3) Leafy snyflorescence, subtended by successively smaller pairs of leaf-like bracts, Cotton et al. 2014, p.14, Figure 3A
3. **Number of flowers –** evaluated on photos, herbarium specimens and in the field, following Cotton et al. 2014 (possibly relevant for floral display)
   0) Few (1-10 flowers per inflorescence)
   1) Moderate (11-25 flowers per inflorescence)
   2) Rich (>26 flowers per inflorescence)
4. **Position of inflorescence in relation to foliage –** evaluated on photos, herbarium specimens and in the field (possibly relevant for how pollinators can approach flowers)
   0) Not projected
   1) Projected (flowers clearly extended from foliage e.g. by an elongated inflorescence stalk or terminal positioning in vine (*Adelobotrys*), easily visible)
5. **Orientation of flowers in inflorescence -** evaluated on photos and herbarium specimens and considering the majority of flowers (traditional pollination syndrome character)
   0) Multiple
   1) Upright-horzontal
   2) Nodding
6. **Merisem –** evaluated on photos, herbarium specimens and in the field; if individuals with variable merosity were present, the most common condition was coded unless different types of merosity were equally abundant (an increase in merisem was mostly observed in bee pollinated species)

0) 4
1) 5
2) 6
3) 5 - 7

7. **Hypanthial stomata –** assessed on hypanthia prepared for SEM (the hypanthium has been proposed as site of nectar secretion (Varassin et al. 2008))
   0) Yes
   1) No
8. **Number of stomata** in $1/10^{th}$ of the hypanthium counted on samples prepared for SEM (numeric, 0-349); (the hypanthium has been proposed as site of nectar secretion (Varassin et al. 2008))
9. **Maximal corolla opening –** maximal opening of petal tips, measured on 3D-models of flowers in AMIRA (numeric (mm)); (traditional pollination syndrome character, flower size)
10. **Corolla height –** measured on longitudinal sections of 3D-models of flowers in AMIRA from the hypanthium rim to the highest point of the corolla (numeric (mm)); (traditional pollination syndrome character)
11. **Ratio between corolla diameter (9) and corolla height (10) –** numeric (traditional pollination syndrome character, indicative of flower shape or tube width)
12. **Corolla shape -** assessed at mid-anthesis (thus excluding opening buds (which at first will all resemble cupule/funnel shapes) and senescent flowers (which will have opened more in certain species)), evaluated on photos and pickled material (traditional pollination syndrome character, important for fit with pollinator and physical restriction of flower access in many other plant lineages)
    0) Bowl-shaped without overlapping margins (*Axinaea*s with corolla more widely open)
    1) Bowl shaped to flat (Meriania species)
    2) Campanulate (bell-shaped, pendant corollas)
    3) Campanulate-salverform (slightly campanulate with reflexed petal tips)
    4) Solanum type (*Graffenrieda*; similar to *Solanum*-type flower with central circle of stamens and reflexed petals)
    5) Urceolate *(Axinaea*s, bell-shaped flowers with an opening narrower than the maximum corolla diameter)
13. **Corolla shape change** over anthesis **-** estimated on photos, in the field and on pickled material (this could potentially change the accessibility to rewards (e.g. in a pseudo-campanulate flower, large bees could be limited in finding optima buzzing positions)
    0) Weak (hardly any change/some spreading of the corolla but only within a shape category)
    1) Strong (i.e. change from one shape category to another (e.g. from cupule to basin))
14. **Corolla colour change over anthesis -** evaluated on photos and in the field (could influence pollinator attraction, compare Brito et al. 2015)
    0) No
    1) Yes
15. **Corolla colour -** evaluated on photos and in the field, using X-rite Colour Checker as a reference (traditional pollination syndrome character)

0) White
1) cream pink
2) Red
3) Salmon
4) Fuchsia
5) Orange
6) Lilac

16. **Colour contrast between corolla and stamens –** based on photos (traditional pollination syndrome character, important for pollinator attraction)
    0) Yes
    1) No
17. **Petal gloss -** evaluated on flowers in the field and if high quality photos were available (traditional pollination syndrome character, pollinator attraction)
    0) Matt
    1) Gloss
18. **Petal surface -** SEM was used to assess the shape of epidermis cells on the ventral petal surface (with bee pollinated flowers usually having conical cells (mostly long papillate, enhancing grip and visibility), and bird pollinated flowers usually having flat surface cells (see Papiorek et al. 2014 for more details))
    0) Smooth
    1) Short papillate
    2) Long papillate
19. **Scent –** evaluated in the field (smelling with the human nose; traditional pollination syndrome character, pollinator attraction)
    0) Flowery
    1) Heavy-sweet
    2) No
    3) Weak (if not all test persons could perceive a smell, but 50% claimed to smell something)
20. **Number of stamens –** evaluated on photos and observations of pickled material (an increase in stamen number was mostly observed in bee pollinated species)
    0) 8
    1) 10
    2) 12
    3) 10-14
21. **Stamen shape dimorphism –** evaluated on photos and observations of pickled material (heteranthery is known to be an important trait in buzz-pollination (Vallejo-Marín et al., 2010)
    0) Isomorphic
    1) slightly dimorphic (small differences in shape or size, but no heteranthery)
    2) strongly dimorphic (heteranthery)
22. **Dimorphism in filament length –** evaluated on pickled material (heteranthery is known to be an important trait in buzz-pollination (Vallejo-Marín et al., 2010)
    0) Yes (if filaments bring the two stamen whorls to different heights)
    1) No

23. **Dimorphism in appendage volume –** evaluated on pickled material (heteranthery is known to be an important trait in buzz-pollination (Vallejo-Marín et al., 2010)
    0) Yes
    1) No
24. **Dimorphism in anther length –** evaluated on pickled material (heteranthery is known to be an important trait in buzz-pollination (Vallejo-Marín et al., 2010)
    0) Yes
    1) No
25. **Stamen colour dimorphism –** evaluated on photos and in field (heteranthery is known to be an important trait in buzz-pollination (Vallejo-Marín et al., 2010)
    0) Yes
    1) No
26. **Stamen arrangement relative to corolla -** the corolla is divided into 5 sections (following the petals in pentamerous species, extrapolating this pattern in hexa- and heptamerous species) and stamen arrangement is classed into these 5 sections by evaluating how many fifth are covered by the appendage tips, evaluated on pickled material and photos (possibly relevant for where the pollinator positions itself on the flower)
    0) 2/5
    1) 3/5
    2) 4/5
    3) 5/5
    4) 3/4
27. **Level of anther pore -** height of the anther pores relative to the style length (measured from style base), evaluated on pickled material (determines site of pollen release in relation to other floral organs)
    0) Top (anther pores close to stigma)
    1) Middle (anther pores located higher than 1/3 of style length but lower than 90% of style length)
    2) Bottom (anther pores located close to style base)
    3) Top/middle (in strongly dimorphic species)
28. **Change of androecial arrangement over anthesis –** evaluated on pickled material, photos and in field (possible change of site of pollen release)
    0) No – androecium remains more or less constant in position during anthesis
    1) Weak – irregular spreading during anthesis
    2) Strong – strong reflexive movement of stamens and migration of pores towards stigma during anthesis
29. **Secondary dorsal stamen appendage shape –** evaluated on pickled material (stamen appendages are sites of interaction with the pollinator (to obtain the reward) at least in bee and passerine pollinated species (Renner 1989, Dellinger et al. 2014))
    0) Bifurcate (bifurcated, often elongated)
    1) Knob (protrusion bending upwards (away from connective strand, not towards pore (compare "nose")), sitting on connective strand; found in *M. tomentosa* group)

2) Nose (rounded structure bending towards pore, sitting on connective strand; found e.g. in *M. haemantha*)
3) Absent (no secondary appendage present)

30. **Shape of primary stamen appendage –** evaluated on pickled material (stamen appendages are sites of interaction with the pollinator (to obtain the reward) at least in bee and passerine pollinated species (Renner 1989, Dellinger et al. 2014))
    0) Acuminate (*Graffenrieda*; small spine, separate from thecae)
    1) Bulbous-acuminate (*M. macrophylla*)
    2) Bulbous (in *Axinaea*, similar width:length, ratio 0.5 to > 1)
    3) Crown (severals *Merianias*, similar to pyramidal but ending in a rugged tip (instead of an acuminate one))
    4) Fusiform (elongated, width:length < 0.25; more direct transition into thecae)
    5) Pyramidal (triangular acuminate pyramid, width:length > 0.33, including species with more distant thecae (e.g. *M. sanguinea* but also *M. haemantha ssp haemantha*)
31. **Known mode of pollen expulsion –** evaluated in the field by pollinator observations and experimental manipulation using tweezers (to mimick birds' bills, compare Dellinger et al. 2014) and tuning forks (to mimick buzzing bees)
    0) Buzzing
    1) Bellows-mechanism
    2) Salt-shaker like pollen release
32. **Location of thecae on connective –** evaluated on pickled material (location is related to the mechanism of pollen release, pollen is released more easily on laterally attached thecae)
    0) Ventral (thecae restricted to dorsal side of connective strand)
    1) Lateral (thecae attached at sides of connective strand, pollen chambers supinated)
33. **Location of thecal end (end of pollen chambers) in relation to appendage –** evaluated on pickled material (possibly related to pollen release)
    0) Base (thecae end at appendage base, actual end of pollen chamber often only visible in cross-sections)
    1) Offset (thecae end a few mm/cm away from appendage base, only connective strand reaches appendage base)
34. **Anther shape –** evaluated on pickled material (possibly related to pollen release/pollen dosing)
    0) Acuminate (continuous narrowing towards the pore, width at pore considerably less than on top)
    1) Oblong (oblong anther which only narrows just before the pore but remains more or less the same thickness)
    2) Acuminate/oblong (dimorphic stamens)
35. **Recurving of anther -** curvature from adaxial to abaxial side (to differentiate more or less straight, cannon-like anthers from curved anthers (mostly at the apex); careful, this should not be confused with anthers elevated due to reflexion of the filament), evaluated on pickled material (possibly related to pollen release/pollen dosing)
    0) Yes

1) No

36. **Spatulate broadening of thecae around anther pore –** evaluated using SEM (possibly related to pollen release/pollen dosing)
    0) Yes
    1) No
37. **Structure of adaxial thecal wall –** evaluated on pickled material and SEM (possibly related to pollen release/pollen dosing)
    0) Ruminate (sturdy and strongly folded, made up by more than one tightly arranged cell layer (possibly a remaining)
    1) Smooth (sturdy but NOT folded, made up by one tightly arranged cell layer and strong cuticle and remnants of tapetum)
    2) Crumpled (soft and flexible, made up by one more loosely arranged cell layer)
38. **Thecae separated into two pollen sacs by septum–** evaluated on cross sections of stamens using microtome sectioning/light microscopy and cross-sections of stamens of HRXCT-scans of flowers in AMIRA (possibly related to pollen release/pollen dosing)
    0) Yes
    1) No
    2) Reduced wall between pollen sacs (in some *Graffenrieda* species)
39. **Number of stamen pores–** evaluated on SEM (possibly related to pollen release/pollen dosing)
    0) 1
    1) 2
    2) 1 or 2 (rare, found in some strongly heterantherous species)
40. **Location of pore on anther–** evaluated on SEM (possibly related to pollen release/pollen dosing)
    0) Apical (the pore is strictly apical with no inclination)
    1) Dorsal (the pore is on the dorsal side with a lip hindering pollen from flying into the apical direction)
    2) Dorsal/Apical (in some strongly heterantherous species, stamen whorls differ in the inclination of the pore)
    3) Dorsal tip (the pore is dorsally inclined but mostly opens to the front, the lip (compare with dorsal) is lacking)
    4) Ventral (the pore is ventrally inclined)
41. **Pore width –** 10 stamens/species measured on 3D models of flowers in AMIRA, mean taken (numeric (mm)); (possibly related to pollen release/pollen dosing)
42. **Pore height –** 10 stamens/species measured on 3D models of flowers in AMIRA, mean taken (numeric (mm)); (possibly related to pollen release/pollen dosing)
43. **Pollen grain diameter –** 10 pollen grains/species measured in 70% ethanol using a fluorescence microscope, mean taken (numeric (mm)); (possibly related to pollen release/pollen dosing)
44. **Structure of stamen filaments –** filaments have been found to constitute the location of nectar secretion, evaluated using light microscopy and SEM; (filament ruptures have been detected as sites of nectar secretion (Dellinger et al., unpublished data))
    0) Dorsal ruptures (necrotic horizontal slits on the dorsal side)

1) Small intercellular holes on proximal lateral side of filament and/or rupture on filament/connective joint
2) Smooth
3) Punctures (rounded necrotic surface damages; down to vascular bundle in some species)

45. **Structure of stamen appendage surfaces–** evaluated on SEM (appendage surface structures may influence the grip for pollinators applying vibrations)
    0) Smooth (no protrusions or groves)
    1) Smooth-pitted (generally smooth, but some depressions)
    2) Cauliflower (both horizontal and vertical grooves, like cauliflower)
    3) Mixed-bumpy (in M. *tomentosa*-group, appendages that have features of suclate/cauliflower but also smooth parts and a generally bumpy surface)
    4) Sulcate (mainly vertical grooves but overall even surface (without cauliflower protrusions)
    5) Papillate (papillae on appendage)
46. **Inflation at thecal base –** evaluated on SEM (possibly related to pollen release/pollen dosing)
    0) Yes
    1) No
47. **Stomata on stamen appendage–** evaluated on SEM (these could potentially be related to nectar or scent emission, Varassin et al., 2008, Dellinger et al., unpublished data)
    0) No
    1) Occasional (sometimes up to five)
    2) Regular (more than five in all stamens)
48. **Ratio vascular bundle:filament width –** numeric (measured on sections of CT-scans, 5 stamens per specimen, at the base of the filament; coronal plane); (thick vascular bundles have been detected in nectar releasing Melastomataceae by Varassin et al., 2008)
49. **Colour stamen appendage** (traditional pollination syndrome character, visual attraction)
    0) Colour appendage
    1) Cream
    2) Yellow
    3) Blue
    4) Fuchsia
    5) Dark violet
50. **Colour thecae** (traditional pollination syndrome character, visual attraction)
    0) Cream
    1) Yellow
    2) White
    3) Red
    4) fuchsia
    5) Dark violet

51. **Colour contrast thecae and stamen appendage –** evaluated on photos and in field (traditional pollination syndrome character, visual attraction)
    0) Yes
    1) No
52. **Relative position of style and corolla –** evaluated on pickled material, viewed from the front/side (traditional pollination syndrome character, related to fit between flower and pollinator)
    0) Free (style usually visible in its full length)
    1) Partly enclosed (upper quarter of the style usually visible)
    2) Enclosed (style mostly enclosed by petals, not (or only tip of stigma) visible)
53. **Style curvature –** evaluated on pickled material (possibly governs pollen pick-up from pollinator; e.g. a hooked style would only pick up pollen if the pollinator positioned itself directly underneath)
    0) Curved (variable curvature, slightly curved to almost straight in 90% of flowers)
    1) Hooked (strong hook at tip in > 90% of flowers)
54. **Stigma diameter –** measured on 3D scans of flowers, mean taken (numeric (mm)); (possibly related to pollen pick-up, Cruden 2000)
55. **Stigma shape -** interpreted when placing the style upright and looking at the stigma from the side in SEM (possibly related to pollen pick-up)
    0) Corymbose (umbrella-shape, overarching the width of the style but usually shorter than wide, sometimes almost rounded like a ball)
    1) Convex (bump, shorter than wide, but not overarching style width)
    2) Conical (elongated, as long or longer than wide, not overarching style width)
    3) Stamp (almost flat, about as wide as the style, neither narrowing nor widening)
56. **Stigma surface -** evaluated on SEM (possibly related to pollen pick-up)
    0) Densely papillate (papillae heads attach closely to each other)
    1) Scarcely papillate (space between papillae)
57. **Colour of style –** evaluated on photos and in the field (visual attraction)
    0) White
    1) Light pink
    2) Fuchsia
    3) Red
    4) Lilac
    5) Salmon
58. **Colour of stigma –** evaluated on photos and in the field (visual attraction)
    0) White
    1) Light pink
    2) Fuchsia
    3) Red
    4) Lilac
    5) Dark purple
59. **Colour contrast style – corolla –** evaluated on photos and in the field (visual attraction)
    0) No

1) Yes
2) Weak

60. **Colour contrast androecium – gynoecium –** evaluated on photos and in the field (visual attraction)
    0) No
    1) Yes
    2) Weak
61. **Colour contrast between stigma and style –** evaluated on photos and in the field (visual attraction)
    0) No
    1) Yes

Notes S2. Detailed description of Merianieae pollination syndromes

Bee syndrome flowers in Merianieae are characterized by a pollen reward, which is released by high-frequency buzzes applied by bees to the stamens. Flowers are often upright or horizontally oriented with wide bowl-shaped to deflexed corollas, with a mean diameter:height ratio of 8.7. Corolla shape changes markedly in the first hours/day of anthesis when corollas gradually reflex. Petal epidermis cells were found to be conical in shape. Flower colours range widely from white to different shades of pink and lilac, with stamens usually forming a strong colour contrast. Stamens may be arranged either on one side of the flower, giving the flowers a distinct monosymmetric architecture (*Meriania, Adelobotrys, Macrocentrum*), or the stamens are distributed more or less regularly in the flower, leading to almost polysymmetric flowers (*Graffenrieda*). Anthers can be erect (*Graffenrieda*), bringing pores close to the stigma, or remain geniculate (the condition found in bud-stage in all species) with pores remaining close to the base of the style in the floral centre. Stamen appendages are usually very conspicuous and variable in shape, pyramidal to weakly acuminate, sometimes bearing secondary appendages, and often have strongly ornamented surfaces. Weak to strong heteranthery is found in all *Adelobotrys* and some *Meriania* species. Thecae are located on the ventral side of the connective and usually have strongly corrugated and rigid walls consisting of two cell layers and an endothecium. A septum separating the thecae into two pollen sacs is present. Pores may be located on the dorsal (*Meriania*, partly *Adelobotrys*) or ventral (*Graffenrieda*, *Macrocentrum*) side of the anther. Styles are usually exserted from the rest of the flower and often strongly curved right beneath the stigma. In many species, stigmas are small and punctiform. Flowery, pleasant scents have been noticed in some species in *Meriania* and *Adelobotrys* (ASD pers. obs.). Anthesis usually starts in the early morning and may last from a single to multiple days (ASD pers. obs.). Bees have been observed in four large flowered *Meriania* species orientating their bodies in parallel to individual stamens, with their head at the appendage and their abdomen pointing towards the pores. They bite into the appendage and vibrate individual stamens at a time. In smaller flowered *A. adscendens*, bees were seen to crouch above the entire androecium (instead of single stamens), head pointing towards the flower centre, and applying vibrations to the entire androecium. Thus, the bee-syndrome encompasses various types of interactions between flowers and buzzing bees.

Flowers belonging to the 'MV' syndrome provide nectar rewards secreted from the stamens and aggregating on the petals (Dellinger et al., unpublished). Flowers are usually pendant and

pseudo-campanulate, with a diameter:height ratio of 1.0. Petal epidermis cells are usually flat, petals glossy and colours range from white, pinkish, salmon to scarlet red. All species have androecia arranged on one side of the flower and stamens undergoing a strong deflexion movement in the early phase of anthesis, bringing pores close to stigmas (anthers erect). Stamen appendages are smaller than in bee-pollinated *Meriania* species, crown shaped and relatively inconspicuous in colouration in some species (e.g., hummingbird/bat pollinated *M. tomentosa*), but larger and more vividly coloured in others (e.g., hummingbird/rodent pollinated *M. sanguinea*). Heteranthery is absent in most of these species, it is present, however, in the Antillean *M. angustifolia* and *M. albiflora*, both of which showed considerable inconsistency in pollination syndrome assignment (alternative: bee; see below). In many species, thecae are attached laterally to the connective. They have a soft, easily deformable (e.g. by a hummingbird's bill) wall made up of the epidermis only. The septum separating the thecae has collapsed. Apical anther pores are usually directed towards the stigma. Styles are often straight, not exceeding the corolla length, and often bear enlarged, slightly flattened stigmas. Floral scent can range from scentless (for the human nose, e.g. *M. furvanthera*) to emitting a flowery perfume-like scent (e.g. *M. tomentosa*) or strong, glue/plastic-like scents in *M. sanguinea* (for details see Dellinger et al., unpublished). Flowers become anthetic in mornings and/or evenings and usually remain open for approximately three days. Mixed diurnal and nocturnal pollinator assemblages have been observed drinking nectar in five species. When the animals insert their bills or tongues/heads into the pseudo-campanulate corollas, they push through the densely arranged anthers to lick nectar aggregated beneath the stamens. They thereby touch the soft, laterally attached thecae and cause pollen release. As all stamens are arranged with the pores pointing downwards, out of the pendant flower, this mechanism is termed 'salt-shaker' like pollen release.

The passerine pollination syndrome is characterized by staminal food body rewards, which at the same time function as pollen expulsion mechanism ('bellows'-mechanism). Passerine syndrome flowers are usually oriented in various directions (upright, horizontal, pendant) with mostly urceolate corollas with a diameter:height ratio of 1.5, which does not change much during anthesis in most species (compare with 'bee' syndrome). Petal epidermis cells were flat to slightly conical and petals were matte matt, colours range from light pink to red, and yellow corollas are also known. In all species with passerine pollination, the brightly coloured stamen appendages form a strong colour contrast with the corolla. Stamens are arranged on one side of the flower (monosymmetric) and in contrast to the 'MV' syndrome, they do not

deflex during anthesis so that the pores remain more or less around the mid length of the style. All species are united by characteristic bulbous stamen appendages with smooth surfaces. Most species show moderate heteranthery mostly in appendage volume and colour. Only *Meriania macrophylla* has strongly dimorphic stamens, a trait otherwise only found in the 'bee' syndrome (see estimation results below). Thecae are located on the ventral side of the connective and have a smooth, sturdy wall, composed of the epidermal cell layer and an endothecium. As in the 'MV'-syndrome, the septum has collapsed. Pores are located on the dorsal side of the anther. Styles are usually partially exserted from the urceolate corollas, with relatively small, conical stigmas. No scents have been noticed with the human nose (ASD, pers. obs.). Anthesis starts in the early morning and lasts for several days up to a week (ASD, pers. obs.). Passerines (tanagers, flowerpiercers) have been observed feeding on the bulbous stamen appendages in three species. The appendages contain high amounts of sugars (food body reward) and also function as a pollen expulsion mechanism: when passerines bite the appendages for consumption, the compression forces contained air into and through the thecae, dusting the birds with pollen grains that are ejected out of the apical pores.

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4. CHAPTER II: BIMODAL POLLINATION SYSTEMS IN ANDEAN MELASTOMATACEAE INVOLVING BIRDS, BATS AND RODENTS

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Bimodal pollination systems in Andean Melastomataceae involving birds, bats and rodents

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Online Appendix A: Table A1, A2, A3, A4, A5, A6, A7, A8, A9, A10, A11, A12, A13, A14 Figure A1, A2, A3, A4, Video A1, A2, A3.

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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 conundrum rarely investigated. We tested if floral scent and nectar traits of species visited by two functional pollinator groups indicate specialization on either one of the two or (intermediate) 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; flowerpiercers/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 and lack adaptation to a single functional pollinator group. The discovery of rodent pollination is particularly important given its rarity outside of South Africa.

