Evolution of stamen dimetrism in Melastomataceae, a large radiation of pollen flowers

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ABSTRACT

Changes in floral traits across evolutionary time are expected in response to selective pressures imposed by pollinators. Stamen dimetrism (here defined as size differences between stamens within the same flower) represents an important strategy to decrease pollen loss during bee-flower interactions in pollen flowers. However, the evolutionary history of stamen dimetrism, and the links between this and other reproductive traits across long periods of time are still poorly understood. Here we investigate the evolution of stamens dimetrism and the evolutionary correlation of this trait and other floral structures and reproductive strategies in Melastomataceae. Floral traits were scored from 336 species and reproductive biology data was gathered for 81 species. Stamen dimetrism is a labile trait, appeared several times throughout the evolutionary history of this clade and is evolutionarily correlated to floral size. Among the 81 species analyzed, we observed that the lineages that depend on pollinators to reproduce correspond to those that evolved the highest stamen dimetrism. The evolutionary lability of stamen dimetrism has probably contributed to the maintenance of the buzz pollination adaptive plateau in possibly the largest radiation of pollen flowers in angiosperms.

1. Introduction

Understanding the origin and diversification of flowering plants and their floral structures has been one of the most recurrent topics in studies of plant evolution (Darwin, 1879; Cardinal and Danforth, 2013; Vasconcelos et al., 2019). The specialized interactions between flowers and pollinators are considered one of the major drivers in the rapid diversification of angiosperms (Stockey et al., 2009; but see Katz, 2018; Hernández-Hernández and Wiens, 2020). Currently, about 90 % of the angiosperm species depend on animals for pollination (Ollerton et al., 2011). Bees are recognized as the most important guild among them and are often involved in specialized interactions with these plants (Williams, 1994; Westerkamp, 1997; Klein et al., 2007; Ollerton et al., 2011). Pollen obtained from flowers is a crucial protein resource for bee larval nutrition and over 20,000 plant species across 65 angiosperm families have specialized into providing pollen as the sole resource to bee pollinators (Buchmann and Hurley, 1978; Buchmann, 1983; Hargreaves et al., 2009; De Luca and Vallejo-Marín, 2013). This reproductive strategy, that occurs in so-called "pollen flowers" (Vogel, 1978), relies almost exclusively on pollination by female bees that are able to vibrate their wing muscles during pollen gathering, in a specific process named buzz pollination (Buchmann, 1983; Vallejo-Marín et al., 2010; De Luca and Vallejo-Marín, 2013; Vallejo-Marín, 2019).

Pollen flowers often present a series of morphological adaptations linked to buzz pollination. The main reason for this is that the production of pollen responds to conflicting selection forces: the pollen collected and used by bees to feed their larvae also contains plant male gametes that are a requirement for sexual reproduction (Vogel, 1978; Vallejo-Marín et al., 2009; De Luca and Vallejo-Marín, 2013; Lunau et al., 2014). Therefore, producing pollen as the sole resource to pollinators may represent an "evolutionary dilemma" to the plant, and opposite selective forces (i.e. producing pollen for bee feeding and for plant reproduction) should favour the evolution of mechanisms that decrease excessive pollen loss during bee-flower interactions (Thorp,

1979; Harder and Thomson, 1989; Westerkamp, 2004a).

For instance, some pollen flowers present stamen dimorphism, also called heteranthery, which refers to the morphological differences among stamens within the same flower (Müller, 1881; Forbes, 1882). According to the division-of-labour hypothesis, stamen dimorphism results from conflicting selection processes that result in the evolution of structures with different functions (Darwin, 1899; Vogel, 1978; Luo et al., 2008; Vallejo-Marín et al., 2009). One of the consequences of stamen dimorphism is that stamens in different whorls in the flower often have different sizes, which we define here as stamen dimetrism. In this system, long stamens would be responsible for producing pollen grains for plant reproduction, while short stamens would produce pollen grains mainly intended for feeding the bees (Luo et al., 2008; Vallejo--Marín et al., 2010, 2014). Notwithstanding, little is known about the emergence and maintenance of stamen dimetrism across large evolutionary time scales (Vallejo-Marín et al., 2010).

An increased understanding on this regard can be achieved by exploring the role of floral ecology in the evolution of stamen dimetrism. Given that size differences among stamens is one effective way to solve the pollen dilemma (Luo et al., 2008; Vallejo-Marín et al., 2009), selective pressures imposed by bees towards stamen dimetrism should be stronger in plants that depend on pollinators for their reproduction. On the other hand, in plants that do not depend on pollinators for reproduction, this selective pressure should be weaker. Therefore, the degree of stamen dimetrism is probably evolutionarily linked to the extent to which these species depend on bees for their reproduction. Furthermore, these selective pressures may also affect other floral traits promoting correlated evolution. The investigation of the correlations between this and other floral traits may evidence allometric changes that are related to the evolution of stamen dimetrism. For example, pollen flowers should also evolve larger and more attractive petals to improve their display for bees, and styles that are as long as the longer stamens and far from grooming areas of the bee's body (Koch et al., 2017).

Here we explore the morphological diversity of floral traits in Melastomataceae, possibly the largest radiation of pollen flower and one of the most diverse angiosperms plant families (Reginato et al., 2020). Even though the vast majority of species in Melastomataceae share a single pollination syndrome (buzz pollination *sensu* Vallejo-Marín et al., 2010), reproductive biology studies have shown that the degree to which different species depend on their pollinators for reproduction is variable (Santos et al., 2012; Brito et al., 2017a). While some species strongly depend on pollinators for reproduction (Brito and Sazima, 2012), others can present asexual routes to seed development (Caetano et al., 2018), thus depending much less on pollinators to achieve reproduction. Due to its size, age and widespread distribution, Melastomataceae provide a good model to understand the evolution of stamen dimetrism across numerous lineages along deep time.

We performed several analytical tests to explore the routes and triggers involved in the evolution of stamen dimetrism in Melastomataceae. First, we checked whether the evolution of this trait was labile (or conserved) throughout the evolutionary history of this clade. Then, we analysed how it evolutionarily correlates with other floral traits that are also involved in reproduction, such as petals and style length. Finally, we investigated whether the degree in which flowers depend on pollinators is related to stronger stamen dimetrism using different trait evolution models.

2. Material and methods

2.1. Model system

The Melastomataceae (Order Myrtales *sensu* APG IV) are a large angiosperm family of 5159 accepted species with pantropical distribution (Renner, 1993; Renner et al., 2013 onwards; Chase et al., 2016; Bacci et al., 2019; Reginato et al., 2020) and stamens that vary widely in their length (Renner, 1989; Almeda, 2009; Reginato and Michelangeli,

2016). Almost all Melastomataceae species have stamens disposed in two whorls that are ontogenetically linked to and presented in the same number as the parts of the perianth (i.e. sepals and petals) (Basso-Alves, 2016). In species with stamen dimetrism, the antesepalous stamens are usually relatively long and involved in reproduction, while the antepetalous stamens are relatively short and involved in feeding bee pollinators (Luo et al., 2008). During pollen gathering, a "morphological fit" between bees and dimetric stamens, i.e. those where length differences are conspicuous, make the pollen grains from the long stamens to be placed on the back of the bees in areas called "safe sites" where it is difficult for the bees to remove them (Koch et al., 2017). Throughout this study, we will differentiate stamens in different whorls as: (1) antepetalous stamens, which are usually short and also known as feeding stamens (for often producing pollen grains that are used for bee feeding), and (2) antesepalous stamens, usually long and also known as pollination stamens (for frequently producing pollen grains that are used mainly in reproduction) (Fig. 1).

2.2. Stamen dimetrism index for Melastomataceae flowers

Because all analyses of morphological data were performed in a phylogenetic context (see below), we selected the species for this study based on a list for which genetic data was also available in GenBank (consulted in February 2018). Genetic information was found for 1842 species (~36 % of the 5159 accepted species), of which 336 had detailed taxonomic descriptions of floral morphology in the literature. These included species representing 14 tribes of Melastomataceae (~78 % of the tribe's diversity) and ~33 % of the accepted genera (57 out of 171).