Introduction

Specialization in plant-pollinator interactions 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 which assumes convergent floral evolution in adaptation to a specific (most effective) functional pollinator group (Faegri & 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 relatively ineffective pollinators in addition to the most effective pollinator can evolve if this results in an overall fitness gain (Aigner 2001, 2006) and pollinator-mediated adaptive trade-offs are minimal (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 & Goldblatt 2005). While pollination of one species by two distinct functional pollinator groups has given grounds to doubt the concepts of pollination syndromes and specialization (e.g. *Delphinium*, Waser 1996), bimodal systems have also been interpreted as special cases of "specialized" systems (e.g. *Tritoniopsis*, Manning & Goldblatt 2005).

Mixed pollination systems (with more than one functional pollinator group) often include a plant lineage's ancestral pollinator, which functions as an additional secondary pollinator (Rosas-Guerrero et al. 2014). In the New World tropics, a small number of mixed hummingbird-bat systems has been described (e.g. Muchhala et al. 2008 and references therein, Amorim et al. 2013, Queiroz et al. 2016). Given obvious differences in

their morphology, activity patterns and sensory abilities, general hypotheses on floral adaptation to a single most effective functional pollinator group versus adaptation to two pollinator groups (possibly with adaptations to both groups) can be tested.

Crucial features of (bimodal) hummingbird-bat-systems 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. 2008). Studying nectar sugar composition can be particularly informative as comparative studies have found strong associations between relative sucrose content, pollinator group, and pollinator specificity (e.g. Baker & Baker 1983, Dupont et al. 2004, Johnson & Nicolson 2008). Flowers pollinated by large bees, specialized nectar-feeding birds, and hawkmoths tend to present nectar rich in sucrose, while nectar of flowers pollinated by short tongued bees, flies, and generalist birds is mostly dominated by hexoses; bat-pollinated flowers in the New World have been found to be intermediate in sucrose and hexose levels (Baker et al. 1998, Johnson & Nicolson 2008, Abrahamczyk et al. 2017).

While visual attractiveness is generally associated with diurnal pollination (e.g., red corollas in bird systems (Faegri & van der Pijl, 1979)), floral scent is regarded as important attractant particularly for nocturnally active pollinators and less important in diurnal bird pollination systems (Raguso et al. 2003, Dobson 2006; as well as deterrent, e.g., of herbivores). Similar to nectar sugar compositions, specific scent bouquets have been related to different functional pollinator groups in some plant lineages (Knudsen & Tollsten 1995, Dobson 2006, Knudsen et al. 2006).

The plant family Melastomataceae (ca. 5000 sp.) is functionally specialized on bee-buzz pollination and characterized by nectar-less flowers, anthers opening by small apical pores, and pollen as sole reward (Buchmann 1983). However, nectar secretion and concomitant pollinator shifts from pollen-collecting bees to non-buzzing insects or

vertebrates have been documented in ca. 100 Neotropical Melastomataceae species scattered across four tribes (e.g. Lumer 1980, Wester et al. 2016, Kriebel & Zumbado 2014, Brito et al. 2017, Vogel 1997, Muchhala & Jarrín-V. 2002, Lagerheim 1899). Although ambiguity remains as to where and how nectar is secreted (Stein & Tobe 1989, Varassin et al. 2008), the shift from pollen to nectar rewarding clearly opened up the specialized buzz-bee pollination syndrome to multiple functional pollinator groups (Brito et al., 2017, Dellinger et al. 2018). Despite this finding, a recent study on the Melastomataceae tribe Merianieae found support for classifying nectar secreting species all visited by different combinations of two functional pollinator groups (e.g. hummingbirds/bats, hummingbirds/rodents) into a single 'mixed-vertebrate' syndrome (Dellinger et al., 2018). This syndrome is characterized by the visitors' shared interest in the nectar reward and their ability to cause pollen ejection via a 'salt-shaker' mechanism, activated when they insert their mouthparts into the pendant, pseudo-campanulate corollas to take up nectar and thereby push against the thecae (Fig. 1A-D, I, J). It remains unclear, however, if this 'mixed-vertebrate' syndrome points toward truly bimodal systems with two equally effective pollinator groups or rather systems with a single most effective primary and an additional secondary pollinator group. 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 buzz-bee pollination to alternative pollinators (shift 1: *M. furvanthera, M. tomentosa*; shift 2: *M*. aff. *sanguinea*, *M. sanguinea*; Dellinger et al., 2018). 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 Norther Peru (Wurdack 1967). The northern population has generally been treated as *M. sanguinea*, but given clear morphological and molecular differences (Dellinger et al., 2018), we treat it as separate taxon in this study.

Meriania species are shrubs or treelets, mostly growing in small, isolated populations in montane rainforests (1.500 m – 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 Oct/Nov 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, the total sampling area spanning a minimum air-line distance of 500 m at each site which should 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; online appendix Table A1 for details).

Pollinator 'quantity' and 'quality'

To assess visitation rates ('quantity'), flowers of multiple individuals (2-10) per species were monitored using video cameras (SONY Camcorder HDF-CX 190, Table A3 details

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 night-time (18:00-00:00). In each video, a minimum of three 30 minute intervals (beginning/middle/end of video) was replayed using the PlayMemoriesHome Sony software, yielding a total of 108 reviewed hours (Table A3). Floral visitors were scored as pollinators if their morphology fit with the flower and their behaviour 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; see Muchhala et al. 2008 for similar approach). Pollinators were identified with the help of literature (Ridgely & Greenfield 2001, Tirira 2017).

In order to understand the contribution to pollination of diurnal vs. nocturnal visitors ('quality'), we manipulated the timing of flower exposure to visitors over a seven-day period in *Meriania* aff. *sanguinea*, *M. sanguinea*, and *M. tomentosa* (Table A1, A4; too few individuals with accessible flowers in *M. furvanthera*). In order to obtain virgin flowers for later exposure to either diurnal or nocturnal visitors, inflorescences were bagged using bridal veil (mesh density < 1 mm) either during day- (ca. 5:45 until 18:00) or night-time (ca. 18:00 until 5:45; Table A4 for details on sample sizes; total flower n = 80) and exposed to visitors at the other time interval, respectively. From day one to four, consecutively opening flowers within each inflorescence were added to the exclusion trials; flowers opening on days 5-7 were not considered. After three days or nights of pollinator exposure (which also marks the end of the flower's lifespan), styles were collected in 70% ethanol. We can rule out pollen deposition on stigmas by bagging/un-bagging as pollen is retained within the poricidal anthers and major amounts that would significantly alter the outcome of the exclusion experiment are only released when

pressure is applied to the thecae directly (but also see Table A5). Un-manipulated control flowers from inflorescences not used in exclusion experiments or neighbouring individuals (when not enough inflorescences were present on individuals used for exclusion trials, Table A5) 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 & Inouye 1993). The entire squashed stigmatic area was measured at 10x magnification and pollen grains were counted at 20x (entire field of view) in three areas from the edge to the centre 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 µm². For each species, a GLMM (Generalized Linear Mixed Effects Model) was used to test for differences between diurnal and nocturnal stigma pollen loads and between controls and exclusion trials, including plant individual as random effect (*lmerTest* package in R, Kutznetsova et al. 2017).

Localization of nectaries

In order 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 from the two shifts (online appendix 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 (Scanning Electron Microscopy) or light microscopy to localize areas of nectar secretion. For SEM, hypanthia and stamens were

dehydrated over an ethanol series, transferred to acetone, critical point dried (CP Autosamdri-815), mounted on stubs, coated with gold using a Sputter Coater (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) and embedded in 2-hydroxyethyl methacrylate (Technovit 7100, hardener II, Heraeus Kulzer, Wehrheim, Germany) and sectioned at 5 µm with a Microm HM rotary microtome 355 (Walldorf, Germany). Sections were stained with 0.2% – Ruthenium red – 0.5% – Toluidine (RT-stain). Images of selected sections were taken with a Nikon digital sight DS-Fi1 camera (Nikon Corporation, Tokyo, Japan) on an Olympus BX50 system microscope (Olympus Optical Corporation, Tokyo, Japan).

Nectar collection and analyses

To assess differences in nectar properties between day and night, flowers of any age were bagged in the early morning (5:30-7:00) or early evening (17:30-18:45) after removing all nectar, if present (Table A7). Flower age 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, presence of nectar, volume and concentration were recorded. Nectar was extracted with 10 µl micro-capillaries and concentration measured by an Eclipse Refractometer 45-81 (Bellingham & Stanley). Volume was estimated from the number of filled 10 µl capillaries per flower. A subset of flowers was re-bagged to assess nectar replenishment at 12 hour intervals (Table A7). For *M. furvanthera*, nectar volume could not be measured due to small sample sizes, concentration was measured from un-bagged flowers. Summary statistics were calculated for all species from all measurements and 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 fixed factor and flower ID as random effect (*M.* aff. *sanguinea* excluded due to small n). A GLMM was run on all measurements on nectar concentration (n = 105) to assess significant differences between species and D/N, treating plant individual as random effect.

10 µl of nectar collected at day/night sampling times was stored in 70% ethanol for sugar analyses using HPLC (a total of 87 samples, Table A7). Nectar sugar samples were dried in a vacuum concentrator centrifuge to remove ethanol and re-dissolved 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 /Thermo) using anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). Sugars were separated on a CarboPac PA1 column (2x 250 mm separation column, 2x 50 mm guard column) 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 & Baker 1982). Bray-Curtis dissimilarity matrices were calculated in R-package *vegan* (Oksanen et al. 2018) and 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 used to test for significant differences in disparity between species.

Volatile collection and analyses

Floral volatiles were collected *in situ* during day (6:00-8:00) and night (18:00-21:00) time using dynamic headspace methods (Dötterl et al., 2005; total n = 113, Table 3). Individual anthetic flowers (age and pollination status not considered) were enclosed in polyester

oven bags (10 × 15 cm; Toppits R©, Germany) and volatiles were collected for 10 min-30 min (depending on strength of perceived scent) through small adsorbent tubes (Varian Inc. ChromatoProbe quartz micro vials; length: 15mm, inner diameter: 2mm) using a membrane pump (G12/01 EB, Rietschle Thomas Inc., Puchheim, Germany; flow rate: 200 ml/min). The tubes contained 1.5mg Tenax-TA (mesh 60–80) and 1.5mg Carbotrap B (mesh 20–40; both Supelco) fixed by glass wool plugs (Heiduk et al., 2015; Mitchell et al., 2015). Three scent samples of leaves at approximately 5 m distance from flowers were collected for each species as negative controls using the same method. Trapped volatiles were analyzed by GC-MS using an automatic thermal desorption (TD) system (TD-20, Shimadzu, 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 was initially 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 (EI mode) from m/z 30 to 350. GC/MS data were processed using the GCMSolution package, Version 4.11 (Shimadzu Corporation 1999-2013). 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 analyses. For quantitative analysis of VOCs, known amounts of monoterpenes, aliphatic, and aromatic compounds were injected into the GC/MS system and mean peak areas were used to determine the total amount of scent (see 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 NMDS (*vegan*) and stacked barplots.

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): *M*. aff. *sanguinea*, *M. tomentosa*; hummingbirds and rodents (nocturnal): *M. sanguinea* Video 1; flowerpiercers (diurnal) and rodents: *M. furvanthera* Video 2, 3). 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 'salt-shaker' mechanism. While hummingbirds and bats mostly hovered, flowerpiercers (passerine birds) and rodents perched. Rodents were observed running along branches and spent up to 10 seconds on a single flower to drink nectar. Wasps and lepidopterans were seen as occasional nectar robbers in all species. Only on a single sunny day, small bees were observed robbing pollen in *M. sanguinea*. The insects' contribution to pollination likely is negligible as they could either not activate the 'salt-shaker' mechanism (wasps, lepidopterans) or did not touch the stigmas due to their small body size (bees). From here onwards, the different pollinator assemblages are grouped as follows: HB (hummingbird/bat), HR (hummingbird/rodent) and FR (flowerpiercer/rodent).

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 open simultaneously (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-value 0.716, df = 27, p = 0.48) and *M. sanguinea* (HR: t-value -0.343, df = 14, p = 0.737) but nocturnal stigmatic pollen loads were higher in *M*. aff. *sanguinea* (HB: t-value 3.038, df = 11, p = 0.01). 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 in day samples of *M*. aff. *sanguinea* (HB, Table A6).

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: a) secretion by dorsal filament ruptures along the entire length of the filament (Figure 1 E, F, online appendix Figure A1 A, B); b) secretion by small ruptures at the ventral side of the joint between filament and anther connective (online appendix Figure A1 E, F, G, H), both in a and b the ruptures are formed during anthesis; and c) secretion by porous tissue on the proximal lateral sides of the filament (Figure 1G, online appendix Figure A1 C, D), already present in pre-anthetic flowers (Table A2 for results on additional species). Accordingly, nectar droplets were found oozing out of dorsal filament ruptures (visible as dark necrotic cavities) in *Meriania tomentosa* (HB, Figure 1E, F, type a) but sitting at the filament-connective joint/upper part of the filament in *M. furvanthera* (FR, Figure 1G, type b and c) and *M.* aff. *sanguinea* (HB) and *M. sanguinea* (HR, type b). Regardless of

the exact site of secretion, nectar pooled between the stamens and petals and is freely accessible to all functional pollinator groups (Figure 1 I, J).

Nectar secretion: timing and volume

Nectar secretion started within the first six hours of anthesis in *M. tomentosa*, while it only started after approximately 24 hours in *M. sanguinea* and *M.* aff. *sanguinea* (online appendix Figure A2). Nectar was secreted throughout anthesis from the first secretion onwards 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-value -1.82, df = 31, $p = 0.08$; *M. sanguinea* (HM) t-value -0.52, df = 28, $p = 0.61$).

Nectar concentration and sugar composition

Nectar sugar concentration ranged between 10.9° and 13.6° BRIX in *Meriania* aff. *sanguinea*, *M. tomentosa* (both HB) and *M. sanguinea* (HR) while it was significantly higher (up to 20° BRIX) in *M. furvanthera* (FR; Table 2, Table A8). Only *M. sanguinea* showed significant differences in nectar concentration between day and night (GLMM: *M. sanguinea* (HM) t-value 3.56, df = 17, $p < 0.01$).

Sugar composition differed significantly among species (F 114, df = 3, r^2 0.787, $p=0.001$, Table A9), 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, Figure 3). *M. furvanthera* differed significantly from all other species (Table A10). Nectar sugar composition did not differ between day and night in any species or the interaction of species and day/night (Table A9). Variability of nectar composition differed

significantly between species ($F = 6.53$, $df = 2$, $p < 0.01$, Figure 3, online appendix Figure A3) and was significantly higher in *M. tomentosa* (HB) than in *M. sanguinea* (HR; Table A11).

Scent composition

Flowers of *Meriania sanguinea* (HR) released a strong solvent-like odour and flowers of *M. tomentosa* (HB) produced weak flowery odours at all times. No odour detectable by the human nose was noted on flowers of *M*. aff. *sanguinea* (HB) and *M. furvanthera* (FR). The GC/MS analyses revealed flower-specific components in all species, however. Independent of species and day-time, scent was detected only in half or less of the samples analyzed. In *M. furvanthera* only diurnal samples contained scents, whereas in the other species scent was detected in both diurnal and nocturnal samples (Table 3). Median total amounts of scent per flower per hour were significantly higher in day samples of *M. tomentosa* ($W = 110$, $df = 20$, $p < 0.01$) while differences were not significant in other species (Table A12). Scent profiles were significantly different between species ($F = 10.8$, $df = 3$, $p < 0.001$, Table A13). *M. 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 A14). 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 like Sabinene and Delta-3-Carene and unknown compounds (Fig. 4, Fig. A4).

Discussion

Taken together, 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, e.g. often reddish flowers, day and night availability of nectar, easy reward access by widely open pseudo-campanulate corollas, staminal nectar release and nectar aggregation beneath the stamens, common pollen expulsion mechanism (Dellinger et al., 2018). On a finer scale, certain differences in adaptation to the distinct functional pollinator groups become apparent: nectar sugar composition follows typical diurnal bird pollinator preferences (Johnson & Nicolson 2008) and scent profiles partially show adaptations to the different nocturnal pollinators.

Our finding of effective rodent pollination in *M. sanguinea* and *M. 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 & Sérsic 1998; Proteaceae, Cárdenas et al. 2017). Both species with rodent pollination show modifications in their inflorescence architecture (short-pedicelled flowers in leaf axils in *M. furvanthera*, Figure 1B) or growth form (procumbent habit of *M. sanguinea*, Figure 1C), which facilitate access to flowers by perching pollinators. This is in contrast to flowers protruding on long inflorescence stalks in *M. tomentosa* and *M.* aff. *sanguinea* (Figure 1A), which are only visited by pollinators capable of hovering while drinking nectar (HB). Although rodent visitation rates were ten 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. 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 seemed more effective pollinators than hummingbirds. However, there are clearly more aspects to pollinator 'quality' than just pollen deposition (but see Muchhala et al. 2008 for a similar approach to ours). 'Quality' differences between pollinators also encompass differences in the efficiency of removing pollen that then gets deposited (and not lost), the 'purity' of deposited pollen (e.g. amount of heterospecific pollen, see Morales et al. 2008, Queiroz et al. 2015) as well as genetic compatibility/viability of deposited pollen (e.g. self-/outcross pollen and consequently fitness of offspring, Ne'eman et al. 2010). Manual pollination experiments in *M. sanguinea* and *M. tomentosa* showed self-compatibility (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.

Our study detects the stamens as nectar secreting organs which contradicts findings on hypanthial nectar secretion in Merianieae (Varassin et al. 2008, but also see Stein & 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. 1H-J) leads to high pressure in the tissue which causes tissue rupture and phloem sap leakage (Vogel 1997, de la Barrera & Nobel 2004). Generally, invertases can change sucrose rich phloem composition in the nectary (Nicholson 2001) 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 & Nicolson 2008), with 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 in *M. furvanthera*, Figure 3). The hexose-rich nectar of *M. furvanthera*, however, indicates the presence of nectary invertases despite the unspecialized nectar leakage (de la Barrera & Nobel 2004; also see Dellinger et al., 2014 for hexose-rich food bodies in closely related passerine pollinated *Axinaea*). 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 adaptation to bat and rodent attraction (Dobson 2006), nocturnal scents were not significantly stronger or even weaker in *M. tomentosa* (HB). 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 aliphatics were dominant in rodent pollinated *M. sanguinea* and *M. furvanthera* (Fig. A4, Knudsen et al. 1995, Pettersson et al. 2004, Dobson et al. 2006). *M. sanguinea* is particularly interesting in this context: 1-Hexen-3-one (mostly confined to nocturnal scent samples) is only known as flower scent 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 behavioural assay, but had no negative effects when tested in combination with the strong attractant 3-Hexanone, also released by *C. visseri* (Johnson et al. 2011). In *M. sanguinea*, however, 3-Hexanone was only detected during

daytime when rodents are not active. Thus, the role of 1-Hexen-3-one in attraction of pollinators 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 towards convergence in the evolution of this compound to communicate with ground dwelling mammals. Given the lack of detectible 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 are usually brightly colored but scentless (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 *Meriania* species, summarized into a 'mixed-vertebrate' pollination syndrome, indeed represent bimodal pollination systems with adaptations to different functional pollinator groups. While studies on nectar secreting Melastomataceae from other tribes (e.g. Miconieae) report an increased "generalization" (e.g. Kriebel & Zumbado 2014, Brito et al. 2017), our 'mixed-vertebrate' syndrome is better described as "specialized bimodal" (compare Manning & Goldblatt 2005). Such bimodal systems have been considered as labile, possibly representing evolutionary transitions between distinct pollination syndromes (Manning & Goldblatt 2005). Given the ancestral buzz-bee pollination syndrome in Merianieae, one could expect such transitions between (ancestral) bees 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 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)) and convergence into the 'mixed-vertebrate' pollination syndrome further supports the idea of a stable pollination strategy. The direction of transitions within the bimodal systems (e.g. from HB to FR or from FR to HB), however, remains unclear and awaits more detailed phylogenetic comparative analyses.

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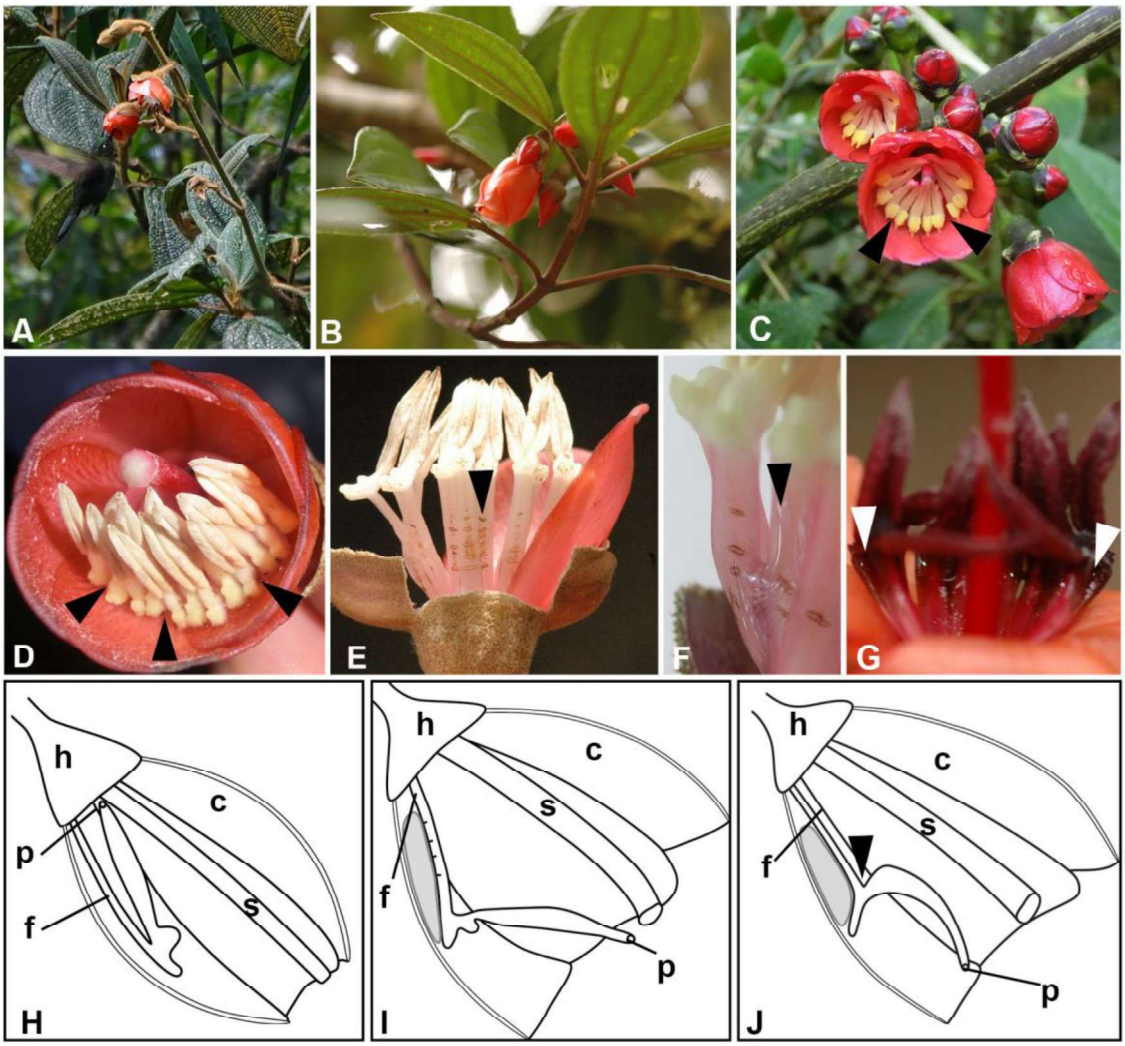

Figure 1. Inflorescences, flowers and nectar secretion in *Meriania* species of the 'mixed-vertebrate' syndrome. A) *M. tomentosa* with protruding inflorescence and Flame-throated Sunangel visiting a flower. B) *M. furvanthera* with flowers arranged in a simple dichasium allowing flowerpiercers and rodents to perch close to flowers. C) Multi-flowered inflorescence on a procumbent branch of *M. 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). H) Generalized schematic drawing of a *Meriania* flower at the beginning of anthesis; stamen is bent with anther tip pointing towards 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. h = hypanthium, c = corolla, s = style, f = filament, p = pore.

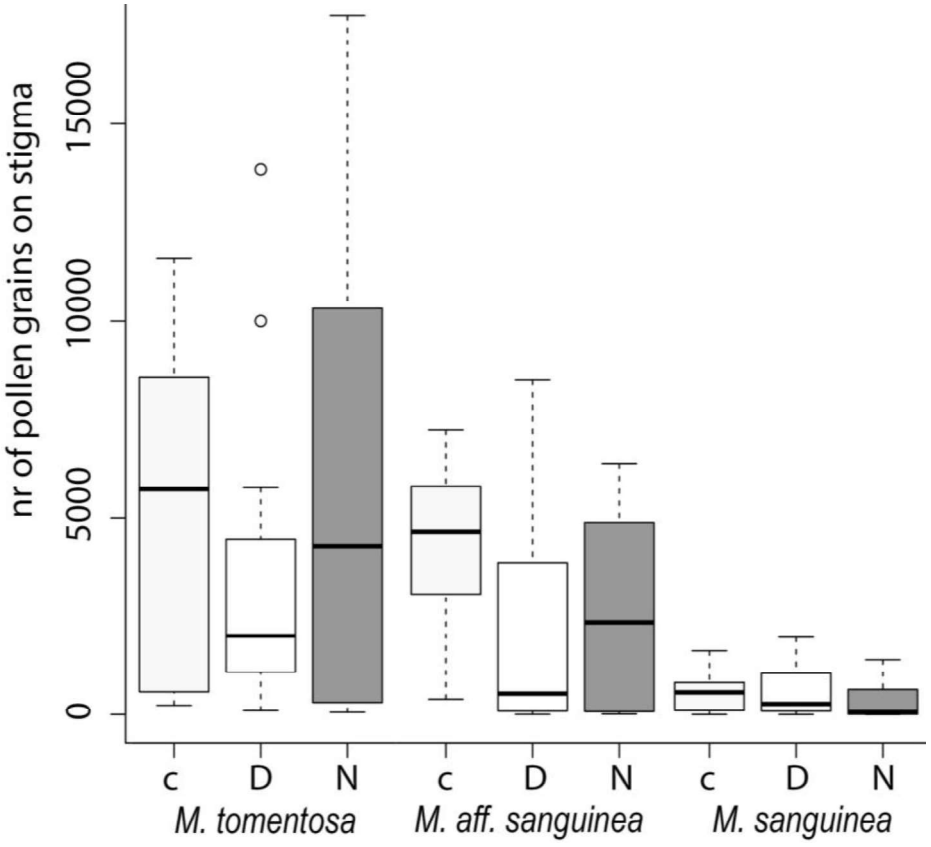

Figure 2. Boxplot showing pollen deposition loads on stigmas of pollinator exclusion experiments in *Meriania*: open flower access (light grey), day access only (white), and

night access only (dark grey); no significant differences in *M. tomentosa* (HB), and *M. sanguinea* (HR); control and night pollen loads significantly higher in *M*. aff. *sanguinea* (HB).

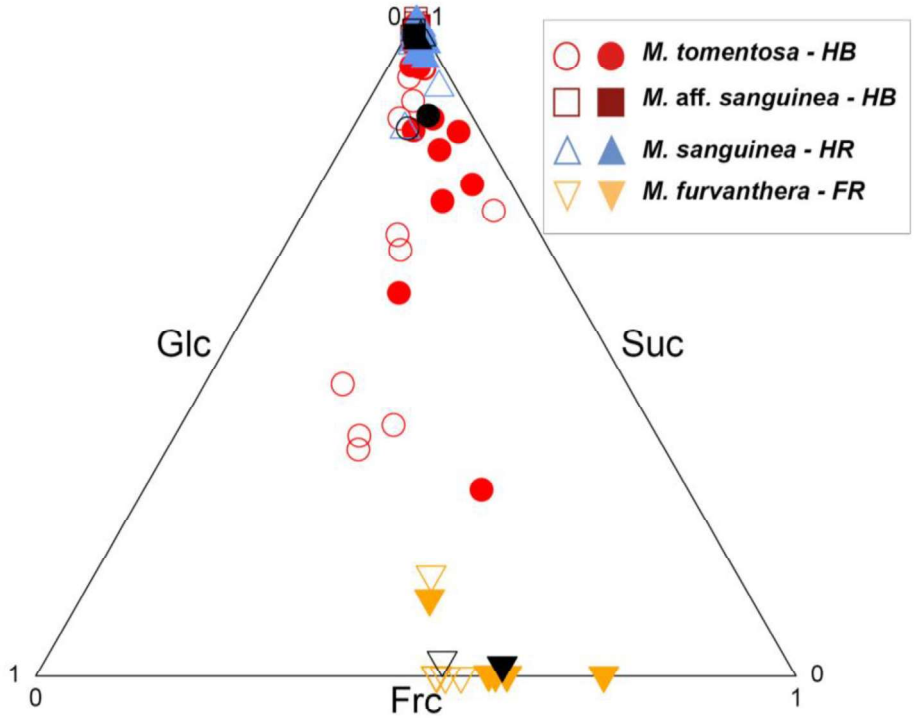

Figure 3. Triangle plot showing relative nectar sugar composition of day-nectar (unfilled symbols) and night-nectar (filled symbols) in the four *Meriania* species. Note the clear separation following bird pollinator preferences: sucrose prevalence in hummingbird-pollinated *M. sanguinea, M.* aff. *sanguinea* and *M. tomentosa* and hexose dominance in flowerpiercer pollinated *M. furvanthera*. Black symbols present species means (white fill – day, black fill – night); Suc – sucrose, Glc – glucose, Frc - fructose.

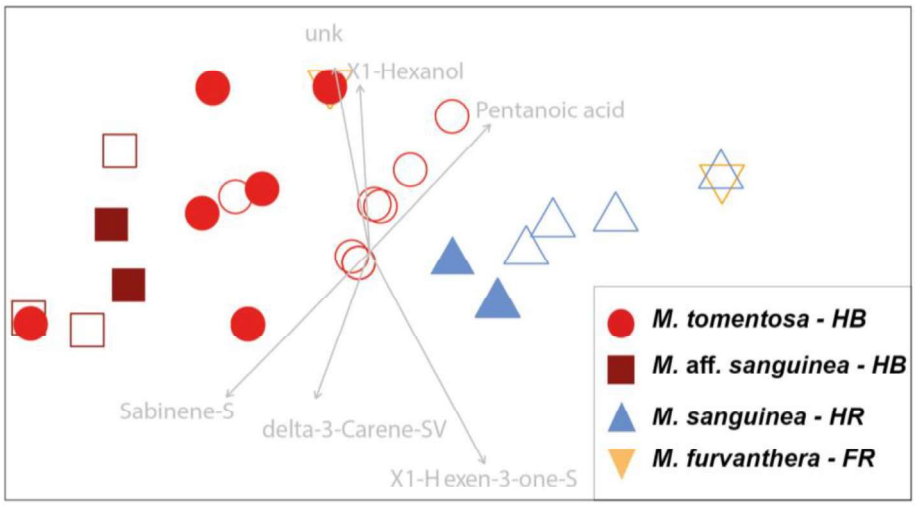

Figure 4. Non-metric multidimensional scaling (NMDS), based on a Bray-Curtis dissimilarity matrix to display semi-quantitative differences in day and night scent profiles of the four *Meriania* species. The stress value of 0.018 indicates a good representation of the observed similarities among scent samples. The six compounds correlating best with the coordinates are given.

Video 1. *Thomasomys* sp. visiting a flower of *M. sanguinea* and drinking nectar. Note deep head insertion of rodent into the flower and the long duration of rodent visit.

Video 2. Rodent visiting multiple flowers of *M. furvanthera* to forage on nectar.

Video 3. Passerine (Masked Flowerpiercer) visiting flowers of *M. furvanthera* for nectar uptake.

Table 1. Pollinator assemblages and visitation rates per flower per hour of the four *Meriania* species and total number of flower observation hours in brackets (for details see online appendix Table A3). Pollinator group: HB – hummingbird/bat, HR – hummingbird/rodent, FR – flowerpiercer/rodent.

species	group	diurnal pollinators	diurnal visitation rate/flower/hour	nocturnal pollinators	nocturnal visitation rate/flower/hour
M. aff. sanguinea	HB	*Eriocnemis derbyi* (Black-thighed Puffleg) *Metallura tyrianthina* (Tyrian Metaltail) *Schistes geoffroyi* (Wedge Billed hummingbird)	1.7 *(50)*	*Anoura* cf. *peruviana* (bat)	0.88 *(45.2)*
M. 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)	0.73 *(20.5)*
M. sanguinea Wurdack	HR	*Heliangelus micraster* (Flame Throated Sunangel)	0.25 *(52)*	*Thomasomys* sp. (rodent)	0.03 *(63.7)*
M. furvanthera Wurdack	FR	*Diglossa cyanea* (Masked Flowerpiercer)	0.11 *(94)*	rodent, unidentified	0.46 *(43.6)*

Table 2. Nectar volume, sugar content and mean relative sugar proportions in day and night samples of the four *Meriania* species. N – measured after night, D – measured after day. Details on sample sizes are given in online appendix Table A7.

species	mean nectar volume (µl)		mean °BRIX		rel. amount glucose (%)		rel. amount fructose (%)		rel. amount sucrose (%)		S/(F+G)		F/G	
	N	D	N	D	N	D	N	D	N	D	N	D	N	D
M. aff. *sanguinea* (HB)	59.6	-	11.8	13.1	0.8	1.0	0.5	1.0	98.7	98.0	71.9	53.9	0.7	1.5
M. 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
M. sanguinea (HR)	73.7	46.8	10.9	13.6	1.6	0.7	2.1	2.0	96.3	97.3	55.5	52.1	2.1	4.1
M. furvanthera (FR)	-	-	20	18.3	43.7	37.0	52.2	60.4	4.1	2.6	0.04	0.02	1.2	1.7

Table 3. Scent composition of the four *Meriania* species with total median amount of scent released per flower per hour (ng) and median (minimum-maximum) relative amount of the specific volatile compounds detected in diurnal (D) and nocturnal (N) samples. The compounds are arranged according to chemical class, and within class sorted according the Kovats retention index (RI, ZB5-column). The number of samples which contained compounds and total number of samples collected for D and N are given in brackets next to D/N.

		M. aff. sanguinea **HB**		***M. tomentosa*** **HB**		***M. sanguinea*** **HR**		***M. furvanthera*** **FR**
Components	**RI**	**D (6/3)**	**N (4/2)**	**D (25/12)**	**N (23/12)**	**D (23/10)**	**N (24/9)**	**D (8/4)**
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(0.1-15.0)	31.9(0.9-184.1)	31.3(2.0-87.4)	24.4(2.2-58.6)
Aliphatic components								
Methyl butanoate*	721					0.0(0.0-0.6)		
1-Hexen-3-one*	775					100.0(0.0-100.0)	100.0	
3-Hexanone*	785					0.0(0.0-78.4)		
1-Hexanol	866			0.0(0.0-30.9)	0.0(0.0-100.0)			0.0(0.0-63.7)
Pentanoic acid	869					0.0(0.0-100.0)		0.0(0.0-34.2)
3-Octanol	995			2.1(0.0-36.1)				
1-Octanol	1069							0.0(0.0-15.4)
Octyl acetate*	1210	0.0(0.0-42.3)	35.0(10.2-59.7)	0.0(0.0-36.1)				
N-bearing compounds								
2-Methylbutanenitrile	723			0.0(0.0-10.3)				
Terpenoids								
Sabinene*	979	57.7(0.0-90.2)	21.3(13.6-29)					
delta-2-Carene	1006							0.0(0.0-100.0)

delta-3-Carene	1017				54.6(0.0-100.0)
(*Z*)-4,8-Dimethyl-1,3,7-nonatriene	1099			0.0(0.0-100.0)	
(*E*)-4,8-Dimethyl-1,3,7-nonatriene	1119			81.6(0.0-100.0)	
p-1,3,8-Menthatriene	1134			0.0(0.0-100.0)	
β-Elemene	1408				0.0(0.0-90.7)
(*E*)-ß-Caryophyllene*	1437	0.0(0.0-100.0)	15.0(3.0-26.9)	0.0(0.0-69.1)	0.0(0.0-55.4)
(*E*)-β-Farnesene*	1463		1.2(0.0-2.5)		
α-Humulene*	1481		5.8(0.1-11.6)		
Unknowns					
m/z 40, 41, 55, 69, 107, 119	1084			0.0(0.0-1.4)	
m/z 55, 69, 81, 109, 135, 161	1487			0.0(0.0-100.0)	
m/z 40, 41, 69, 94, 120, 135	1493		8.7(4.4-12.9)		
m/z 40, 55, 69, 135, 187, 223	1505		1.0(0.4-1.6)		
m/z 40, 69,105, 121, 136, 177	1508		1.6(0.0-2.1)		
m/z 41, 55, 69, 102, 121, 136	1518		0.9(0.0-18.6)		

Appendix A from "Bimodal pollination systems in Andean Melastomataceae involving birds, bats and rodents"

Details on sampling localities

Table A1. Details on study sites, altitudinal range sampled for each species, number of individuals used for pollinator exclusion experiments, the minimum and maximum air-line distance between sampled individuals and experimental period.

species	locality	altitudinal range sampled	no individuals	air-line distance between individuals (min – max)	experimental period
M. aff. *sanguinea*	Ecuador, Guanderas Reserve	3260 m – 3400 m	7	30 m – 505 m	25.10.2017 – 01.11.2017
M. furvanthera	Ecuador, Podocarpus National Park, Cajanuma	2700 m – 2760 m	-	200 m – 960 m	04.11.2017 - 10.11.2017
M. sanguinea	Ecuador, Podocarpus National Park, Cajanuma	2740 m – 3100 m	19	2 m – 1120 m	04.11.2017 - 10.11.2017
M. tomentosa	Ecuador, Bellavista Cloudforest Reserve	2160 m – 2250 m	7	25 m – 502 m	19.11.2017 – 25.11.2017

Table A2. Additional nectar secreting *Meriania* species included in the structural study on nectar secreting tissues of stamens, and phylogenetic position (shift 1 or shift 2 to 'mixed-vertebrate' syndrome; Dellinger et al., 2018), and type of nectar secretion. Herbarium vouchers of these species have been deposited at QCA, FLAS, and WU.

species	locality	shift	type
Meriania costata	*Ecuador, PN Podocarpus, Cajanuma, 2900m*	1	b, c
Meriania loxensis	*Ecuador, PN Podocarpus, Cerro Toledo, 2900m*	1	b
Meriania phlomoides	*Costa Rica, Monteverde Cloud Forest Reserve, 1800m*	1	a
Meriania pichinchensis	*Ecuador, El Pahuma Reserve, 1930m*	1	a
Meriania radula	*Ecuador, Reserva Tapichalaca, 3180m*	2	b
Meriania tetragona	*Ecuador, Tapichalaca Reserve, 2860*	2	b

Details on video observations ('pollinator quantity')

Table A3. Details on video observations for the four *Meriania* species. Below each species name, the total number of monitored individuals is given. Each line corresponds to a single video, sorted by day (D) or night (N), the number of flowers filmed in each video, the number of hours reviewed in each video (the number in brackets is the total amount of flower hours reviewed), the total number of flower visits within the reviewed time, the total number of visits to the plant plus information of how many of these visits were to more than one flower. At the end of each species section, sums are given. Note that in five videos, less than three 30 minute intervals were reviewed; we stopped reviewing when heavy rainfalls or storm prevented clear vision.

species	treatment	no flowers filmed	no hrs reviewed	total no flower visits	total no plant visits	cases of multiple flower visits	% of multiple flower visits
M. furvanthera	D	4	4 (16)	12	2	2	100
Individuals: 2	D	13	6 (78)	1	1	0	0
	N	2	3.8 (7.6)	1	1	0	0
	N	12	3 (36)	19	2	2	100
sum	**D**	**17**	**10 (94)**	**9**	**2**	**1**	
	N	**14**	**6.8 (43.6)**	**20**	**3**	**2**	
M. aff. sanguinea	D	3	2 (6)	4	1	1	100
Individuals: 6	D	7	2.5 (17.5)	33	10	10	100
	D	2	5 (10)	27	19	5	26
	D	3	2.5 (7.5)	8	2	2	100
	D	6	1.5 (9)	13	6	6	100
	N	7	3.6 (25.2)	26	14	3	21
	N	5	2.6 (13)	3	5	0	0
	N	2	3 (6)	11	9	2	22
	N	1	1 (1)	0	0	-	
sum	**D**	**20**	**13.5 (50)**	**85**	**34**	**20**	
	N	**15**	**10.2 (45.2)**	**40**	**20**	**5**	
M. sanguinea	D	3	1.5 (4.5)	5	2	2	100
Individuals: 10	D	3	0.5 (1.5)	2	1	1	100
	D	2	2 (4)	0	0	-	
	D	3	2 (6)	1	1	0	0
	D	2	2 (4)	2	1	1	100
	D	1	1.5 (1.5)	0	0	-	
	D	2	2.5 (5)	0	0	-	
	D	3	2 (6)	3	1	1	100
	D	7	1.5 (10.5)	0	0	-	
	D	3	3 (9)	0	0	-	
	N	1	1.5 (1.5)	0	0	-	
	N	3	1 (3)	0	0	-	
	N	5	6 (30)	1	1	0	0
	N	1	4 (4)	0	0	-	

	N	1	4.8 (4.8)	0	0	-	
	N	1	4.5 (4.5)	1	1	-	
	N	8	1.8 (14.4)	0	0	-	
sum	**D**	**29**	**18.5 (52)**	**13**	**6**	**5**	
	N	**20**	**24.1 (63.7)**	**2**	**2**	**0**	
M. tomentosa	D	1	1 (1)	9	9	-	
Individuals: 7	D	2	2 (4)	15	8	7	88
	D	3	1.5 (4.5)	15	6	5	83
	D	2	1.5 (3)	17	9	4	44
	D	1	2 (2)	9	9	-	
	D	1	1 (1)	3	3	-	
	D	2	2 (4)	2	0	-	
	D	2	2 (4)	2	1	1	100
	N	2	2 (4)	1	1	0	0
	N	3	2 (4.5)	9	3	1	33
	N	1	2 (2)	4	4	-	
	N	3	2 (6)	1	1	0	0
	N	2	2 (4)	0	0	-	
sum	**D**	**14**	**13 (23.5)**	**75**	**45**	**17**	
	N	**11**	**10 (20.5)**	**15**	**9**	**1**	

Details on exclusion experiments ('pollinator quality')

Table A4. Details on sample sizes in the exclusion experiment on pollen deposition by the different diurnal and nocturnal functional pollinator groups for *M*. aff. *sanguinea*, *M. sanguinea* and *M. tomentosa*. In *M*. aff. *sanguinea* and *M*. *tomentosa* some individuals presented multiple inflorescences, while *M. sanguinea* individuals only presented one multi-flowered inflorescence; column heading: c – controls, un-manipulated flowers exposed to pollinators for three days; D – flowers bagged during night time, allowing visits of diurnal pollinator only; N – flowers bagged during daytime, allowing visits of nocturnal pollinators only).

species	plant ID	inflos per treatment per plant			flowers per treatment per plant		
		c	**D**	**N**	**c**	**D**	**N**
Meriania* aff. *sanguinea	1	2	1	1	4	3	3
	2	0	1	0	0	1	0
	3	1	0	0	1	0	0
	4	1	1	2	3	3	5
	5	0	1	0	0	1	0
	6	1	0	1	1	0	2
	7	1	0	0	1	0	0
sum	**7**	**6**	**4**	**4**	**10**	**8**	**10**
Meriania sanguinea	1	-	-	-	2	3	0

	2	-	-	-	0	0	3
	3	-	-	-	0	2	0
	4	-	-	-	1	0	5
	5	-	-	-	1	0	0
	6	-	-	-	0	2	0
	7	-	-	-	0	1	0
	8	-	-	-	2	0	0
	9	-	-	-	0	2	0
	10	-	-	-	0	0	1
	11	-	-	-	0	0	1
	12	-	-	-	0	0	2
	13	-	-	-	0	5	0
	14	-	-	-	0	7	0
	15	-	-	-	1	0	1
	16	-	-	-	2	0	3
	17	-	-	-	4	0	1
	18	-	-	-	0	0	1
	19	-	-	-	2	0	0
sum	**19**	**-**	**-**	**-**	**15**	**22**	**18**
Meriania tomentosa	1	1	2	1	1	3	2
	2	2	0	4	4	0	7
	3	0	0	1	0	0	2
	4	1	6	2	1	7	3
	5	0	1	0	0	2	0
	6	0	1	1	0	2	2
	7	1	0	0	3	0	0
sum	**7**	**7**	**10**	**9**	**9**	**14**	**16**

Table A5. Median number of pollen grains on stigmas summed up for D+N treatments (D+N pollen) in comparison to un-manipulated flowers (control pollen) for each species. These values indicate that accidental pollen deposition on stigmas by bagging/unbagging flowers, although highly unlikely given the strict pollen dosing by poricidal anthers, should be ruled out and did not affect experimental outcome.

species	median D+N pollen	median control pollen
M. aff. *sanguinea*	3542	4650
M. sanguinea	331	569
M. tomentosa	6301	5736

Table A6. Results of GLMMs on differences in stigmatic pollen loads of controls versus exclusion trials for the three species, treating individual ID as random effect.

species	t-value	df	p
M. aff. *sanguinea D*	-3.262	20	0.004
M. aff. sanguinea N	-1.762	18	0.094
M. sanguinea D	-1.45	50	0.153
M. sanguinea N	-0.64	50	0.525
M. tomentosa D	-0.538	35	0.594
M. tomentosa N	0.11	35	0.913

Details on nectar measurements

Table A7. Number of flowers for the nectar measurements of *Meriania* species. N – measurements taken at sunrise after nights (night nectar), D – measurements taken at sunset after days (day nectar). Sample sizes for measures of nectar sugar concentrations (BRIX, measured with a refractometer), total nectar volume (measured after 12h bagging of flowers), and sample sizes for nectar analyses by HPLC are given. For *M. sanguinea* and *M. tomentosa*, some flowers were re-bagged after the first measurement to assess if nectar was replenished; the number of re-bagged flowers are given in brackets.

species	no flowers concentration (remeasure)		no flowers volume (remeasure)		no flowers sugar types	
	N	D	N	D	N	D
Meriania aff. *sanguinea*	15	5	24	9	4	2
Meriania furvanthera	7	3	-	-	5	5
Meriania sanguinea	16 (5)	14 (7)	23 (11)	19 (8)	14	14
Meriania tomentosa	22 (11)	23 (10)	20 (12)	28 (12)	22	21

Table A8. Results from generalized linear mixed-effects model on nectar concentration between species and day/night and interaction of factors, treating plant ID as random effect. Comparisons of species against *M. furvanthera*; and of each species' day nectar against N – night nectar.

Factor	estimate	t-value	p-value
M. sanguinea	**-4.396**	**-2.577**	**0.012**
M.* aff. *sanguinea	**-3.533**	**-2.06**	**0.042**
M. tomentosa	**-3.152**	**-1.889**	**0.619**
N: M. furvathera	2.952	1.578	0.118
N: *M. sanguinea*	**2.952**	**2.69**	**0.008**
N: M. aff. *sanguinea*	1.300	0.928	0.355
N: *M. tomentosa*	0.253	0.313	0.755

Table A9. Summary table for PERMANOVA results on relative nectar sugar composition of between Meriania species, day/night and individuals.