In order to increase the reach of our sample, we collected floral morphology data from the selected species through a careful search in the specialized literature. We also extended our search to theses, dissertations and reviews that contained detailed taxonomic descriptions. The average trait length was collected from the values presented in the species descriptions made by taxonomists. The lengths of the following traits were collected: antesepalous and antepetalous stamens, including filament, anther length and pedoconnective (if present), petals and styles, all in millimetres. These structures were chosen given their key roles in attraction, pollination and reproduction in Melastomataceae (Table S1).

Stamen dimetrism was characterized as the difference in length between stamen sets (filament and anthers together, and pedoconnective if present). We are aware that other stamen differences that characterize stamen dimorphism in its broadest sense, such as colour and/or shape, may also be present in the species analyzed here. However, such traits were not considered here given the lack of such information in many taxonomic descriptions. Moreover, stamen length is the most recurrent stamen trait for the majority of species descriptions and allows a simple and direct comparison among different studies since it can be objectively measured.

To describe the difference in length between stamens in different whorls, we calculated a stamen dimetrism index (SDI) given by:

$$SDI = \frac{S - s}{S + s}$$

Where *S* is the length of the antesepalous stamen, and *s* is the length of the antepetalous stamen. This index ranges from -1 to 1; 0 indicates strict isometrism between stamens and values close to -1 and 1 indicate extreme values of stamen dimetrism. Negative values are found when antepetalous stamens are longer than antesepalous stamens. In the genus *Rhynchanthera*, stamen dimetrism occurs within the antesepalous stamen whorl while the antepetalous whorl exhibits staminodia (Renner, 1990). In this case, we considered the length difference between the long and the short stamens of the antesepalous whorl to estimate the stamen dimetrism index. In the SDI, the difference in the stamen lengths is divided by their sum. In this way, the SDI corrects possible bias
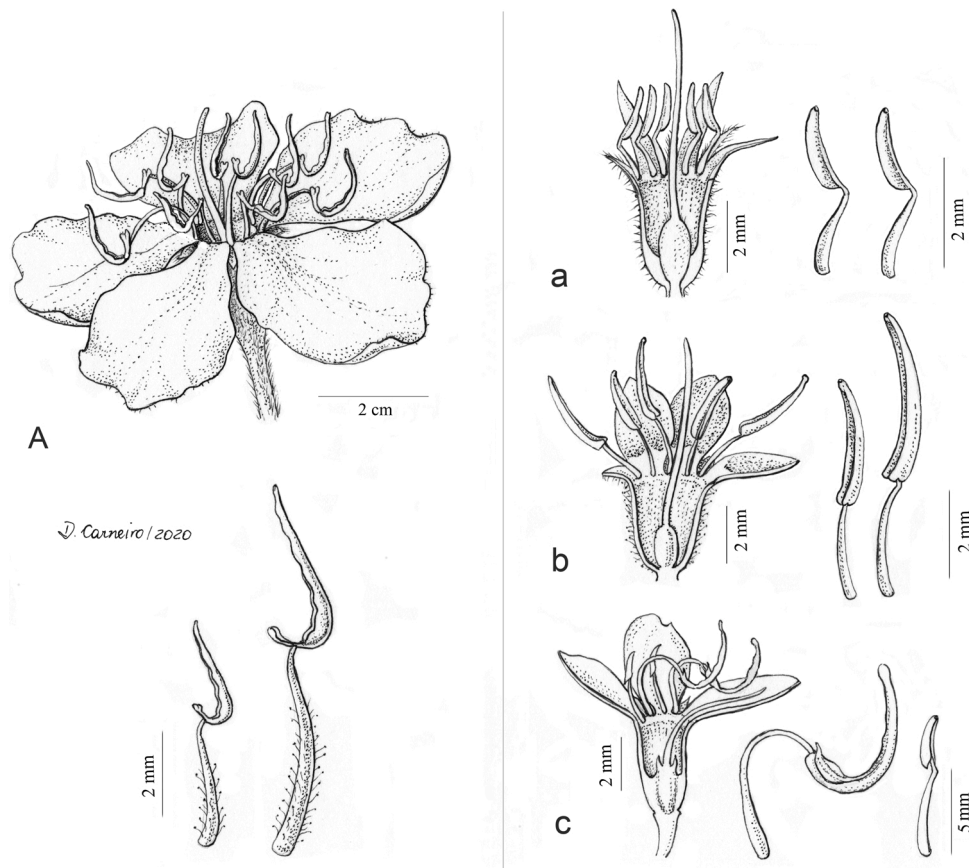
Fig. 1. (A) Illustration of the *Pleroma raddianum* Gardn. a Melastomataceae flower with emphasis on the dimetric stamens. In this case, the largest stamen (right) is antesepalous and the smallest is antepetalous (left). In (a) *Leandra eichleri* Cogn. representing isometric stamens, (b) *Cambessedesia eichleri* Cogn. showing subdimetric stamens, and (c) *Fordiophyton chenii* (S. Jin Zeng & X. Y. Zhuang) representing dimetric stamens.

resulting from morphological descriptions by different groups of taxonomists. The SDI also provides a value for stamen dimorphism that is independent of floral size, which is more suitable for our analyses.

SDI data was treated as a continuous trait in all analyses except in one of the analysis of ancestral state reconstruction, where a mixed approach was used, and the data was categorized to estimate transition rates among states. The categories used in this study (see below) already exist in the taxonomic literature, but the thresholds between categories may vary depending on the author. Thus, for some of the data analysis, we divided SDI values into 3 arbitrary categories for improving the visualization of the results: *isometric*, where there is no significant difference between the stamen sizes (- $0.1 < SDI < 0.1$), *subdimetric*, where one stamen is up to twice the size of the other ($0.1 < SDI < 0.3$ or - $0.3 < SDI < -0.1$) and *dimetric*, where one stamen is over twice the size of the other ($SDI > 0.3$ or $SDI < -0.3$).

2.3. Phylogenetic inference

We built a molecular dataset by filtering and including the most common molecular markers for the 1842 Melastomataceae species available on *GenBank*, resulting in a matrix with seven plastid and two nuclear regions. The plastid dataset encompasses three intergenic spacers (*accD-psaI, atpF-atpH, psbK-psbI* and *trnS-trnG*), two protein-coding genes (*ndhF* and *rbcL*) and one intron (*rpl16*), and the nuclear data is based on two ribosomal spacers (nrETS and nrITS). Nine species belonging to the CAP clade (Crypteroniaceae, Alzateaceae, Penaeaceae), sister to the Melastomataceae within the Myrtales (APG IV, 2016), were also selected as outgroups. These were *Alzatea verticillata* (Alzateaceae)*, Brachysinphon acutus* (Penaeaceae)*, Cryteronia griffithii, Cryteronia paniculata* (Crypteroniaceae)*, Olinia ermaginata, Olinia ventosa,*

Rhynchocalyx lawsonioides, Penaea mucronata and *Saltera sarcocolla* (Penaeaceae). Genbank accession numbers for all sequences included in this analysis are available at the Supporting Information (Table S2).

The individual loci were aligned with MAFFT 7.3 (Katoh and Standley, 2014) using the G-INS-i strategy. The best scheme of DNA partitioning and best substitution models were estimated in PartitionFinder2 (Lanfear et al., 2012) by comparing values of BIC. The best scheme resulted in five partitions (1. *accD-psaI, psbK-psbI* and *trnS-trnG*; 2. *atpF-atpH, ndhF* and *rpl16*; 3. *rbcL*; 4. nrETS; 5. nrITS) and the model GTR + G was recovered as the best fit for all partitions (Table S3).

Tree inference and time divergence among clades were estimated under a Bayesian framework implemented in BEAST 2.5.0 (Bouckaert et al., 2014). Molecular clock was adjusted for lognormal uncorrelated and tree priors followed the Yule model. Fossil constrains and secondary calibration points were positioned in three nodes, including: 1. The MRCA (most recent common ancestor) of Melastomataceae (prior = lognormal, mean = 1, s.d. = 1, offset = 56), based on *Melastomites montanensis* a fossil leaf from the Paleocene (Brown, 1962); 2. *Rhexia* + *Arthrostemma* clade (prior = lognormal, mean = 1, s.d. = 1, offset = 20), based on seed fossils from the Miocene (Collison & Pingen, 1992) and 3. CAP clade (prior = normal, mean = 52.7, s.d. = 6), based on a secondary calibration point recovered from a broader analysis of Myrtales (Berger et al., 2016). We ran two independent analyses of 50 million generations each, sampling every 1000 generations and combined the stable posterior distributions from both runs using LogCombiner v.1.8.0. Convergence was analysed using Tracer v.1.6 (Rambaut et al., 2014) and considered satisfactory when ESS values were above 200. The maximum clade credibility (MCC) tree was generated in TreeAnnotator v.1.8.0. This tree was used as a base for all of the analyses performed in this study. The relationships recovered are

in accordance with other molecular phylogenetic trees recently published for other groups of the family (Clausing and Renner, 2001; Ionta et al., 2007; Goldenberg et al., 2008; Meirelles, 2015; Zeng et al., 2016; Veranso-Libalah et al., 2017).