Factor	d.f.	*F*	r^2	*p-value*
Species	**3**	**114.7**	**0.787**	**0.001**
Species*Daytime	4	0.687	0.006	0.593
Species*Individual	21	1.533	0.074	0.125

Table A10. Posthoc tests of PERMANOVA (table A9) on differences in nectar sugar composition between species. As daytime and individual did not result as significant, these factors were dropped from post hoc analyses.

pairs	F.Model	R2	p.value	p.adjusted
Meriania furvanthera vs Meriania aff. sanguinea	**615.74**	**0.9778**	**0.001**	**0.006**
Meriania furvanthera vs Meriania tomentosa	**146.46**	**0.7417**	**0.001**	**0.006**
Meriania furvanthera vs Meriania sanguinea	**2157.09**	**0.9835**	**0.001**	**0.006**
Meriania aff. sanguinea vs Meriania tomentosa	2.86	0.0574	0.068	0.408
Meriania aff. sanguinea vs Meriania sanguinea	1.54	0.0458	0.195	1.0
Meriania tomentosa vs Meriania sanguinea	**10.35**	**0.131**	**0.003**	**0.018**

Table A11. Bonferroni-adjusted p-values from pairwise species comparison by TukeyHSD test on significant differences in variability in nectar sugar composition. *M. tomentosa* (HB) was significantly more variable than *M. sanguinea* (HM) but not *M. furvanthera* (FR).

pairs	p adj
Meriania sanguinea-Meriania furvanthera	0.569
Meriania tomentosa-Meriania furvanthera	0.339
Meriania tomentosa-Meriania sanguinea	**0.002**

Details on scent analyses

Table A12. Summary table for differences in hourly scent release between day and night for M. aff. *sagnuinea*, *M. sanguinea* and *M. tomentosa*; Mann-Whitney U-tests (data not normally distributed as tested by Shapiro test). No test was run for *M. furvanthera* as only diurnal samples contained compounds.

species	**W**	**df**	**p**
M. aff. sanguinea	0	3	0.2
M. sanguinea	38	17	0.604
M. tomentosa	110	20	<0.01

Table A13. Summary table for PERMANOVA on the relative odour composition of the four *Meriania* species between D/N.

Factor	d.f.	*F*	r^2	*p-value*
Species	**3**	**10.844**	**0.3747**	**0.001**
Species*Daytime	**3**	**4.427**	**0.1529**	**0.001**

Table A14. Posthoc tests of results from PERMANOVA (Table A12) on scent composition between species and day/night (D/N) with Bonferroni correction.

pairs	F.Model	R2	p.value	p.adjusted
Maffsanguinea D vs Maffsanguinea N	0.836	0.218	0.5	1
Maffsanguinea D vs Msanguinea D	5.531	0.335	0.005	0.105
Maffsanguinea D vs Msanguinea N	21.280	0.680	0.004	0.084
Maffsanguinea D vs Mtomentosa D	**4.967**	**0.311**	**0.002**	**0.042**
Maffsanguinea D vs Mtomentosa N	2.652	0.169	0.022	0.462
Maffsanguinea D vs Mfurvanthera D	1.491	0.332	0.4	1
Maffsanguinea N vs Msanguinea D	5.610	0.359	0.013	0.273
Maffsanguinea N vs Msanguinea N	47.010	0.839	0.02	0.42
Maffsanguinea N vs Mtomentosa D	4.982	0.333	0.018	0.378
Maffsanguinea N vs Mtomentosa N	2.741	0.186	0.021	0.441
Maffsanguinea N vs Mfurvanthera D	1.814	0.476	0.333	1
Msanguinea D vs Msanguinea N	2.934	0.147	0.097	1
Msanguinea D vs Mtomentosa D	**14.045**	**0.438**	**0.001**	**0.021**
Msanguinea D vs Mtomentosa N	**9.543**	**0.323**	**0.001**	**0.021**
Msanguinea D vs Mfurvanthera D	3.437	0.256	0.05	1
Msanguinea N vs Mtomentosa D	**28.920**	**0.630**	**0.001**	**0.021**
Msanguinea N vs Mtomentosa N	**17.122**	**0.474**	**0.001**	**0.021**
Msanguinea N vs Mfurvanthera D	19.279	0.682	0.017	0.357
Mtomentosa D vs Mtomentosa N	**8.201**	**0.291**	**0.001**	**0.021**
Mtomentosa D vs Mfurvanthera D	3.465	0.257	0.031	0.651
Mtomentosa N vs Mfurvanthera D	1.895	0.136	0.083	1

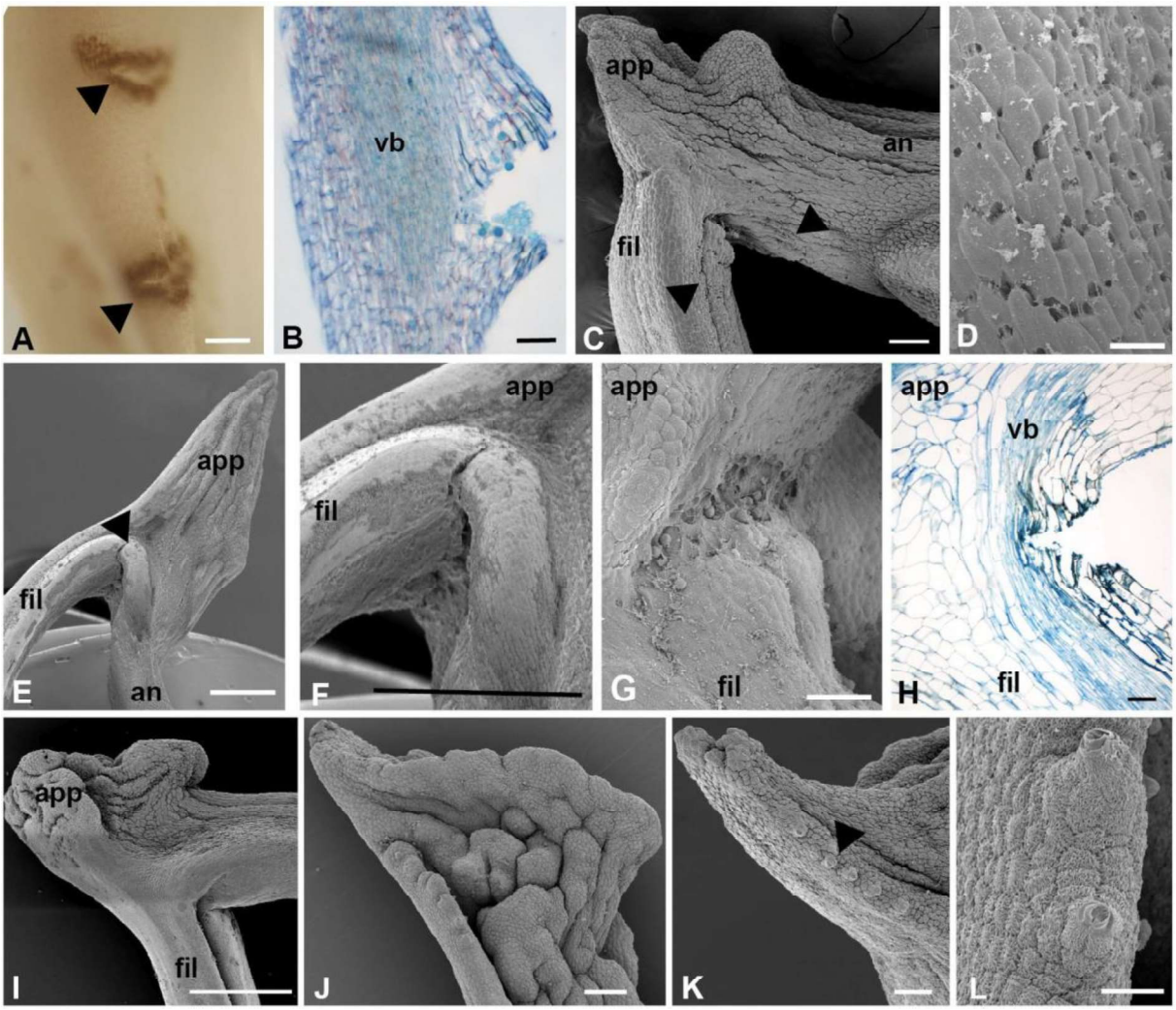

Figure A1. SEM and light microscope images and microtome sections of *Meriania* stamens. A) Type a: dorsal filament ruptures of old stamen of *M. phlomoides*. B) Type a: longitudinal section of medial part of filament showing dorsal rupture reaching vascular bundle in old stamen of *M. tomentosa*. allowing phloem sap to ooze out. 100µm. C) Type c: stamen of *M. furvanthera* with porous tissue at lateral distal part of filament and ventral side of connective (arrows). 200µm. D) Type c: detail of C. 50µm. E) Type b: *M. loxensis* with rupture at ventral filament-connective joint (arrow). 1mm. F) Type b: detail of E. 1mm. G) Type b: small rupture at ventral side of filament-connective joint in old stamen of *M. sanguinea.* 100m. H) Type b: longitudinal section of rupture at ventral filament-connective joint reaching vascular bundle in *M. costata*. allowing phloem sap to ooze out. 100µm. I) Sculptured stamen appendage without stomata of *M. pichinchensis*. 1mm. J) Strongly sculptured appendage of anther connective of *M. tetragona.* 200µm. K) Line of stomata on appendage (arrow) of *M. sanguinea*. 100µm. H) Raised stomata on connective appendage of *M. tetragona.* 50µm. an – anther. app – appendage. fil – filament. vb – vascular bundle.

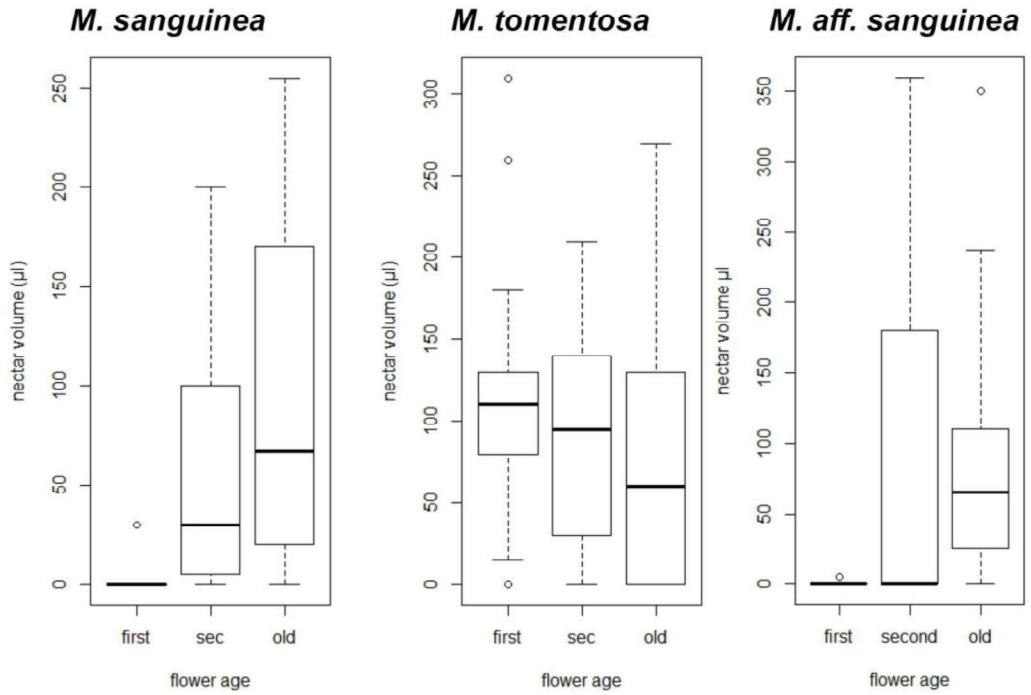

Figure A2. Total nectar volume (after 12 hours of bagging) secreted by first day flowers (first. <24h). second day flowers (second. 24h-48h) and flowers older than that (old. >48h) in *M. sanguinea* (hummingbird/rodent). *M. tomentosa* and *M*. aff. *sanguinea* (both hummingbird/bat).

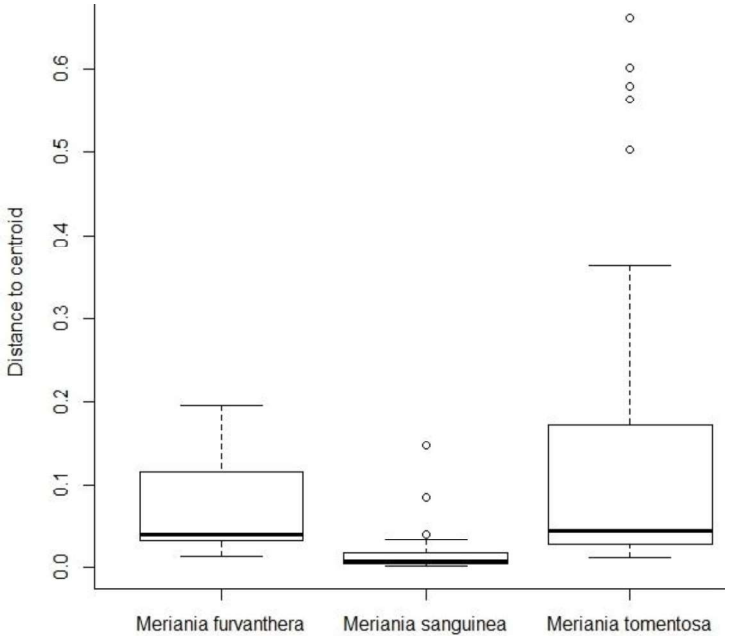

Figure A3. Variation in nectar sugar composition (Sucrose, Glucose, Fructose) calculated as distance to centroid in the four species *M.* aff. *sanguinea*. *M. tomentosa* (both HB), *M. sanguinea* (HR) and *M. furvanthera* (FR).

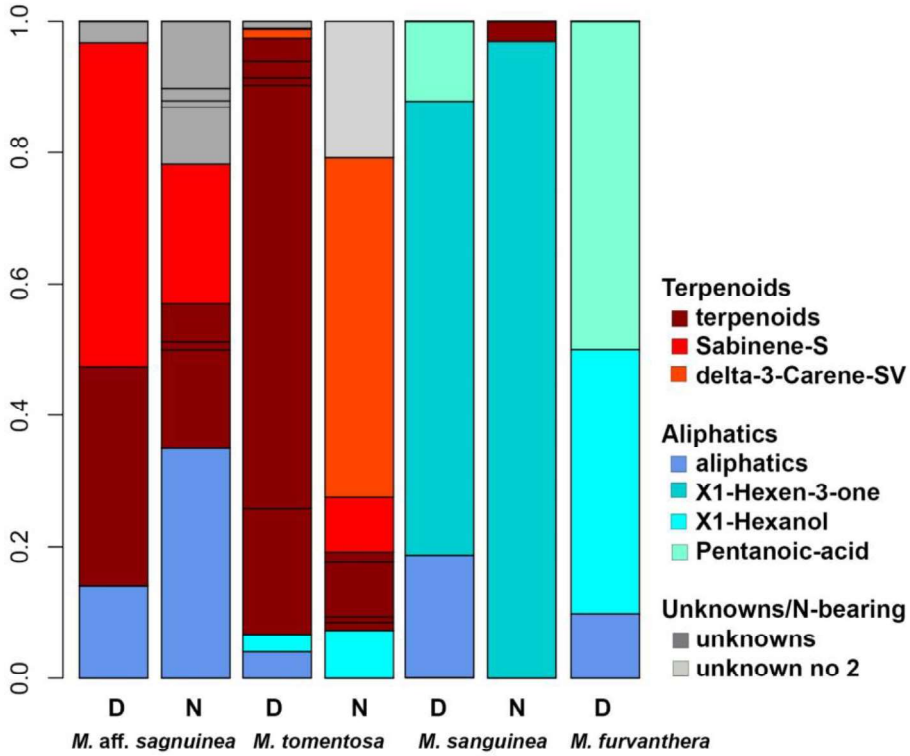

Figure A4. Diurnal (D) and nocturnal (N) scent profiles for the four different species; colours represent the main odour classes (Terpenoids – red tones. Aliphatics – blue tones; unknowns – grey), with compounds correlating best with the NMDS ordination analysis highlighted (see Fig. A4), *M.* aff. *sanguinea* and *M. tomentosa* HB, *M. sanguinea* HR, *M. furvanthera* FR.

Video A1. *Thomasomys* sp. visiting a flower of *M. sanguinea* and drinking nectar. Note deep head insertion of rodent into the flower and the long duration of rodent visit.

Video A2. Rodent visiting multiple flowers of *M. furvanthera* to drink nectar.

Video A3. Passerine (Masked Flowerpiercer) visiting flowers of *M. furvanthera* for nectar uptake.

5. CHAPTER III: IS MODULARITY THE KEY TO ADAPTIVE SUCCESS? TESTING HYPOTHESES ON MODULARITY IN FLOWERS OF MERIANIEAE (MELASTOMATACEAE)

Authors: **Agnes S. Dellinger, Silvia Artuso, Susanne Pamperl, Fabián Michelangeli, Darin S. Penneys, Diana Fernández-Fernández, Scott W. Armbruster, Yannick Staedler, Christian Klingenberg, Jürg Schönenberger**

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Title: Is modularity the key to adaptive success? Testing alternative hypotheses on modularity in flowers of Merianieae (Melastomataceae)

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Keywords: floral modularity, shape evolution, geometric morphometrics, pollinator shifts

Abstract

Modularity in organisms is shaped by genetic and developmental constraints and natural selection on functionally related traits. While hypotheses on shape evolution have been tested extensively in animals, patterns of modularity in plants remain severely understudied. Animal pollinated flowers are particularly interesting in this context as they comprise distinct developmental units (perianth organs, stamens, and carpels) and underlie strong selection by pollinators. We employ High Resolution X-ray Computed Tomography (HRXCT) and 3D geometric morphometrics to study the flowers of 33 species with different pollinators to test five competing hypotheses on floral modularity at a macroevolutionary scale (tribe Merianieae, Melastomataceae). We find that pollinator mediated selection has led to the evolution of functional floral modules that span across floral developmental units. These functional modules differ significantly between species with different pollinators and are best explained by distinct floral adaptations to optimize fit to the different pollinators. We detect the strongest modularity in the functionally highly specialized ancestral buzz-bee pollination system of Merianieae and a decrease in modularity in species, which shifted to vertebrate pollination. Our results indicate that the high degree of modularity in the ancestral system may be the key to the adaptive success of buzz pollination in the group, making the system flexible to explore different areas on an 'adaptive plateau'. At the same time, this high degree of floral modularity may also have facilitated shifts to novel vertebrate pollination systems.

Significance Statement

Understanding the diversity of organismal shapes remains a major challenge in evolutionary biology. While various hypotheses have been tested in animals, patterns of modularity in plants remain largely unclear. We test competing hypotheses on developmental and functional modularity using 3D flower models in a clade of Neotropical angiosperms that is characterized by a broad diversity of pollination systems including bees, birds, bats, and rodents. We find that functional modules were apparently optimized in each pollination system independently and that floral modularity may be key to the adaptive success of our study group. Our work presents a novel approach to the study floral diversification by testing different modularity hypotheses at a

macroevolutionary scale and including species underlying different pollinator selection regimes.

Introduction

Understanding the evolution of organismal shape is key to understanding diversity on Earth. The morphological structures of animals and plants are integrated to function as a whole, but parts of these structures may be modular and change relatively independently of each other (Olsen & Miller 1958, Klingenberg 2009, Esteve-Altava 2016, Klingenberg 2014). The extent to which modularity is shaped by genetic and developmental constraints or results from natural selection on functionally related traits remains an open question in evolutionary biology (Lande 1979, Murren 2002, Armbruster et al. 2004, Cheverud 2004, Claverie & Patek 2013). While the study of modularity has a long tradition in anthropology and zoology (e.g. modularity of the cranium, human brain, mandibles, insect wings), comparatively little is known about patterns of modularity in plants (Berg 1960, Diggle 2014, Pérez et al. 2008, Esteve-Altava 2016). This is surprising since plants, and particularly flowers, lend themselves to test competing hypotheses on modularity and the evolution of shape.

Flowers, the defining structures of angiosperms (flowering plants), are made up of different developmental categories, i.e., the different organ types that are present in a typical flower organized in whorls, including sterile perianth organs (tepals, sepals, petals) and the fertile male (stamens) and female organs (carpels). The different organ whorls of a flower represent distinct developmental modules (Irish 2017). In order to achieve reproduction, these floral organs function in synorganization (Endress 1994, Kay et al. 2006, Specht & Bartlett 2009, Endress 2016, Sauquet et al. 2017). Particularly in animal pollinated plants (ca. 87.5% of angiosperms, Ollerton et al. 2011), flowers underlie strong selection by pollinators with organs (co-)functioning to achieve pollinator attraction and successful pollen transfer (Berg 1960, Murren 2002, Armbruster et al. 2004, Alcantara et al. 2013, van der Niet et al. 2014; for discussion on selection by antagonistic and abiotic factors also see Strauss & Whitall 2006, Harder & Johnson 2009). Thus, the evolution of flower shape is likely constrained by developmental and genetic linkage on the developmental modules (Herrera et al. 2002, summary of floral pleiotropy by Smith 2016), but pollinators could potentially select for alternative functional modules across developmental categories (e.g. Ordano et al. 2008, Rosas-Guerrero et al. 2011, Armbruster et al. 2014, Baranzelli et al. 2014, Pérez-Barrales et al. 2014, Fornoni et al. 2016). Such (partially overlapping) functional modules have been proposed for traits involved in pollinator attraction ("attraction module", e.g. showy

petals), reproductive organs ("reproductive module", stamens and carpels) or mechanical fit-traits mediating efficient pollen transfer ("efficiency module", e.g. a module comprised of all floral organs involved in the monosymmetric construction of the flower; Benitéz-Vieyra et al. 2006, Fenster et al. 2009, Rosas-Guerrero et al. 2011, Diggle et al. 2014, Endress 2016, Esteve-Altava 2016, Chartier et al. 2017, Fig. 1). Generalizations on modularity prove to be difficult, however, as type and strength of selection are not necessarily uniform across the flower (e.g. corolla shape mediating attraction, fit to pollinators or avoidance of herbivores, Armbruster 1999, Strauss & Whitall 2006).

Evolutionary modularity is defined as the interaction of genetic, developmental and functional modularity across macroevolutionary timescales (Claverie & Patek 2013, Klingenberg 2014). Congruency between functional and evolutionary modules has been found in vertebrates (Monteiro et al. 2005, Goswami & Polly 2010). In theory, such evolutionary modularity could increase rates of evolution and evolvability, as each module can potentially respond independently to selection (Claverie & Patek 2013, Diggle 2014, Felice & Goswami 2018, Larouche et al. 2018, Opedal 2018). In flowers, this should become particularly apparent in the comparison of closely related plant taxa that have repeatedly shifted functional pollinator groups. Functional pollinator groups are defined as groups of pollinators imposing similar selective pressures on flowers (Fenster et al. 2004, summarized into 'pollination syndromes', Grant & Grant 1965, Stebbins 1970, Johnson 2006). Thus, per definition, shifts in functional pollinator groups (e.g. from bee to hummingbird) result in changes in phenotypic selection regimes on flowers, and could translate to shifts in floral phenotype (Harder & Johnson 2009, van der Niet et al. 2014, Smith & Kriebel 2018). To date, only few studies have assessed the impact of pollinator shifts on floral modularity. They suggest possible independent evolution of floral modules (e.g. corolla tube versus stamen/style length in *Nicotiana*, Bissell & Diggle 2010), changes or loss in function of modules with pollinator shifts (e.g. corolla as landing platform in *Schizanthus*; Pérez et al. 2007) or stasis of floral structure through evolutionary time (Bignonieae, Alcantara et al. 2013). To our knowledge, this is the first study to test competing hypotheses of floral modularity using 3D landmark-based geometric morphometrics across a tribe of species pollinated by different functional pollinator groups to understand patterns of flower shape evolution.

The tribe Merianieae (Melastomataceae) exhibits an extraordinary diversity of functional pollinator groups (bees, passerines, hummingbirds, bats and rodents) and repeated shifts

from bee to vertebrate pollination (Dellinger et al. 2018). All species have tubular anthers releasing pollen only by a small apical pore and when triggered by pollinators (Renner 1989). Marked differences in pollen expulsion mechanisms have recently been identified as one of the major traits differentiating Merianieae into three pollination syndromes: 'buzz-bee', 'mixed-vertebrate' and 'passerine' (Dellinger et al. 2018). Stamen appendages represent the key for activating pollen expulsion in the 'buzz-bee' and 'passerine' syndrome ('buzz-bee': handles for applying buzzes (vibrations) to shake out pollen; 'passerine': bellows to eject pollen clouds; Dellinger et al. 2014), while they have lost their function in the 'mixed-vertebrate' syndrome (pollen expulsion by a salt-shaker mechanism when pressure is applied to the thecae by nectar foraging pollinators). The functionally highly specialized 'buzz-bee' syndrome clearly is ancestral in Merianieae and reflects an exceptional evolutionary success (an 'adaptive plateau') at the family level as ca. 98% of the 5000 Melastomataceae species are buzz pollinated (Renner 1989, Berger et al. 2015, Dellinger et al. 2018).

We use 3D-geometric morphometrics on High Resolution X-ray Computed Tomography (HRXCT) scans of flowers of 33 Merianieae species and comparative phylogenetic methods to test five competing hypotheses on floral modularity and shape evolution in the three Merianieae pollination syndromes. We find significant restructuring of floral functional modularity with pollinator shifts across developmental categories and partial congruence between functional and evolutionary modularity. Pollinator shifts went along with significant changes in floral phenotypic optima in Merianieae. The high degrees of modularity through evolutionary time that we find for Merianieae possibly explain both the diversity of floral shapes of the 'adaptive buzz-bee pollination plateau' and the potential to evolve into new areas of shape space in connection with pollinator shifts.

Results

Testing hypotheses on floral modularity in Merianieae pollination syndromes

We found significant differences in patterns of floral modularity between the three different pollination syndromes based on our geometric morphometric assessment (Fig. 1, Table 1). Flowers within the 'buzz-bee' syndrome were overall highly modular and the only ones to show significant modularity in all five hypotheses, including the developmental hypothesis (Hyp. 1). We found no modularity in flowers of the 'mixed-

vertebrate' syndrome, indicating a non-independence of corolla shape, stamen appendages and the pore/stigma complex (Table 1). For flowers of the 'passerine' syndrome, however, our analyses identify significant functional modularity as suggested by hypotheses 3 and 4, into 'attraction and 'efficiency' modules. In order to compare strengths of modularity among syndromes, we calculated effect sizes (z-scores, Adams & Collyer 2016). For each pollination syndrome, we found effect sizes to be highest for hypothesis 4 (corolla and stamen pores/stigma as one module) and second highest for the Merianieae-specific hypothesis 5 (Table 1). Accordingly, hypothesis 4 is the only one where the degree of modularity differed significantly among all three pollination syndromes (Table S5). Results are congruent with the resampled datasets (Table S6).

We assessed model fit (EMMLi, Goswami & Finarelli 2016) in order to understand which of the five hypotheses of modularity fits the data best. An additional 0-hypothesis of no modularity was included in the test. The Merianieae-specific hypothesis 5, partitioning the flower into three independent functional modules, resulted as best fit for the 'buzz-bee' syndrome (AICc -1360.7, posterior probability of Hyp. 5 > 97%; Fig. 1F). For the 'mixed-vertebrate' and 'passerine' syndrome, hypothesis 4, partitioning the flower into an 'attraction module' (appendages) and an 'efficiency module' (corolla shape, pore/stigma complex) resulted as best fit ('mixed-vertebrate' AICc -803.2, posterior probability of Hyp. 4 49.4%, Fig. 1E; 'passerine' AICc -591.8, posterior probability of Hyp. 4 68.5%, Fig. 1E), despite an overall lack of significant modularity in the 'mixed-vertebrate' syndrome. For both shifted syndromes, the Merianieae-specific modularity hypothesis (Hyp. 5) resulted as second best fit (Table S7).

Evolutionary floral modularity in Merianieae

In order to evaluate the relative evolutionary independence of floral modules, we tested the five modularity hypotheses (Fig. 1) in an evolutionary framework using a phylogeny of the 33 species included in this study. We found highest support for the three functional hypotheses indicating effects of pollinator mediated selection. The two hypotheses partitioning the flower into attraction and efficiency functional modules (Hyp. 3, 4), as well as the Merianieae-specific hypothesis (Hyp. 5), separating an attraction from a pollen expulsion and pollen transfer functional module, were significant across the phylogeny (Table 1). Neither the developmental hypothesis (Hyp. 1) nor the perianth vs. reproductive organ hypothesis (Hyp. 2) were supported (Table 1). These results indicate a

significant degree of modularity in Merianieae flowers that is apparently the result of pollinator mediated selection. Accordingly, the Merianieae-specific hypothesis proposing three independent floral functional modules (Hyp. 5) results as best fitting (AICc -1442.0) with more than 99% posterior probability (Table S7).

In addition, our analyses show that the three floral functional modules of hypothesis 5 evolve at significantly different rates of morphological evolution (under Brownian motion, R = 4.963, p = 0.001). Corolla shape apparently evolved at a significantly higher rate (sigma 6.56 x 10^{-4}) than the pore/stigma complex (sigma 3.09 x 10^{-4}) and the stamen appendages (sigma 1.32 x 10^{-4}).

Flower shape evolution in Merianieae

To understand flower shape evolution in connection with pollinator shifts across Merianieae, we constructed a flower shape space using PCA (variation explained: PC1 31.6 %, PC2 16.5 %). PC1, which captures differences in corolla shape ranging from reflexed open corollas to urceolate/pseudo-campanulate corollas, separates the 'buzz-bee' syndrome flowers from the 'mixed vertebrate' and 'passerine' syndrome flowers (Fig. 3). PC2 separates the two shifted syndromes and describes differences of androecial arrangement ranging from geniculate stamens with pores close to the base of the style in the flower centre ('buzz-bee' and 'passerine' syndrome) to reflexed stamens with pores close to the stigma (some 'buzz-bee' and 'mixed vertebrate' syndrome).
Despite this clustering in relation to pollination syndrome rather than phylogenetic relatedness, there is a strong phylogenetic signal in the data, indicating that flowers of closely related taxa are more similar than expected by chance (K_{mult} 0.457, *p = 0.001*). We used the newly developed penalized likelihood framework (Clavel et al. 2018) to estimate the fit of four different models of evolution (Brownian motion (BM), Lambda, Early-burst (EB), Ornstein-Uhlenbeck (OU)) directly on the landmark data. We found the best fit with the OU model (lowest GIC, Table S9), which assumes evolution towards different phenotypic means as could be expected under selection mediated by different functional pollinator groups (Smith & Kriebel 2018). In order to test if these shifts in floral shape coincide with pollinator shifts, we estimated regime shifts on the phylogeny (l1ou, Khabbazian et al. 2016). As this method does not support highly multivariate landmark data, we estimated regime shifts on PC1 and PC2. We found support for three independent shifts, all of which coincide with pollinator shifts (Fig. 4, Figure S1). We

found no significant shift along the branch leading to *M. inflata* (‘passerine’ syndrome) or along any of the clades with ‘buzz-bee’ syndrome species. The model allowing for convergence in these shifts had the best fit (pBIC ‘shifts-model’ -37.0, pBIC ‘convergence-model’ -42.1, Fig. S1, Table S10). This result did not change when we incorporated intraspecific phenotypic variability by resampling the shape data (66% best fit of ‘convergence-model’, Table S10, S11).
These results are further supported by visualizing the shape space over evolutionary time (Video 1). Whilehe ’buzz-bee’ syndrome lineages remain in theancestral area of shape space (possibly corresponding to the ‘adaptive plateau’), the four lineages with vertebrate pollinators explore new areas of the morphospace and converge either into the ‘mixed-vertebrate’ or the ‘passerine’ syndrome.

Discussion

Pollinator shifts in Merianieae are clearly linked to significant shifts in patterns of floral modularity and mean floral phenotypic shape. Our analyses show that evolutionary floral modularity across Merianieae is best explained by a functional hypothesis partitioning the flower into three modules characteristic for this ancestrally buzz-pollinated group.

Our assessment of five alternative hypotheses on floral modularity shows that pollinator mediated selection can generate functional modules across developmental modules (i.e. across floral whorls and organ types, defined in Hyp. 1). While studies on modularity in animals report similar importance of developmental and functional factors as source of modularity (29.8% and 27.1%, respectively, less importance of genes and environment; reviewed in Esteve-Altava 2016; Klingenberg et al. 2003, Goswami et al. 2009), modularity in plants it most often explained by function (38.2%) rather than development (14.7%; reviewed in Esteve-Altava 2016; Ordano et al. 2008, Rosas-Guerrero et al. 2011, but see e.g. Herrera et al. 2002 for support of developmental modules in flowers). Our results in Merianieae support the view that function is the most important factor structuring floral modularity. Pollinator shifts in Merianieae are accompanied by major changes in trait function (e.g. bees alight on flowers to buzz (vibrate) single stamens or entire androecia while hummingbirds hover in front of flowers to drink nectar; for details see Dellinger et al. 2014, 2018). It is thus difficult to partition all Merianieae flowers consistently into ‘attraction’, ‘reproduction’ or ‘efficiency’ modules as proposed by

literature (Hyp. 2-4, Rosas-Guerrero et al. 2011, Diggle et al. 2014, Esteve-Altava 2016). Instead, each syndrome was best characterized by a distinct functional modularity hypothesis (Hyp. 5 in 'buzz-bee' and Hyp. 4 in 'mixed-vertebrate and 'passerine') and a functional modularity hypothesis specific to Merianieae (Hyp. 5) resulted as best fit across the entire phylogeny. Thus, our results are in line with other studies arguing that floral integration and modularity is likely too complex to consistently partition the floral traits into functional modules across clades (Armbruster 1999, Baranzelli et al. 2004). The colourful perianth, for example, is usually understood as 'attraction module'. However, the perianth may not only function in attracting pollinators but also in mediating flower and pollinator fit or in deterring less efficient pollinators or herbivores and, hence, may underlie conflicting selection pressures (Strauss & Whitall 2006). Also in Merianieae, the corolla underwent prominent changes in shape and function during pollinator shifts (summarized by PC1 in shape space). The function as a landing platform in many 'buzz-bee' syndrome species was lost with shifts to vertebrate pollination (see Pérez et al. 2008 for similar example in *Schizanthus* with shift to moth pollination). Instead, corollas apparently have acquired a novel 'efficiency' function in that their urceolate or pseudo-campanulate shapes mediate the mechanical fit with the pollinators. This idea is supported by hypothesis 4 resulting as best fit in the 'mixed-vertebrate' and 'passerine' syndrome, defining an 'efficiency' module made up by the corolla shape and the pore/stigma complex (Fig. 2C,D).

Theory suggests that high degrees of modularity increase evolutionary adaptive potential (evolvability) in organisms through reduced pleiotropy (Wagner 1996, Claverie & Patek 2013). Differences in evolutionary rates of the three floral modules that we found for Merianieae support this idea. Corolla shape evolved at a significantly higher rate (double to six-fold) than the other two Merianieae specific modules, which is particularly important in the light of pollinator shifts. Attraction traits (e.g. signalling and reward), which presumably are the first "filters" for acquiring novel pollinators, have been hypothesized to change first, followed by efficiency traits (Thomson & Wilson 2008, Opedal 2018). In Merianieae, reward type (pollen, nectar or food bodies) is a key trait in differentiating the different pollination syndromes and possibly was one of the first traits to change (Dellinger et al. 2018). Corolla shape apparently also responded relatively quickly to pollinator mediated selection, while stamen appendage position and the pore/stigma complex were more conserved. Our shape analyses show that in the 'buzz-

bee' syndrome, the androecium is tightly aggregated beneath the style, rendering the flower functionally monosymmetric (Fig. 3C; note that Merianieae flowers are not structurally monosymmetric, see SI Methods). This tight arrangement possibly represents an ancestral efficiency function of the appendage and pore/stigma module, which underlay relatively strong stabilizing selection to optimize bee pollinator fit on the stamens for buzzing (Nielsson 1988, Cresswell 1998, Benítez-Vieyra et al. 2006, Opedal 2018). Monosymmetry alone may not have been strong enough to assure efficient pollen transfer when Merianieae species underwent pollinator shifts, but was supplemented by changes in corolla shape (formation of 'pseudo-tubes') restricting access directions to floral rewards.

Monosymmetry and floral tubes or nectar spurs have been identified as pre-adaptations to shifts from bee to bird pollination in many lineages (Kay et al. 2006, Cronk & Ojeda 2008, Fenster et al. 2009, but also see 'brush-flowers' e.g. in Proteaceae or *Acacia* or tube-less flowers in Loasaceae, Strelin et al. 2016). While functional monosymmetry is mainly expressed in the androecium of 'buzz-bee' pollinated Merianieae (see above), they lack a floral tube. A crucial pre-adaptation facilitating pollinator shifts, however, may lie in the modular organization (bauplan) of Merianieae flowers and represent an evolutionary 'line of least resistance' (Stebbins 1970). It is striking that our analyses consistently found higher modularity in the 'buzz-bee' syndrome than in any of the two shifted syndromes (Table 1). This suggests that floral diversification in Merianieae started from an ancestrally three-modular system (best fit of Hyp. 5 in the 'buzz-bee' syndrome). With pollinator shifts, modularity patterns changed (best fit of Hyp. 4 in the 'mixed-vertebrate' and 'passerine' syndrome) and strength of modularity decreased significantly or even was lost in the 'mixed-vertebrate' syndrome (Table 1). The Merianieae lineages that underwent pollinator shifts did by no means evolve completely novel shapes. Their flowers rather represent different combinations of the modules that wre likely already present in their 'buzz-bee' pollinate ancestors and are seen in extant 'buzz-bee' syndrome relatives. These modules include, for instance, the pseudo-campanulate corolla that is characteristic for the 'mixed-vertebrate' syndrome but is also present in 'buzz-bee' *Adelobotrys*. Another example are the reflexed stamens that are present in the 'mixed-vertebrate' syndrome but are also found in the 'buzz-bee' pollinated genus *Graffenrieda*. Thus, the new areas of shape space explored by the shifted syndromes mirror

combinations of floral traits and modules that have not been realized in the 'buzz-bee' syndrome, but whose components were there.

Taking this idea ahead, our findings suggest that the modular floral bauplan of Merianieae both may have allowed significant shifts in floral phenotype in response to changed selection regimes by pollinator shifts and at the same time may have enabled adaptive wandering on the 'buzz-bee' syndrome plateau. This may be particularly important considering an apparent 'line of strong resistance' in Merianieae: the tubular anther structure making the system functionally specialized on pollinators capable of triggering complex pollen release mechanisms (Buchmann 1983, Dellinger et al. 2018). Such high degrees of specialization have been related to evolutionary dead-ends in other systems (Tripp & Manos 2008), but is apparently not the case in Merianieae. Several other speciose plant lineages represent comparable 'adaptive plateaus', including Malpighiaceae (Davis et al. 2014), *Mimosa* (Fabaceae, Barneby 1991), *Croton* (Euphorbiaceae, Webster 1993), *Myrcia* and *Eugenia* (Myrtaceae, Vasconcelos et al. 2018) as well as the buzz-pollinated genus *Solanum* (Solanaceae, Symon 1979) and the Melastomataceae tribe Miconieae (Renner 1989, Reginato & Michelangeli 2016). In these systems, pollination strategies range from generalist (bee-)pollination (*Myrcia*, Vasconcelos et al. 2018, *Miconia*, de Brito et al. 2017) to specialized oil-flowers (Malpighiaceae, Davis et al. 2014) and buzz pollination (*Solanum*, Knapp 2010; Miconieae, Renner 1989). Testing whether maintenance of these 'adaptive plateaus' is facilitated by high floral modularity, allowing for considerable flexibility to accommodate changeable environmental conditions, or the result of stabilizing selection conserving floral integration patterns (Alcantara et al. 2013), provides a fruitful challenge for future investigations.

In conclusion, our study exemplifies a novel approach to studying floral evolution by testing competing hypotheses on floral modularity at a macroevolutionary scale. We demonstrate that pollinator mediated selection can disrupt both patterns and degree of floral modularity. The high degree floral modularity detected in the ancestral pollination syndrome possibly explains how diversification could occur even in functionally highly specialized pollination system such as buzz-pollination.

Material and Methods

Taxon sampling and pollination syndrome classification. Alcohol preserved floral material of 33 Merianieae species (ca 11% of Merianieae) was collected during various sampling trips to South and Central America, encompassing both the morphological and taxonomic diversity of the tribe (Supplementary Table S1). For 15 of the 33 species, pollinators are documented and include bees (seven species), passerines (three species) and mixed assemblages of hummingbirds, bats, rodents and/or flowerpiercers (five species, Dellinger et al. 2018). For the 18 species with unknown pollinators included in this study, the syndrome classification of Dellinger et al. (2018) was used, resulting in a total of 19 'buzz-bee' syndrome species, eight 'mixed-vertebrate' and six 'passerine' syndrome species.

Phylogeny and ancestral pollination syndromes. Our analyses of evolutionary modularity and flower shape evolution are based on a recently developed phylogeny which includes 150 tips (Dellinger et al., 2018). Bayesian Analyses were performed in BEAST2 (v2.5.0, Drummond & Bouckaert 2014) under a seven partition scheme (best fit; SI Methods for details). Based on previous analyses across the Melastomataceae, calibrated with fossils across the Myrtales, we fixed the age of the Merianieae at 29.25 MY and ran three independent analyses of 60 million generations each (20% burn-in). We combined the stable posterior distributions with LogCombiner v2.5.0 (Rambourt & Drummond 2018a) and summarized the maximum clade credibility tree (MCC-tree) with TreeAnnotator v2.5.0 (Rambourt & Drommond 2018b). The phylogeny was then pruned to only include the 33 tips present in the current study using *drop.tips* (PHYTOOLS, Revell 2012). Ancestral pollination syndromes were reconstructed using ML methods ('equal-rates' and 'all-rates-different' models tested, function *ace* in APE; Paradis et al. 2004) and the 'equal-rates' model selected by a likelihood-ratio test (Table S8). Stochastic character mapping (1000 iterations) with the empirical Bayes method was then run on the 'equal-rates' model to validate ML estimation (*make.simmap* PHYTOOLS; Revell 2012).

HRXCT scanning, 3D-models, landmarking. 147 flowers (a mean of four flowers per species) were prepared for HRX-CT scanning by putting them into a contrasting agent for four weeks (1% Phosphotungstic Acid (PTA) – 70% EtOH, Supplementary Table S1, Staedler et al. 2013, Staedler et al. 2017). Fully contrasted flowers were mounted in

plastic cups (Semadeni Plastics Group) and stabilized by acrylic-pillow foam to prevent movement during the scanning process. Samples were HRX-CT scanned using the Xradia MicroXCT-200 system, raw scan data has been deposited on the open source platform Phaidra (https://phaidra.univie.ac.at/). Three-D models of flowers were reconstructed (XMReconstructor XRadia Inc.) and 37 landmarks placed (AMIRA 5.5.0) to capture aspects of flower shape possibly under pollinator mediated selection (Figure 1A, Table S1). All landmarks were placed by SA in order to minimize variation due to observer error (SI Methods).

All subsequent data analyses were performed in R (R Core Team 2018). Generalized Procrustes superimposition of landmarks was performed in GEOMORPH (Adams & Otárola-Castillo 2013) to remove variation in position, orientation and size (e.g. Bookstein 1991, Mitteroecker & Gunz 2009). The mean floral shape of each species was calculated and shape space visualized by Principal Component Analyses (PCA). Shape change along PC1 and PC2 was visualized by wireframes. To incorporate aspects of intraspecific variability, 100 resample datasets were constructed where species with more than one specimen available were resampled at random and results were compared with results from analyses on mean shape.

Modularity analyses. We explored five different hypotheses on floral modularity (Figure 1, Table S4), including a developmental hypothesis (Hyp. 1), three functional hypotheses derived from the literature (Hyp. 2-4) and a hypothesis specifically designed to capture trait functioning in Merianieae (Hyp. 5).

The covariance ratio (CR) was chosen as metric to test the five modularity hypotheses as it generates robust results even with small and variable sample sizes (Adams 2016). The five hypotheses were tested for each pollination syndrome separately but on joint Procrustes fitted landmark coordinates using the function *test.modularity* (GEOMORPH) with 1000 random permutations. For assessing evolutionary modularity, CR coefficients were calculated for all species together while accounting for phylogenetic relatedness using the function *phylo.modularity* (GEOMORPH).

As summary measures of trait correlation are sensitive to various attributes of the data, they cannot be readily compared between different groups (Adams 2016, Bookstein 2016) such as, for instance, the three different pollination syndromes considered here. We thus extend the approach of Adams & Collyer (2016; developed for the Partial Least Squares

correlation coefficient) to calculate effect sizes (z-scores) to statistically evaluate the strengths of modularity between the three different pollination syndromes (see SI Methods for details). Two-sample tests were performed to assess if degrees of modularity differed significantly between pollination syndromes.

To assess the fit of the five competing modularity hypotheses, we used the maximum-likelihood approach proposed by Goswami & Finarelli (2016) using the function EMMLi (EMMLi; Goswami & Finarelli 2016, SI Methods). An additional null-model of no modularity was included.

For the best-fit hypothesis of three floral modules (Hyp. 5), we tested whether these modules evolved at different rates using the compare.multi.evol.rates function under Brownian motion (GEOMORPH).

Flower shape evolution. We calculated phylogenetic signal in flower shape on the landmark data by the K_{mult} statistic, specifically designed for multivariate data (Adams 2014). We then assessed the fit of four different evolutionary models (Brownian motion (BM), lambda, Early Burst (EB), Ornstein-Uhlenbeck (OU)) to the landmark data using the newly developed penalized likelihood framework (Clavel et al. 2018). Based on the clear clustering of the three different pollination syndromes in shape space (assessed by PCA), we used PC1 and PC2 to visualize flower shape change on the phylogeny by constructing a traitgram (PHYTOOLS). We then modelled trait evolution (PC1-2) under an Ornstein-Uhlenbeck (OU) process (Hansen 1997) to screen for different phenotypic optima within Merianieae using the *l1ou* R-package (Khabbazian et al. 2016). We used a LASSO (Least Absolute Shrinkage and Selection Operator) procedure (Tibshirani 1996) to estimate shifts in phenotypic optima from the data without an a-priori definition of where regime shifts may have occurred (*estimate_shift_configuration* function, "estimated shifts-model"). Convergence of these shifts was then evaluated using the *estimate_convergent_regimes* function (L1OU*)*. We evaluated model fit using the phylogenetic Bayesian information criterion (pBIC) and calculated weights (*aicw* from GEIGER, Pennell et al. 2014).

Finally, morphospace evolution through time was reconstructed on PC1 and PC2 using the *evomorphospace* function (EVOMAP, Smaers & Mongle 2018). Ancestral character estimation was done for PC1 and PC2 (ace, method "REML", APE) and branches were coloured according to the estimation of ancestral pollination systems (Fig. 2A, Fig. 3A).

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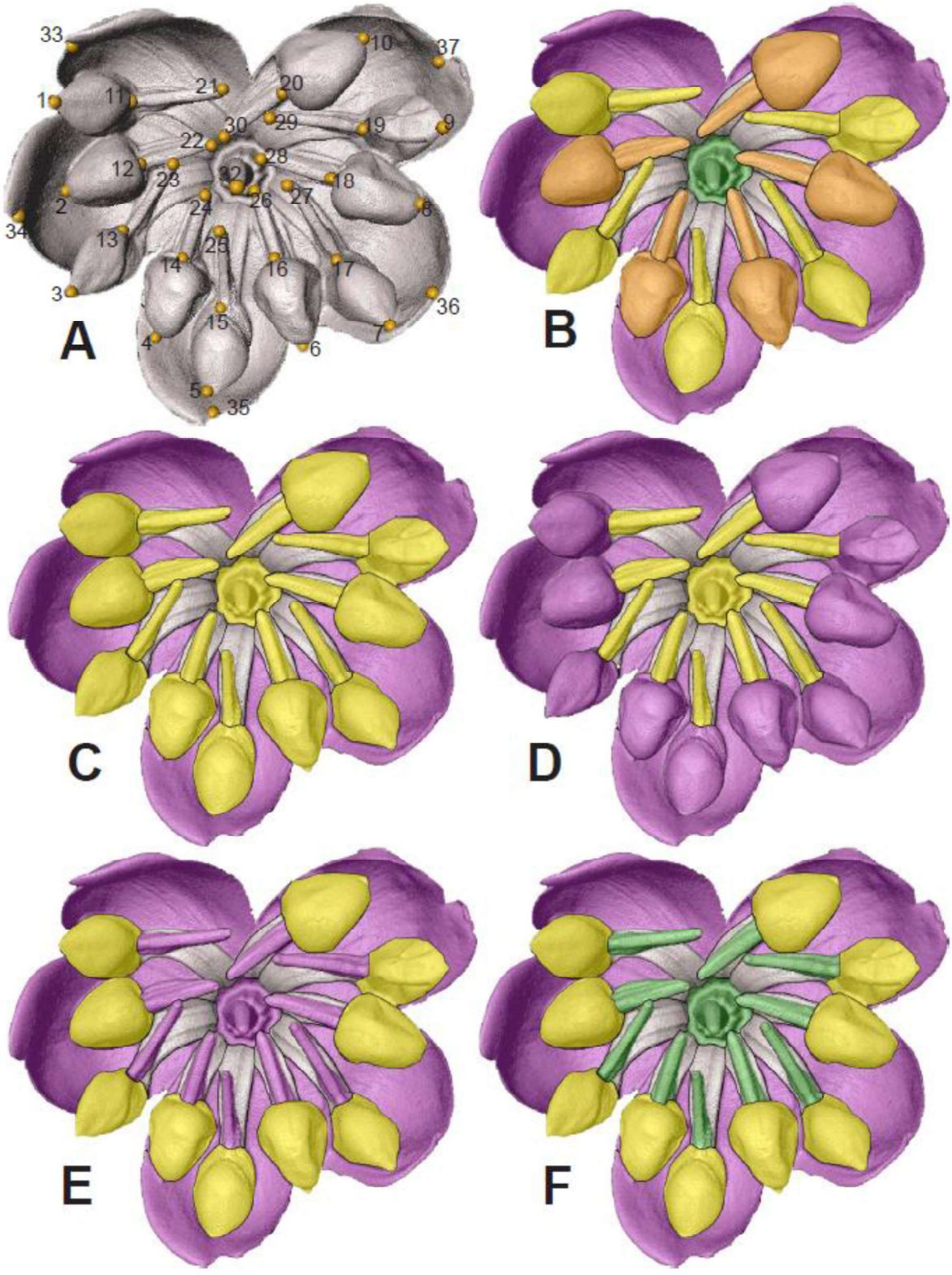

Figure 1. Flower landmark configuration and the five alternative hypotheses on floral modularity tested in Merianieae, visualized on an HRX-CT scan of a flower of *Axinaea costaricensis* ('passerine' syndrome). Colour patterns represent the different hypothesized modules. (A) The 37 landmarks placed on Merianieae flowers: 1-10 – appendage tips, 11-20 – appendage base, 21-30 – stamen pores, 31 – base of style, 32 – stigma, 33-37 – petal tips. (B) Hyp. 1: developmental modules (four organ whorls including the petal whorl in purple, the alternipetalous stamens whorl in orange, the alternisepalous stamens whorl in yellow, and the

carpel whorl in green; the sepals are not landmarked as they are not involved in pollination). (C) Hyp. 2: 'corolla module' in purple and 'reproductive module' in yellow (Esteve-Altava 2016). (D) Hyp. 3: 'attraction module' (corolla and appendages) in purple and 'efficiency module' (pores/stigma) in yellow (Diggle 2014). (E) Hyp. 4: alternative configuration of 'attraction module' (appendages only) yellow and 'efficiency module' (corolla, pore/stigma) in purple (Diggle 2014). (F) Hyp. 5: Merianieae specific modules, 'corolla module' in purple, 'pollen expulsion module' (appendages) in yellow, and 'pollen transfer module' (pore/stigma) in green.

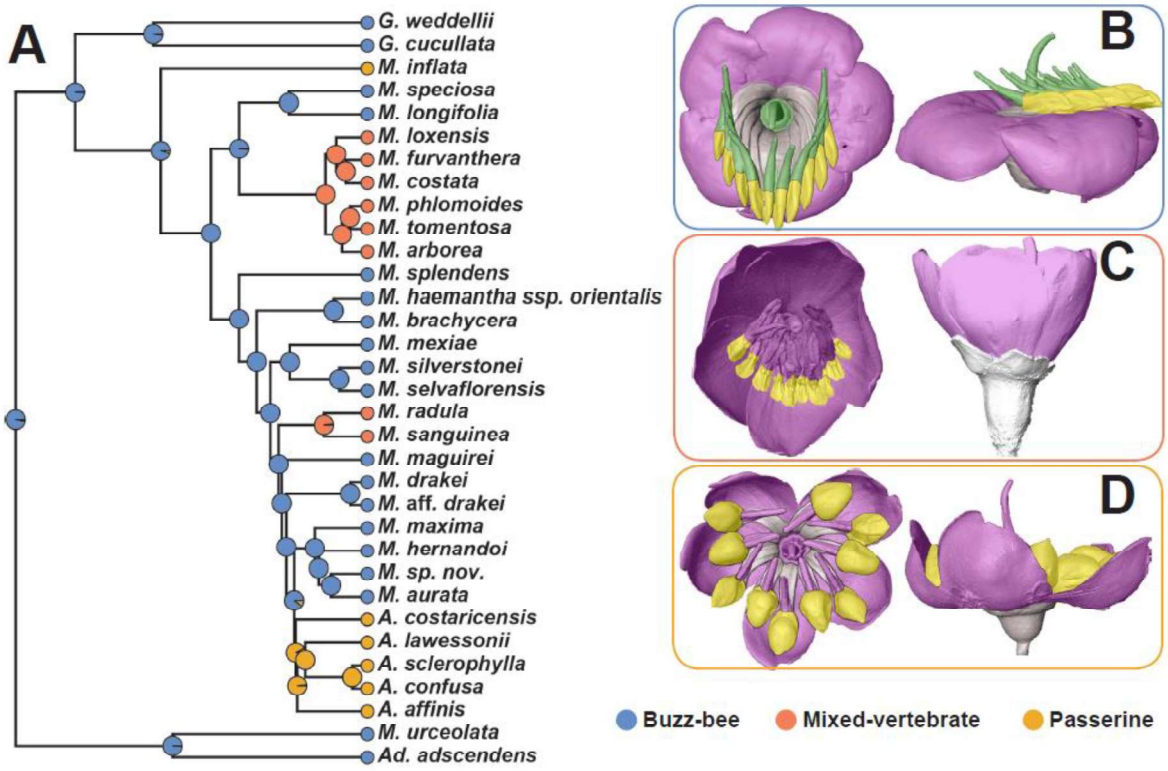

Figure 2. Reconstruction of ancestral pollination syndromes in Merianieae and best-fit modularity hypothesis for each pollination syndrome. (A) MCC-tree of Merianieae with ancestral reconstruction of pollination syndromes showing that 'buzz-bee' pollination is ancestral and that both the 'mixed-vertebrate' and the 'passerine' syndrome each evolved twice independently. (B) 'Buzz-bee' pollination syndrome flower of *Meriania hernandoi* with modularity hypothesis 5 (module 1: corolla in purple, module 2: appendages in yellow, module 3: pores/stigma in green). (C) 'Mixed-vertebrate' syndrome flower of *M. tomentosa* with modularity hypothesis 4 (module 1: corolla in purple, pore/stigma, module 2: appendages in yellow). (D) 'Passerine' syndrome flower of *Axinaea costaricensis* with modularity hypothesis 4 colour-coded as in (D).

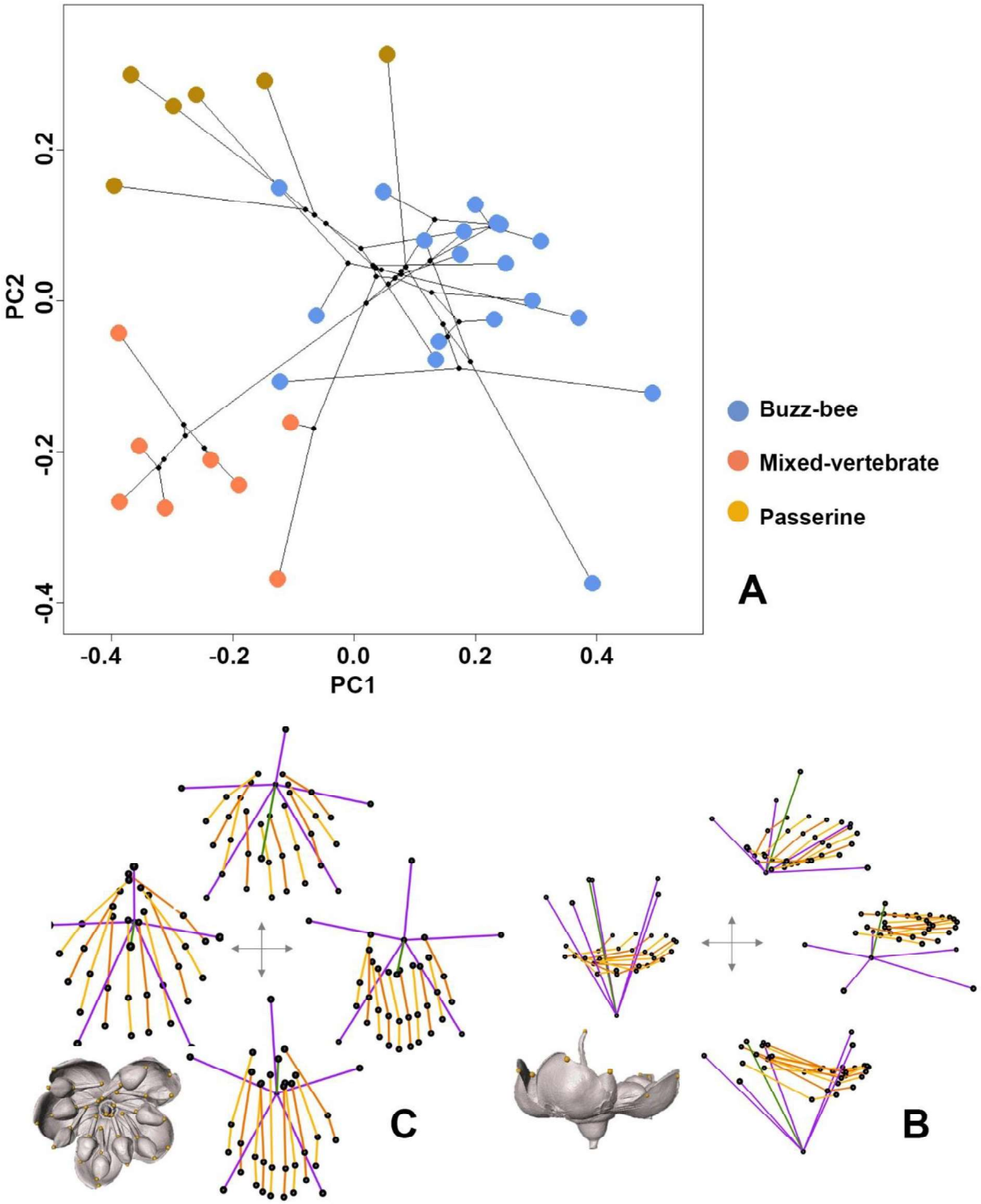

Figure 3. Phylomorphospace of Merianieae and floral shape change on PC1 and PC2. (A) PCA of mean flower shape of 33 Merianieae species with the three pollination syndromes occupying different areas of shape space. (B) Flower shape change (lateral view) along PC1 and PC2, visualized by wireframes. (C) Flower shape change (frontal view) along PC1 and PC2, visualized by wireframes. HRX-CT scanned flower of *A. costaricensis* is shown to facilitate interpretation of wireframes. Wireframe colouration follows floral organ categories (Hyp. 1): purple – petals, yellow/orange – the two different stamen whorls, green – gynoecium (as in Fig. 1B).

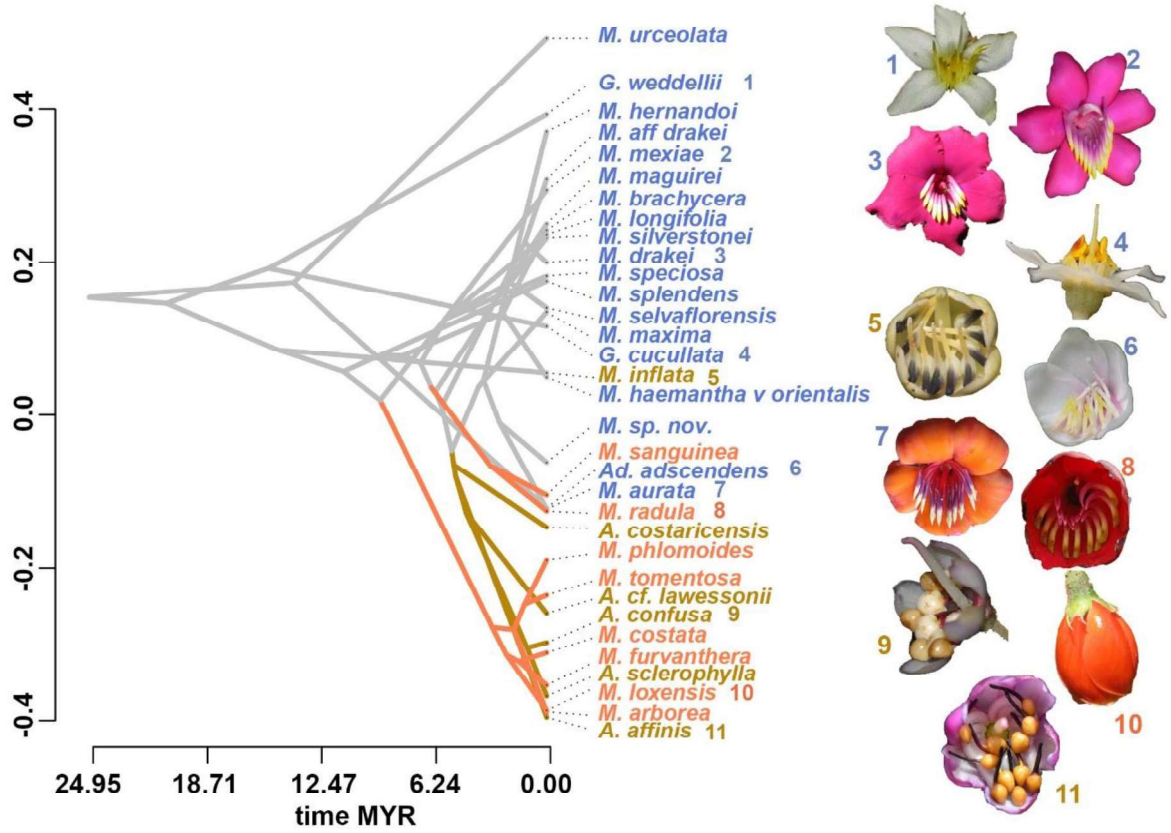

Figure 4. Traitgram showing floral shape evolution as summarized by PC1 through 24.95 million years. The three coloured lineages show significant shifts in floral phenotypic optima as estimated by Ornstein-Uhlenbeck models; grey branches indicated lineages that remained within the same phenotypic optimum (adaptive plateau). To indicate pollination syndromes of extant taxa, species names and flower image numbers are coloured as follows: 'buzz-bee' – blue, 'mixed-vertebrate' – salmon, 'passerine' – ocher. Flowers of extant taxa exemplify Merianieae floral diversity: 1) *Graffenrieda weddellii*, 2) *Meriania mexieae*, 3) *M. drakei*, 4) *G. cucullata*, 5) *M. inflata*, 6) *Adelobotrys adscendens*, 7) *M. aurata*, 8) *M. radula*, 9) *Axinaea confusa*, 10) *M. loxensis*, 11) *A. affinis*.

Table 1. Results from the five different hypotheses on modularity (Fig. 3) for the three pollination syndromes. Highest degrees of modularity are present in the 'buzz-bee' syndrome and lowest in the 'mixed-vertebrate' syndrome. Analyses of evolutionary modularity accounting for phylogenetic relatedness (column "Merianieae") show significant modularity in Hyp. 3, 4 and 5. CR – Covariance Ratio, p – p-value <0.05 indicates significantly smaller CR than expected when no modularity is present, Z – effect sizes of CR.

modularity	buzz-bee			mixed-vertebrate			passerine			Merianieae	
hypothesis	CR	p	Z	CR	p	Z	CR	p	Z	CR	p
Hyp. 1	0.860	*0.002*	2.000	1.112	0.698	0.430	1.079	0.427	0.095	1.578	1.000
Hyp. 2	0.889	*0.019*	1.886	1.012	0.322	0.440	1.025	0.160	0.683	0.987	0.213
Hyp. 3	0.908	*0.014*	2.576	0.994	0.201	0.747	1.012	0.105	1.025	0.882	*0.004*
Hyp. 4	0.774	*0.001*	6.708	0.949	0.054	1.981	0.833	*0.001*	12.999	0.950	*0.038*
Hyp. 5	0.804	*0.001*	3.877	0.978	0.080	1.486	0.918	*0.004*	4.337	0.934	*0.018*

Supplementary Information for

Is modularity the key to adaptive success? Testing hypotheses on modularity in flowers of Merianieae (Melastomataceae)

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This PDF file includes:

Other supplementary materials for this manuscript include the following:

Supplementary Information Text

1. Methods

1.1. Landmark placement

37 landmarks were selected under the criteria of homology and repeatability (ability to accurately locate homologous landmark position in different specimens) to capture patterns of floral shape variation in the three different pollination syndromes. Landmarks were placed as follows: five on the typical notch on the petal tips, one at the base of the style (on top of the syncarpous ovary, not visible in Figure 1a), ten on the stamen appendage tip, ten on the base of the stamen appendages, ten on the anther pores, one on the stigma. All landmarks were placed by SA in order to minimize variation due to observer inconsistencies.

1.2. Estimation of missing landmarks

In 78 of the 147 specimens used for analyses, all landmarks could be placed accurately without problems. The remaining 69 specimens showed minor damages due to handling and transport or damage by herbivores or pollen thieves (e.g. broken tip of one petal, broken style tip, broken stamen or stamen tip chewed up by *Trigona* bees (pollen thieves)) so that one to maximally ten landmarks could not be placed. Most geometric morphometric analyses require the placement of exactly the same number of homologous landmarks in all specimens and are intolerant of missing data (Arbour & Brown 2014). Our dataset includes a number of rare taxa collected at sites with difficult access all over South America and excluding those from our analyses would have greatly reduced the breadth (in terms of taxonomic and morphological diversity) of our study. We thus chose to estimate missing landmarks for the 69 specimens in questions, following methods developed by Arbour & Brown (2014). For these specimens, the missing landmarks were estimated by four different landmark estimation techniques (Baysian PCA (BPCA), mean substitution (MS), thin-plate spline interpolation (TPS) and least-squares regression (REG)) using the R-package 'LOST' (see Arbour & Brown (2014) for a thorough comparison of estimation techniques; J. Arbour provided updated R scripts to run TPS in 3D, currently not implemented in 'LOST'). To improve estimation accuracy, missing landmarks were only estimated from specimens most similar to the specimen for which landmarks should be estimated (Neeser et al. 2009). Thus, the dataset of the 78 intact specimens was divided into six subsets for estimation (first column Table S2). For each of the subsets, a test run was performed by randomly removing one to ten landmarks in one individual 50 times and estimating the missing landmarks. Each estimated set was Procrustes fitted, a PCA was performed and using the function protest() from the R-package 'vegan', PCA-coordinates (first two axes) of the estimated subset and the intact subset were compared to test if the estimation procedure significantly altered relative morphospace occupation patterns. In addition, T- and F-tests were used to test for significant alteration of each landmark position between the estimated and the intact set in all 50 runs. All estimation techniques gave PCA results that were significantly correlated to the respective intact subset but the four techniques differed in the quality of single landmark estimation (Table S3) with MS and REG performing worst. TPS was chosen as method to estimate landmarks in all 69 specimens. In order to keep possible errors due to

missing data small, each specimen with missing data was estimated separately with its respective subset.

1.3. Notes on flower symmetry

Although Merianieae flowers appear symmetric by the androecium (bilateral symmetry) on first glance, symmetry types are not straight forward (Savriami & Klingenberg 2011). Petals present rotational symmetry, while symmetry in the androecium is more complex. Moderate (difference in filament length between stamen whorls) to pronounced (two distinct sets of stamens) heteranthery is present in most species. Thus, the first stamen on the left side is not necessarily a symmetric copy of the last stamen on the right side. We thus refrained from employing procedures commonly usind in geometric morphometrics to remove effects of symmetry from the data.

1.4. Procrustes fitting and shape space calculation

All data analyses were performed in R (R Core Team 2018). Generalized Procrustes superimposition of landmarks was performed in GEOMORPH (Adams & Otárola-Castillo 2013) to remove variation in position, orientation and size (e.g. Bookstein 1991, Mitteroecker & Gunz 2009). The mean shape of each species was calculated and shape space visualized by Principal Component Analyses (PCA). In addition, phylomorphospaces were calculated using the *phylomorphospace* function in PHYTOOLS. Shape change along PC1 and PC2 was visualized by wireframes based on codes from http://rgriff23.github.io/2017/11/10/ plotting-shape-changes-geomorph.html. To incorporate aspects of intraspecific variability, 100 resample datasets were constructed where species with more than one specimen available were resampled at random and results were compared with results from analyses on mean shape.

1.5. Testing hypotheses on modularity

We explored five different hypotheses on floral modularity (Figure 1, Table S4) to understand whether pollinator shifts disrupted modularity patterns as could be expected under the pollinator-shifts model. Hypothesis 1 makes no assumption on floral functions but splits the flower into its developmental units, the petals, the two separate stamen whorls, and the style. Hypotheses 2-4 are based on the literature and are based on flower organ functioning. While hypothesis 2 (Fig. 1) partitions the flower into the petals vs sexual organs (Fornoni et al. 2016), hypotheses 3 and 4 distinguish between ‘attraction’ and ‘efficiency’ function traits (Diggle 2014). As the delimitation of these two functions can be difficult, two alternative hypotheses have been designed, Hyp. 3 classifying petals and appendages into the ‘attraction’ function and Hyp. 4 only classifying appendages into the ‘attraction’ function, while the petals are allocated to the ‘efficiency’ function. Finally, hypothesis 5 is based on specific trait functioning in Merianieae and partitions the flower into three modules: the corolla as landing platform (‘buzz-bee’ syndrome) or guide for bills (‘mixed-vertebrate’ and ‘passerine’ syndrome), the stamen appendages as triggers for pollen expulsion mechanisms (in ‘buzz-bee’ and ‘passerine’ syndrome), and the pore/stigma complex as unit of pollen placement and pickup.

The covariance ratio (CR) was chosen as a metric to test the five modularity hypotheses as it generates robust results even with small and variable sample sizes (Adams 2016). The CR-metric determines the degree of modularity between pre-defined modules (from

our Hyp. 1-5) and estimates if they are significantly more modular than when landmarks are randomly re-assigned to modules (null-hypothesis of random trait association). The CR-coefficient ranges between 0 and positive values, smaller values indicate less covariation between partitions of data and hence modularity. Testing of the five modularity hypotheses was done for each pollination syndrome separately but on joint Procrustes fitted landmark coordinates using the function *test.modularity* (GEOMORPH). 1000 random permutations were used to evaluate the statistical significance of the observed CR-coefficient. The CR-coefficients calculated on the mean shape per species were compared against the CR-coefficients of the 100 randomly resampled datasets to incorporate intraspecific variation.

1.6. Evaluating the strength of modularity between syndromes. Summary measures of trait correlation are sensitive to various attributes of the data and hence cannot be readily compared between different groups (Adams 2016, Bookstein 2016) such as, for instance, the three different pollination syndromes considered here. Adams & Collyer (2016) proposed the "z-score" as a standardized test statistic for the rPLS (Partial Least Squares correlation coefficient) where the rPLS is scaled by its permutation-based sampling distribution ("effect size" of the rPLS is calculated as standard deviates for the permuted samples). Calculating the effect size of the difference between two rPLS effect sizes allows for direct comparison of the strength of morphological integration across datasets (Adams & Collyer 2016). We extended this approach for the CR-coefficient in order to statistically evaluate the strengths of modularity between the three different pollination syndromes. Two-sample tests were performed to assess if levels of modularity differed significantly between pollination syndromes.

1.7. Assessing evolutionary floral modularity. In order to understand if detected floral modules represent relatively independent units also in an evolutionary context, we tested the five different modularity hypotheses across the Merianieae phylogeny. The CR-coefficient was calculated for all species together while accounting for phylogenetic relatedness using the function *phylo.modularity* (GEOMORPH).

1.8. Selecting the best-fit hypothesis of floral modularity. The approaches outlined above allow for detection of modularity and an evaluation of the strength of modularity between the different pollination syndromes. However, they do not permit conclusions on which modularity hypothesis fits the data best. We thus used the maximum-likelihood approach proposed by Goswami & Finarelli (2016) to assess the fit of the five competing hypotheses. First, vector congruence coefficient correlation matrices were calculated on the Procrustes fitted landmark coordinates for each pollination syndrome separately, resulting in three 37x37 element matrices (Goswami 2006) using the *dotcorr* function (PALEOMORPH; Lucas & Goswami 2017). We then ran the function *EMMLi* (EMMLi; Goswami & Finarelli 2016) to detect the best fitting model for each pollination syndrome by comparing the finite-sample corrected Akaike Information Criterion (AICc). EMMLi allows for complex models with different correlation coefficients between and within hypothesized modules, so that a total of 15 different models were tested, including a model of no modularity. The same procedure was repeated for all species together to assess the best-fit modularity hypotheses across Merianieae.

1.9. Flower shape evolution. We calculated phylogenetic signal in flower shape on the landmark data by the K_{mult} statistic, which is an extension of Blomberg's Kappa statistic and designed for multivariate data (Blomberg et al. 2003, Adams et al. 2014). We then assessed the fit of four different evolutionary models (Brownian motion (BM), Lambda, Early Burst (EB), Ornstein-Uhlenbeck (OU)) to the landmark data using the newly developed penalized likelihood framework for highly multivariate datasets (Clavel et al. 2018). Based on the clear clustering of the three different pollination syndromes in shape space as assessed by PCA, we used PC1 and PC2 to visualize flower shape change on the phylogeny by constructing a traitgram (PHYTOOLS). We then modelled trait evolution (PC1-2) under an Ornstein-Uhlenbeck (OU) process (Hansen 1997) to screen for different phenotypic optima within Merianieae using the *l1ou* R-package (Khabbazian et al. 2016). We used a LASSO (Least Absolute Shrinkage and Selection Operator) procedure (Tibshirani 1996) to estimate shifts in phenotypic optima from the data without an a-priori definition of where regime shifts may have occurred (*estimate_shift_configuration* function, "estimated shifts-model"). Convergence of these shifts was then evaluated using the *estimate_convergent_regimes* function (L1OU*)*. We evaluated model fit using the phylogenetic Bayesian information criterion (pBIC) and calculated weights (*aicw* from GEIGER, Pennell et al. 2014).

Finally, morphospace evolution through time was reconstructed on PC1 and PC2 using the *evomorphospace* function (EVOMAP, Smears & Mongle 2018). Ancestral character estimation was done for PC1 and PC2 (ace, method "REML", APE) and pollination syndromes were painted onto branches according to the estimation of ancestral pollination systems (Fig. 1a).

1.10. Phylogeny and Dating. Bayesian analyses were performed in BEAST2 (v2.5.0) (Drummond & Bouckaert 2014), as implemented through the CIPRES portal (http://www.phylo.org/; Miller & al., 2010). The best partition scheme was determined with PartitionFinder 2 (Lanfear et al. 2016), using each loci as a separate probable partition, and in the case of the three coding genes, also allowing for each of the three codon positions to be considered a partition. A seven partition scheme was found to be the best fit for the data (each locus as an independent partition, and in the case of ndhF, first codon position separate from second and third position). Each partition was assigned the GTR+ Γ + i model of sequence evolution and the partitions were unlinked. Rate variation across branches was set as uncorrelated and log-normally distributed, and with tree prior set to the Yule process. Based on previous analyses across the Melastomataceae, calibrated with fossils across the Myrtales, we fixed the age of the Merianieae at 29.25 MY. (Michelangeli et al. Unpublished). We ran three independent analyses of 60 million generations each, sampling every 20,000 generations with a 20 % burn-in. Convergence was assessed using Tracer v.1.6 (Rambaut et al., 2014), and runs were considered satisfactory with effective sample size (ESS) values greater than 200. The stable posterior distributions of the independent runs were combined using LogCombiner v2.5.0 (Rambout & Drummond, 2018a) and a maximum clade credibility tree summarized with TreeAnnotator v2.5.0 (Rambout & Drummond, 2018b).

Supplementary Figures

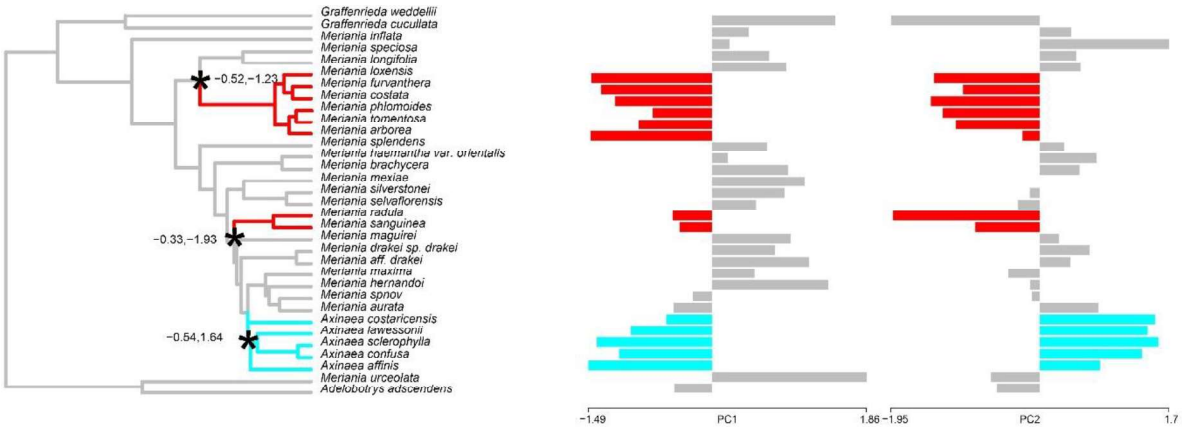


Figure S1. The three estimated shifts in phenotypic optima assessed on PC1 and PC2 of Merianieae floral shape space on the MCC-tree. Significant shifts are indicated by asterisks and represent three of the four transitions from 'buzz-bee' to vertebrate pollination. The red colouration of two shifts indicates convergence in floral shape within the 'mixed-vertebrate' syndrome, the blue colouration indicates shift to the 'passerine' syndrome. Regime shifts were evaluated on PC1 and PC2, PC1 summarizes corolla shape from reflexed ('buzz-bee') to urceolate or pseudo-campanulate corollas in the two vertebrate pollination syndromes. PC2 summarizes stamen arrangement, reflexed in the 'mixed-vertebrate' syndrome and bent-in in the 'passerine' syndrome; this difference is illustrated by the different PC2 values between the two shifted syndromes.

Supplementary Tables

Methods

Table S1. Merianieae species used for study on flower evolution; details on sampling localities, voucher information, number of flowers used for HRX-CT scanning from each locality and pollination syndromes ('buzz-bee', 'mixed vertebrate', 'passerine') are given. For species with known pollinators, references for pollinator observations are given in brackets.

species	collection number	collector	country	state/province	collection date	voucher	no. of flowers	pollinator
Adelobotrys adscendens	FA10230	Frank Alemda	Colombia	Valle del Cauca	04.02.2011	CAS 1120080	9	buzz-bee (Dellinger et al. 2018)
Adelobotrys adscendens	RK5676	Ricardo Kriebel	Panamá	PN Chagres	14.09.2011	NYBG1653386	2	
Axinaea affinis	AD41	Agnes Dellinger	Ecuador	Azuay	29.11.2012	-	1	passerine
Axinaea cf. *lawessonii*	AD112	Agnes Dellinger	Ecuador	Loja, PN Podocarpus	11.09.2016	WU 0092822	1	passerine
Axinaea confusa	AD136	Agnes Dellinger	Ecuador	Loja, PN Podocarpus	18.09.2016	WU 0092808	10	passerine (Dellinger et al. 2018)
Axinaea costaricensis	AD75	Agnes Dellinger	Costa Rica	San José, highway to Cerro de la Muerte	02.02.2015	WU	19	passerine (Dellinger et al. 2018)
Axinaea costaricensis	AD74	Agnes Dellinger	Costa Rica	San José, Cerros de Escazú	12.03.2015	WU	11	
Axinaea sclerophylla	AD109	Agnes Dellinger	Ecuador	Loja, PN Podocarpus	10.09.2016	WU 0092819	1	passerine
Graffenrieda cucullata	AD159	Agnes Dellinger	Ecuador	Pastaza, along path to EcoMinga	16.11.2016	QCNE	4	buzz-bee (Dellinger et al. 2018)
Graffenrieda weddellii	MA1503	Marcela Alvear	Colombia	Risaralda	05.01.2014	CAS 1155724	1	buzz-bee
Meriania arborea	FA10564	Frank Alemda	Colombia	Norte de Santander	28.02.2012	CAS 1128115	1	mixed-vertebrate

Meriania aff. drakei	AD140	Agnes Dellinger	Ecuador	Pastaza, Reserva EcoMinga	13.11.2016	QCNE	4	buzz-bee
Meriania aff. drakei	AD141	Agnes Dellinger	Ecuador	Pastaza, Reserva EcoMinga	13.11.2016	QCNE	3	buzz-bee
Meriania aff. drakei	AD141B	Agnes Dellinger	Ecuador	Pastaza, Reserva EcoMinga	13.11.2016	QCNE	2	buzz-bee
Meriania aff. drakei	AD152	Agnes Dellinger	Ecuador	Pastaza, Reserva EcoMinga	15.11.2016	WU 0092853	2	buzz-bee
Meriania aurata	AD145	Agnes Dellinger	Ecuador	Pastaza, Reserva EcoMinga	13.11.2016	QCNE	2	buzz-bee
Meriania brachycera	FA10593	Frank Alemda	Colombia	Norte de Santander	26.02.2012	CAS 1127903	1	buzz-bee
Meriania costata	AD106	Agnes Dellinger	Ecuador	Loja, PN Podocarpus	10.09.2016	WU 0092833	4	mixed-vertebrate (Dellinger et al. 2018)
Meriania drakei	AD132	Agnes Dellinger	Ecuador	Napo, Cosanga along roadside	14.10.2016	WU 0092810	8	buzz-bee (Dellinger et al. buzz)
Meriania furvanthera	AD23	Agnes Dellinger	Ecuador	Loja, El Tiro	20.10.2012	WU 0072421	1	mixed-vertebrate (Dellinger et al. 2018)
Meriania furvanthera	AD124	Agnes Dellinger	Ecuador	Loja, PN Podocarpus	13.09.2016	WU 0092838	3	
Meriania haemantha ssp. orientalis	FA10651	Frank Alemda	Colombia	Santander	11.03.2012	CAS 1128063	1	buzz-bee
Meriania hernandoi	AD131	Agnes Dellinger	Ecuador	Napo, Cosanga along roadside	14.10.2016	WU 0092801	8	buzz-bee (Dellinger et al. 2018)
Meriania inflata	RG2078	Renato Goldenberg	Brazil	Bahia	13.10.2014	NYBG02286571	1	passerine
Meriania longifolia	FA10536	Frank Alemda	Colombia	Norte de Santander	25.02.2012	CAS 1127902	1	buzz-bee (Renner 1989)
Meriania loxensis	AD115	Agnes Dellinger	Ecuador	Loja, PN Podocarpus	12.09.2016	QCNE	3	mixed-vertebrate
Meriania maguirei	AD110	Agnes Dellinger	Ecuador	Loja	11.09.2016	QCNE	4	buzz-bee (Dellinger et al. 2018)

Meriania maxima	MA1768	Marcela Alvear	Colombia	Narino	16.02.2013	CAS 1155921	4	buzz-bee
Meriania maxima	AD104	Agnes Dellinger	Ecuador	Pichincha, Reserva Bellavista	08.09.2016	WU 0092813	1	(Dellinger et al. 2018)
Meriania mexiae	MA1853	Marcela Alvear	Colombia	Putumayo	16.02.2013	CAS 1156500	1	buzz-bee
Meriania phlomoides	AD78	Agnes Dellinger	Costa Rica	Heredia, Cerro Dantas	07.03.2015	WU 583185	6	mixed-vertebrate
Meriania phlomoides	AD76	Agnes Dellinger	Costa Rica	Puntarenas, Monteverde	22.02.2015	WU	4	(Dellinger et al. 2018)
Meriania radula	AD15	Agnes Dellinger	Ecuador	Loja, PN Podocarpus	11.10.2012	WU 0072420	1	mixed-vertebrate
Meriania sanguinea	AD108	Agnes Dellinger	Ecuador	Loja, PN Podocarpus	10.09.2016	WU 0092832	9	mixed-vertebrate (Dellinger et al. 2018)
Meriania selvaflorensis	MA1465	Marcela Alvear	Colombia	Caldas	02.03.2011	CAS 1119760	1	buzz-bee
Meriania silverstonei	FA10210	Frank Alemda	Colombia	Valle del Cauca	01.02.2011	CAS 1120063	1	buzz-bee
Meriania speciosa	FA10219	Frank Alemda	Colombia	Valle del Cauca	02.02.2011	CAS 1119942	1	buzz-bee
Meriania splendens	MA1690	Marcela Alvear	Colombia	Narino	25.01.2013	CAS 1156411	1	buzz-bee
Meriania sp. *nov*	AD158	Agnes Dellinger	Ecuador	Pastaza, Reserva EcoMinga	15.11.2016	WU 0092855	1	buzz-bee
Meriania tomentosa	AD30	Agnes Dellinger	Ecuador	Loja, PN Podocarpus	30.10.2012	WU 0072422	1	mixed-vertebrate (Vogel 1997, Dellinger et al. 2018)
Meriania tomentosa	AD105	Agnes Dellinger	Ecuador	Pichincha, Reserva Bellavista	08.09.2016	WU 0092814	4	mixed-vertebrate (Vogel 1997, Dellinger et al. 2018)
Meriania tomentosa	AD111	Agnes Dellinger	Ecuador	Loja, PN Podocarpus	11.09.2016	QCNE	2	mixed-vertebrate (Vogel 1997,

									Dellinger et al. 2018)
Meriania urceolata	KR1446	Karen Redden	Guyana	Cuyuni-Mazaruni	08.12.2002	NY02513392	1	buzz-bee	

Table S2: Number of intact and estimated specimens of each subset of specimens used for estimation and for each pollination syndrome.

subset for estimation	no intact specimens	no estimated	total no subset	pollination syndrome	total no syndrome
large flowered *Meriania*	12	36	48	buzz-bee	64
Adelobotrys sp.	4	7	11	buzz-bee	
Graffenrieda sp.	3	2	5	buzz-bee	
M. tomentosa group	16	13	29	mixed-vertebrate	39
M. sanguinea group	9	1	10	mixed-vertebrate	
Axinaea sp., M. inflata	34	10	44	passerine	44

Table S3: Proportion of simulations where one or more landmarks differed significantly between the estimated and the intact set (T-test/F-test) for the four different estimation techniques. TPS was chosen to estimate landmarks in specimens with missing data.

subset for estimation	BPCA	MS	REG	TPS
large flowered *Meriania*	0/0.03	0/0.65	0/0.01	0/0.04
Adelobotrys adscendens	0/0.22	0.01/0.38	-	0/0.15
Graffenrieda sp.	0.27/0.24	0.46/0.41	-	0.16/0.19
Meriania tomentosa group	0/0.01	0/0.87	0/0.01	0/0.01
Meriania sanguinea group	0/0.13	0/0.69	0/0.31	0/0
Axinaea sp., M. inflata	0/0	0/0.8	0/0	0/0

Table S4. Specification of the five hypotheses of modularity, landmark partitioning, source of modularity hypothesis and expectations for the three different pollination syndromes in Merianieae.

Hypotheses	Landmark partitioning	source	expectations for pollination syndromes
Hyp 1: *Developmental units*: corolla (M1), outer stamen whorl (M2), inner stamen whorl (M3), style (M4)	M1: 33-37; M2: 1,3,5,7,9,11,13, 15,17,19,21,23,25,27,29; M3: 2,4,6,8,10,12,14,16,18,20,22,24,26,28,30; M4: 31,32	Esteve-Altava 2016	no expectation
Hyp 2: *Corolla* (M1) versus *reproductive organs* (M2)	M1: 31, 33-37 M2: 1-30, 32	Fornoni et al. 2015; Esteve-Altava 2016	*modularity of corolla as an attraction trait in all systems*
Hyp 3: Attraction (*corolla and stamen appendages*, M1) versus efficiency (*pore locations and stigma,* M2)	M1: 1-20, 31, 33-37 M2: 21-30, 32	Diggle 2014	*modularity in 'buzz-bee' syndrome* (corolla as landing platform and stamens as handles for buzzing) but not in 'mixed-vertebrate' and 'passerine'
Hyp 4: Attraction *(appendages,* M1) versus efficiency (*pore location, stigma and corolla*, M2)	M1: 1-20, M2: 21-37	Diggle 2014	*modularity in 'passerine' syndrome* (corolla mediates fit, appendages trigger bellows) but not in 'buzz-bee' and 'mixed-vertebrate' syndrome
Hyp 5: *Merianieae specific functional modules*: attraction *(corolla,* M1) versus pollen expulsion (*stamen appendages*, M2) versus pollen transfer (*pore locations and stigma*, M3)	M1: 31, 33-37 M2: 1-20 M3: 21-30, 32	this study	*modularity in 'buzz-bee' and 'passerine' syndrome* (appendages as triggers for pollen expulsion mechanisms) but not 'mixed-vertebrate' syndrome

Supplementary Results

Table S5. Pairwise comparison of effect sizes of the five hypotheses on floral modularity for the three different pollination syndromes. The lower off-diagonal values represent the pairwise differences in z-scores (effect sizes) of the CR coefficient, the upper off-diagonal gives their associated p-values (i.e. p<0.05 indicating a significant difference in modularity; significant p-values printed in italics).

Hyp. 1	buzz-bee	mixed-vertebrate	passerine
buzz-bee		*0.024*	*0.043*
mixed-vertebrate	1.979		0.358
passerine	1.719	0.364	
Hyp. 2	**buzz-bee**	**mixed-vertebrate**	**passerine**
buzz-bee		0.067	0.073
mixed-vertebrate	1.498		0.451
passerine	1.456	0.123	
Hyp. 3	**buzz-bee**	**mixed-vertebrate**	**passerine**
buzz-bee		*0.042*	*0.026*
mixed-vertebrate	1.728		0.477
passerine	1.942	0.057	
Hyp. 4	**buzz-bee**	**mixed-vertebrate**	**passerine**
buzz-bee		*<0.001*	*0.049*
mixed-vertebrate	3.873		*<0.001*
passerine	1.658	3.567	
Hyp. 5	**buzz-bee**	**mixed-vertebrate**	**passerine**
buzz-bee		*0.004*	*0.042*
mixed-vertebrate	2.663		0.055
passerine	1.728	1.596	

Table S6. Results from the five different hypotheses on modularity (Fig. 3) for the three pollination syndromes with the resampled dataset. Highest modularity was found in the 'buzz-bee' syndrome and lowest modularity in the 'mixed-vertebrate' syndrome. In contrast to analyses on the mean shape, evolutionary modularity accounting for phylogenetic relatedness (column "Merianieae") was only found in Hyp. 2 in the resampled dataset. CR – Covariance Ratio, p – p-value <0.05 indicates significantly smaller CR than expected when no modularity is present, Z – effect sizes of CR.

modularity	buzz-bee			mixed-vertebrate			passerine			Merianieae	
hypothesis	CR	p	Z	CR	p	Z	CR	p	Z	CR	p
Hyp. 1	0.857	*0.003*	2.185	1.131	0.789	1.016	1.059	0.37	0.330	1.579	0.996

Hyp. 2	0.898	*0.034*	1.819	1.009	0.354	0.502	1.014	0.192	0.678	1.002	0.285
Hyp. 3	0.915	*0.022*	2.519	0.987	0.233	0.87	0.994	0.112	1.39	0.876	*0.015*
Hyp. 4	0.801	*0.001*	6.19	0.931	0.065	2.365	0.813	*0.001*	11.522	0.960	0.078
Hyp. 5	0.824	*0.001*	3.690	0.961	0.085	1.687	0.903	*0.003*	4.449	0.937	0.051

Table S7. Model parameters and log-likelihood fits for the five hypotheses of modularity (Hyp1-5) and a hypothesis of no modularity for all Merianieae species of this study (n=33) and the different pollination syndromes separately. The optimal model for each dataset is highlighted in bold.

Merianieae	LogL	K	n	AICc	dAICc	Model LogL	Model Posterior Probability
Null modularity	676.5	2	666	-1349.1	93.0	0.000	0.000
Hyp1.same.Mod + same.between	680.2	3	666	-1354.4	87.7	0.000	0.000
Hyp1.sep.Mod + same.between	689.9	6	666	-1367.7	74.3	0.000	0.000
Hyp1.same.Mod + sep.between	690.1	8	666	-1364.0	78.1	0.000	0.000
Hyp1.sep.Mod + sep.between	699.8	11	666	-1377.2	64.8	0.000	0.000
Hyp2.same.Mod + same.between	678.8	3	666	-1351.6	90.4	0.000	0.000
Hyp2.sep.Mod + same.between	684.5	4	666	-1361.0	81.0	0.000	0.000
Hyp3.same.Mod + same.between	715.9	3	666	-1425.7	16.4	0.000	0.000
Hyp3.sep.Mod + same.between	719.5	4	666	-1430.9	11.1	0.004	0.003
Hyp4.same.Mod + same.between	702.2	3	666	-1398.3	43.7	0.000	0.000
Hyp4.sep.Mod + same.between	703.3	4	666	-1398.6	43.4	0.000	0.000
Hyp5.same.Mod + same.between	718.7	3	666	-1431.3	10.7	0.005	0.004
Hyp5.sep.Mod + same.between	722.1	5	666	-1434.1	8.0	0.019	0.015
Hyp5.same.Mod + sep.between	724.7	5	666	-1439.3	2.8	0.252	0.197
Hyp5.sep.Mod + sep.between	**728.1**	**7**	**666**	**-1442.0**	**0.0**	**1.000**	**0.781**
'buzz-bee' syndrome							
Null modularity	667.9	2	666	-1331.7	29.0	0.000	0.000
Hyp1.same.Mod + same.between	672.0	3	666	-1338.0	22.7	0.000	0.000
Hyp1.sep.Mod + same.between	673.5	6	666	-1334.8	25.9	0.000	0.000
Hyp1.same.Mod + sep.between	680.9	8	666	-1345.7	15.0	0.001	0.000
Hyp1.sep.Mod + sep.between	682.4	11	666	-1342.4	18.3	0.000	0.000
Hyp2.same.Mod + same.between	671.1	3	666	-1336.1	24.6	0.000	0.000
Hyp2.sep.Mod + same.between	671.6	4	666	-1335.0	25.7	0.000	0.000
Hyp3.same.Mod + same.between	673.9	3	666	-1341.7	19.0	0.000	0.000
Hyp3.sep.Mod + same.between	677.4	4	666	-1346.7	14.0	0.001	0.000
Hyp4.same.Mod + same.between	680.9	3	666	-1355.7	5.0	0.083	0.045
Hyp4.sep.Mod + same.between	680.9	4	666	-1353.8	6.9	0.032	0.017
Hyp5.same.Mod + same.between	**683.4**	**3**	**666**	**-1360.7**	**0.0**	**1.000**	**0.542**
Hyp5.sep.Mod + same.between	684.5	5	666	-1359.0	1.7	0.424	0.230
Hyp5.same.Mod + sep.between	683.9	5	666	-1357.7	3.1	0.216	0.117
Hyp5.sep.Mod + sep.between	685.0	7	666	-1355.9	4.8	0.090	0.049
'mixed-vertebrate' syndrome							
Null modularity	399.3	2	666	-794.6	8.8	0.012	0.004
Hyp1.same.Mod + same.between	399.3	3	666	-792.6	10.8	0.005	0.002

Hyp1.sep.Mod + same.between	399.9	6	666	-787.6	15.8	0.000	0.000
Hyp1.same.Mod + sep.between	402.7	8	666	-789.1	14.3	0.001	0.000
Hyp1.sep.Mod + sep.between	403.2	11	666	-784.0	19.4	0.000	0.000
Hyp2.same.Mod + same.between	400.9	3	666	-795.9	7.6	0.023	0.008
Hyp2.sep.Mod + same.between	401.0	4	666	-793.9	9.6	0.008	0.003
Hyp3.same.Mod + same.between	400.1	3	666	-794.2	9.2	0.010	0.004
Hyp3.sep.Mod + same.between	402.2	4	666	-796.4	7.0	0.030	0.011
Hyp4.same.Mod + same.between	**404.7**	**3**	**666**	**-803.4**	**0.0**	**1.000**	**0.362**
Hyp4.sep.Mod + same.between	404.7	4	666	-801.4	2.0	0.363	0.131
Hyp5.same.Mod + same.between	404.4	3	666	-802.7	0.7	0.711	0.257
Hyp5.sep.Mod + same.between	405.2	5	666	-800.3	3.1	0.214	0.077
Hyp5.same.Mod + sep.between	405.6	5	666	-801.0	2.4	0.299	0.108
Hyp5.sep.Mod + sep.between	406.4	7	666	-798.6	4.8	0.089	0.032
'passerine' syndrome							
Null modularity	294.3	2	666	-584.5	7.3	0.026	0.011
Hyp1.same.Mod + same.between	294.4	3	666	-582.8	9.0	0.011	0.005
Hyp1.sep.Mod + same.between	296.6	6	666	-581.1	10.7	0.005	0.002
Hyp1.same.Mod + sep.between	294.8	8	666	-573.3	18.6	0.000	0.000
Hyp1.sep.Mod + sep.between	297.0	11	666	-571.5	20.3	0.000	0.000
Hyp2.same.Mod + same.between	294.6	3	666	-583.2	8.7	0.013	0.006
Hyp2.sep.Mod + same.between	297.0	4	666	-586.0	5.9	0.053	0.023
Hyp3.same.Mod + same.between	294.8	3	666	-583.5	8.4	0.015	0.007
Hyp3.sep.Mod + same.between	295.1	4	666	-582.2	9.7	0.008	0.003
Hyp4.same.Mod + same.between	**299.0**	**3**	**666**	**-591.9**	**0.0**	**1.000**	**0.435**
Hyp4.sep.Mod + same.between	299.4	4	666	-590.8	1.1	0.576	0.251
Hyp5.same.Mod + same.between	297.3	3	666	-588.5	3.3	0.188	0.082
Hyp5.sep.Mod + same.between	299.0	5	666	-588.0	3.9	0.144	0.063
Hyp5.same.Mod + sep.between	299.1	5	666	-588.0	3.8	0.148	0.064
Hyp5.sep.Mod + sep.between	300.8	7	666	-587.5	4.4	0.112	0.049

K – Model parameters, LogL – raw log-likelihood fits for each model, AICc – finite sample corrected Akaike Information Criterion, dAICc – difference between lowest AICc and each respective AICc, Model LogL – Model log-likelihood.

Table S8. Best-fit model selection for estimation of ancestral pollination syndrome using ML methods. 'ER' – equal rates model; 'ARD' – all rates different model.

model	AIC	LogL	p
ER	40.28	-19,14	
ARD	49.12	-18,56	0.95

Table S9. Comparison of fit of four different models of trait evolution on landmark data as assessed by GIC; estimated parameter values are given.

model	GIC	parameter
Brownian motion	-17 047	-

lambda	-18 245	lambda 1e-5
early burst	-17 045	r 0
Ornstein-Uhlenbeck	**-18 326**	**alpha 10**

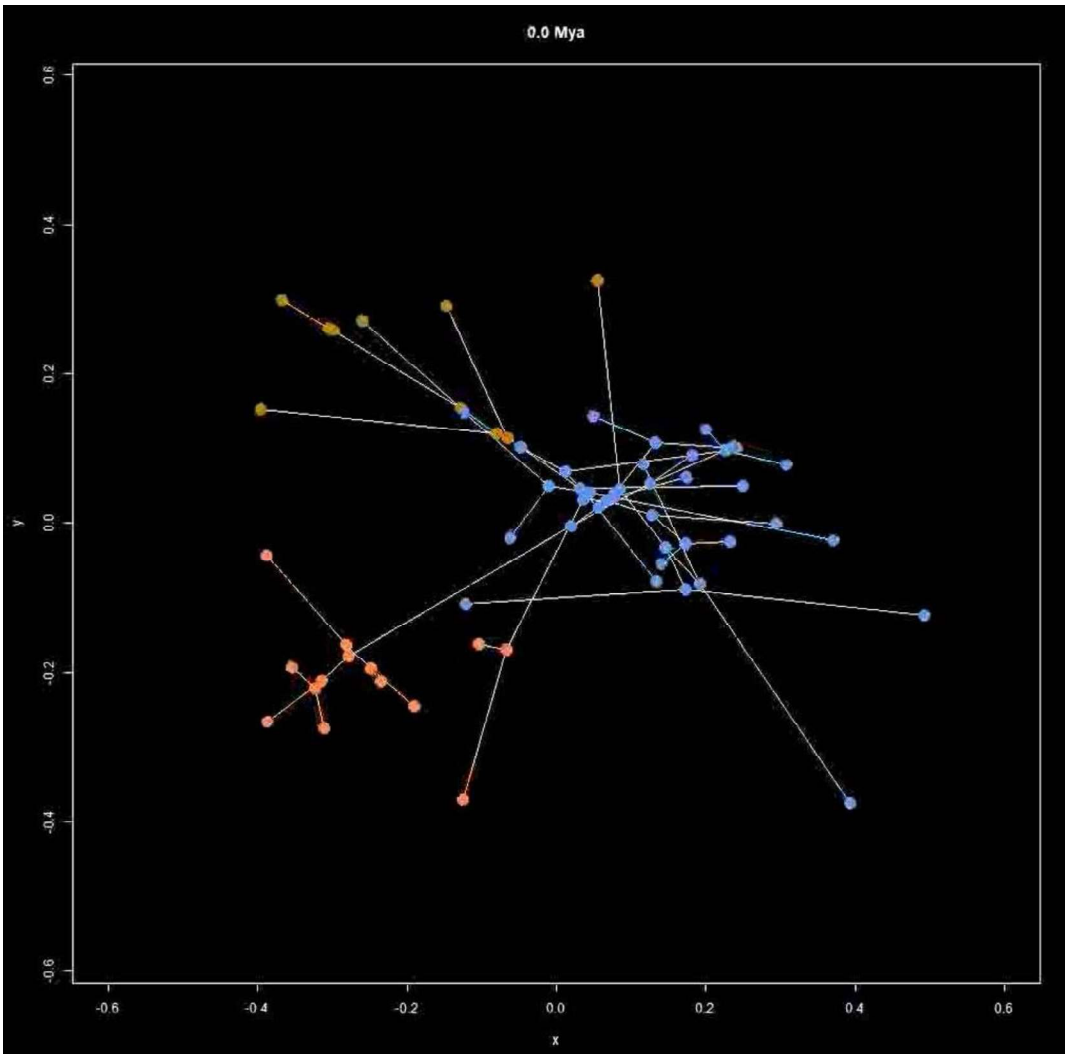
Table S10. Comparison of fit of the two different OU-models on PC1 and PC2 on shape means and best fit model for resampled trait datasets (% of best fit from 100 runs).

model	pBIC	difference pBIC	w	% best fit model
estimated shifts	-37.043	5.077	0.066	29
estimated convergence	**-42.120**	**0.000**	**0.838**	**66**

Table S11. Proportion of times a species was included in a regime shift for the resampled trait dataset. Note that with one exception (*M. inflata*) all species which have shifted pollination syndrome ('mixed-vertebrate' or 'passerine') were also found to have undergone a shift in phenotypic optimum as measured by PC1 and PC2.

species	% shift	pollination syndrome
Axinaea sclerophylla	100	passerine
Axinaea confusa	100	passerine
Axinaea cf. lawessonii	100	passerine
Axinaea affinis	100	passerine
Axinaea costaricensis	87	passerine
Meriania arborea	70	mixed-vertebrate
Meriania tomentosa	68	mixed-vertebrate
Meriania phlomoides	68	mixed-vertebrate
Meriania furvanthera	67	mixed-vertebrate
Meriania loxensis	66	mixed-vertebrate
Meriania costata	66	mixed-vertebrate
Meriania radula	62	mixed-vertebrate
Meriania sanguinea	51	mixed-vertebrate
Graffenrieda weddellii	30	buzz-bee
Meriania aurata	19	buzz-bee
Meriania sp. nov	17	buzz-bee
Graffenrieda cucullata	10	buzz-bee
Meriania maxima	5	buzz-bee
Meriania hernandoi	5	buzz-bee
Meriania aff. drakei	3	buzz-bee
Adelobotrys adscendens	3	buzz-bee
Meriania urceolata	2	buzz-bee
Meriania inflata	2	passerine

Meriania drakei	2	buzz-bee
Meriania splendens	1	buzz-bee
Meriania speciosa	1	buzz-bee
Meriania silverstonei	1	buzz-bee
Meriania selvaflorensis	1	buzz-bee
Meriania mexiae	1	buzz-bee
Meriania maguirei	1	buzz-bee
Meriania longifolia	1	buzz-bee
Meriania haemantha ssp. orientalis	1	buzz-bee
Meriania brachycera	1	buzz-bee



Movie S1. Phylomorphospace of Merianieae flower shape (PC1 and PC2) through evolutionary time. Note the stasis of nodes in the central to right area of shape space occupied by extant 'buzz-bee' syndrome species, while branches where shifts to the 'mixed-vertebrate' or 'passerine' syndrome have occurred explore new areas of flower shape space. Branches are coloured according to reconstructions of ancestral pollination systems ('buzz-bee' – blue, 'mixed-vertebrate' – salmon, 'passerine' – yellow).

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6. Concluding Discussion

The tremendous diversity of angiosperm flowers has primarily evolved in response to pollinator mediated selection (Specht & Bartlett 2009, Sauquet et al. 2017, Campbell et al. 2018), and further evidence of strong pollinator mediated selection is provided in this thesis. In Chapters I to III, I showed how pollinator shifts have affected floral traits in Merianieae and that the bee-vertebrate directionality of pollinator shifts seems to hold true also in this group (Cronk & Ojeda 2008). Despite criticism on pollination syndromes (e.g. Ollerton et al. 2009), my results demonstrate the utility and continued applicability of the concept for a broad understanding of trait functioning in response to pollinator mediated selection in a plant group. Pollination syndromes as a tool for structuring diversity in a group may be particularly useful when floral traits specific to the group in question are considered. I found reduced power of traditional pollination syndrome characters such as colour and scent in differentiating syndromes in Merianieae (Faegri & van der Pijl 1979). Traits specific to Merianieae, however, namely the pollen expulsion mechanism and the reward type, were powerful in correctly classifying species into syndromes. In cases where information on these traits was not available, combinations of other characters such as the relative position of the stigma and the corolla opening or the presence of filament ruptures helped to differentiate syndromes.

In my eyes, my results have important implications for the continued heuristic value of pollination syndromes. First, they serve as a tool to summarize multivariate floral trait responses to pollinators across speciose clades, for which pollinator observations in all species are not feasible. Many studies have focused on a limited set of traits to describe syndromes (Rosas-Guerrero et al. 2014), but, particularly when predicting pollinators, it may be advantageous to consider additional, group-specific traits. In particular, the impact of lineage history and the 'evolutionary starting point' need to be taken into account. In Merianieae, the specialized poricidal anther structure of the 'buzz-bee' syndrome (as starting point) may have prevented evolution towards more traditional syndromes such as tubular hummingbird flowers. Clearly, poricidal anthers have been retained throughout pollinator shifts and entailed the evolution of alternative mechanisms of pollen expulsion. Trends towards traditional syndromes, such as a change in corolla shape to more closed forms (canalizing pollinator access) in shifted syndrome species, are apparent as well, however.

Second, as suggested by Stebbins (1970), generalizations should not be made without detailed investigations of the actual pollination biology of at least some species of the group. The 'mixed-vertebrate' pollination syndrome, which I define in Chapter I and explore in more detail in Chapter II, exemplifies how misleading crude generalizations may be. Using Random Forest Analyses, I did not find any support for splitting the 'mixed-vertebrate' syndrome into the different combinations of diurnal and nocturnal pollinators (hummingbirds and bats or hummingbirds and rodents or flowerpiercers and rodents). Only extensive fieldwork made me understand that all five species (*M.* aff. *sanguinea, M. furvanthera, M. phlomoides, M. sanguinea, M. tomentosa*) that I had the opportunity to study both during day- and night-time actually were pollinated by two functional pollinator groups each. It seems likely that species where I have only performed pollinator observations during daytime so far (e.g. *M. radula*, *M. tetragona*, *M. costata*) are also visited by additional nocturnal pollinators. In this context, the two Cuban species (*M. albiflora, M. angustifolia*) could be particularly interesting. My analyses placed them into the 'mixed-vertebrate' syndrome, but their flowers exhibit strong heteranthery, a trait otherwise only found in the 'buzz-bee' and 'passerine' syndromes. Only fieldwork will clarify whether these species are possibly still visited by buzzing bees, in addition to vertebrates, or whether they have fully shifted towards vertebrate pollinators.

Generally, the 'mixed-vertebrate' syndrome is rather peculiar when viewed in traditional syndrome theory which assumes adaptation to a single most effective functional pollinator group (Faegri & van der Pijl 1979, Fenster et al. 2004). Syndrome theory does not negate the existence of secondary, albeit less efficient pollinator groups (Stebbins 1970, Rosas-Guerrero et al. 2014). However, syndromes are also usually only defined on a single functional pollinator group. Furthermore, functional groups are largely based on pollinator taxonomy (Robertson 1928, Fenster et al. 2004). Fenster et al.'s (2004) definition of functional groups as species behaving in 'similar ways on a flower', however, renders this concept a lot more flexible. Although flowerpiercers, hummingbirds, bats and rodents are generally viewed as different functional groups, they should be considered as a single 'nectar foraging' group in Merianieae. As demonstrated in Chapter II, these groups differ in their selection on certain floral traits such as nectar and scent given obvious differences in their sensory abilities and foraging preferences. These selection pressures are, however, not strong enough to move any of the investigated species out of the broad 'mixed-vertebrate' syndrome. Instead, the 'nectar

foraging' pollinator group is united by its shared interest in the nectar reward and capability of triggering the salt-shaker like pollen release mechanism. I propose that the 'mixed-vertebrate' syndrome may represent a series of stable bimodal pollination systems that are actually specialized on simultaneously exploiting two traditional functional pollinator groups such as hummingbirds and bats or hummingbirds and rodents, respectively.

Reports on truly bimodal systems are scarce in literature, a few examples come from South Africa (e.g. Iridaceae with butterflies and sunbirds, Manning & Goldblatt 2005) and South America (e.g. Bromeliaceae with hummingbirds and bees, Schmid et al. 2011). Detailed assessments of floral traits and pollen transfer efficiency are required to differentiate bimodal systems from systems with ancestral secondary pollinators, which may appear similar at first glance. In Merianieae, all functional pollinator groups clearly deposited large amounts of pollen on stigmas and nectar and scent showed mixed adaptations to either group. In their study on *Aphelandra acanthus*, Muchhala et al. (2008) showed that subtle differences, e.g. in the purity of deposited pollen, may exist between hummingbirds and bats visiting the same species. Such differences possibly also exist in Merianieae, despite clear adaptations to both pollinator groups. However, I do not rule out the existence of nectar secreting Merianieae species or populations visited by either only a diurnal or a nocturnal functional pollinator group. The discovery of such a species or population would enable me to tease apart effects of selection imposed by only one functional group as opposed to the outcome of the 'bimodal' selection.

Furthermore, it is important to differentiate bimodal systems from generalized pollination strategies, which usually involve more than just two functional pollinator groups. In the Melastomataceae tribe Miconieae (ca. 1500 sp), the evolution of nectar secretion in a few species has mostly led to generalization (Brito et al. 2016). These species have retained their open flower shape with easily accessible nectar and are visited by mixed assemblages of non-buzzing bees, wasps and flies. Interestingly, pollinator shifts in these species did also lead to changes in anther morphology, namely an enlargement of the anther pore (Goldenberg et al. 2008). Our continuously growing understanding of pollination strategies and flower trait functioning in different clades of the Melastomataceae may allow for rigorous testing of the evolutionary fine-tuning and different pathways of shifts away from the highly specialized 'buzz-bee' syndrome at the family level in future.

My results of Chapter I and III clearly show that pollinator mediated selection does not act on single traits but affects multiple traits of the floral phenotype (Armbruster et al. 2004, Ordano et al. 2008, Fenster et al. 2009). This is particularly relevant as many earlier studies focus on relatively few traits that can easily be coded for (e.g. colour, reward, size, Smith et al. 2008, Wilson et al. 2017), but may actually only provide relatively limited insights into how pollinator mediated selection affects flowers. The use of HRX-CT scanning enabled me to produce 3-D models of entire flowers and to assess the impact of pollinator mediated selection on floral shape. Until now, this method has been used relatively infrequently in studies on flower evolution, but provides an important advance in our understanding of the three-dimensional structure of flowers (e.g. Niet et al. 2010, Staedler et al. 2013, Wang et al. 2015).

Given that pollination mechanisms are functionally relatively specialized in Merianieae, it is not surprising that I found functional aspects structuring floral phenotypes rather than developmental affinities (compare e.g. Esteve-Altava 2016). Most importantly, I found reduced floral modularity in species which have shifted to vertebrate pollination while the 'buzz-bee' syndrome is characterized by high degrees of modularity. The loss in modularity in shifted syndrome species may possibly be explained by changes in selection on the flower's efficiency function. In the 'buzz-bee' syndrome, efficiency – i.e. the fit between stamens, stigma and pollinator – is mostly mediated by arranging the bees along the median plane of the androecium (Renner 1989, own observations). In many species, bees bite into appendages to convey vibrations. In the two shifted syndromes, however, interactions with the pollinators have changed. In the 'mixed-vertebrate' syndrome, stamen appendages are not involved in the pollen expulsion mechanism at all. Hence, fit cannot be mediated by optimizing the positioning of the appendages and the pore/stigma complex. In the 'passerine' syndrome, stamen appendages are the integral part of the bellows' mechanism of pollen expulsion and are removed from the flowers and consumed by the pollinating birds (Dellinger et al. 2014). My hypothesis is that the apparent change in corolla shape towards more campanulate forms with pollinator shifts was a new way of mediating fit with the larger vertebrate pollinators. The narrower entrance to the flower in the shifted syndromes constrains the directions from which vertebrates can insert their mouthparts into the flower (Muchhala 2007). This hypothesis is supported by the apparent union of corolla shape and the pore/stigma complex into one functional module in the two shifted syndromes. In the larger context, these findings make sense as tubular corollas are common in many bird pollinated plant lineages and

have even been identified as a pre-condition facilitating shifts to hummingbird pollination (e.g. Cronk & Ojeda 2008, Fenster et al. 2009).
With respect to the 'buzz-bee' syndrome, my results support the hypothesis that its high degree of modularity is key to the adaptive success of buzz pollination in Merianieae and possibly also at the family level. As mentioned in the introduction, the functional group of buzzing bees is very diverse (Cardinal et al. 2018). Hence, buzzing bees come in a variety of shapes, sizes and biophysical properties. The evolutionary flexibility (i.e. flexibility to respond to slightly different selection regimes) of the Merianieae flower possibly facilitates adaptive wandering to allow for small phenotypic changes to exploit this bee diversity (Reginato & Michelangeli 2016). The 'adaptive plateau' of buzz-bee pollination in Merianieae is supported by my estimation of shifts in phenotypic optima using Ornstein-Uhlenbeck models. Despite the large floral diversity within the 'buzz-bee' syndrome, there is no indication that these species have actually significantly shifted from their plateau. These findings open up many new questions on the relation between floral modularity and evolutionary potential (evolvability, Campbell et al. 2018, Opedal 2018) and may be applied to a variety of study systems or pollination strategies.

To conclude, I think that my colleagues and I have contributed substantially to advancing our knowledge on the pollination biology of Merianieae and that I have met my target of clarifying basic patterns of floral evolution and pollinator mediated selection in the tribe. Our findings open many future avenues for more detailed experimental studies on drivers of pollinator shifts or for the analysis of relationships between pollinator shifts and biogeographic patterns in the tribe. Also, as indicated above, I hope that my combination of methods and ideas inspires and promotes novel research in pollination biology in other systems.

7. LITERATURE

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