2.4. Ancestral state reconstruction

The ancestral reconstruction of stamen dimetrism was performed using two sets of data. The first set of data treated SDI as a continuous trait. For this analysis, we used the *contMap* function of the R package *phytools* (Revell, 2012) and estimated the evolutionary history of this trait along the phylogeny of Melastomataceae based on a Brownian motion model. The second reconstruction considered three categories of SDI (isometric, subdimetric and dimetric) and was performed to test evolutionary shifts between morphological categories particularly how gradual transitions between morphological extremes are. However, note that both subdimetric and dimetric categories represent stamens that may contribute to the division-of-labour as it will be discussed below.

For this reconstruction, we first tested which of the following evolutionary models best fitted the data using the *fit.discrete* function of the R package *geiger* (Harmon et al., 2008): ER (equal rates), ARD (all different rates), SYM (symmetrical) and "Ordered" (manually configured to prohibit evolutionary changes between extreme states in the Q matrix). The best model was selected by comparing Akaike weights and used to execute a stochastic character mapping with 1000 simulations using the function *make.simmap* from the R package *phytools* (Revell, 2012).

2.5. Correlated evolution between floral traits

We tested for evolutionary correlation between the lengths of the two sets of stamens, between stamens and style length and between stamens and petal length. These correlations were performed to reveal trade-offs and allometric constraints involved in the evolution of stamen dimetrism. For these analyses, we used phylogenetic generalized least squares, using the *gls* function of the R package *nlme*. This method tests the correlation between two or more variables, correcting for phylogenetic relatedness (Pinheiro et al., 2020). These correlations were performed assuming an Ornstein-Ühlenbeck evolutionary model (see below), by setting the *corMartins* function of the R package *ape* (Paradis and Schliep, 2018) as one of the arguments in the analysis. For correlated evolution between floral trait, we considered 209 species (13 tribes and 41 genera of Melastomataceae) for which petal and style length measurements, in addition to stamen length, were available in the literature. In this case, the remaining species were pruned from our main phylogenetic hypothesis. In all of these analyses, we considered the *log* value of the traits, given the non-normal distribution of the data.

2.6. Evolution of stamen dimetrism and dependence on pollinators

To explore correlations and changes in evolutionary rate of SDI associated to dependence on pollinators for reproduction in species of Melastomataceae, we first performed a *phyloANOVA* from the R package *phytools* (Revell, 2012) to test if SDI and dependence on pollinators are correlated. We gathered information about dependence on pollinators for fruit set in 81 species from two previous studies (Santos et al., 2012; Brito et al., 2017a; Table S1). Even though these studies are mostly regional and do not encompass the whole geographical distribution of the family, these species represent 7 tribes and 23 genera thus providing a relatively well-distributed sample in the phylogeny of Melastomataceae. We classified the breeding system of each species as dependent or independent of the pollinators for fruit set following Brito et al. (2017a). In that study, they determined whether fruits were developed in bagged and unbagged flowers in the field (adapted from Radford et al., 1974). Pollinator dependent species encompass cross- and self-compatible species that depend on the action of pollinators, mainly

vibrating bees, to set fruits. The pollinator-independent species encompass autogamous self-pollinating and apomictic species that produce fruits even when flowers were bagged during field experiments. Some of these species may be facultatively pollinated by animals, but they do not rely on pollinators for fruit production (Hokche and Ramírez, 2008; Santos et al., 2012; Brito et al., 2017a). Note that poricidal anthers, a morphological feature that is strongly conserved in Melastomataceae flowers, do not mean necessarily that a species requires interaction with pollinators to achieve reproduction. Pollinator-independent species can set seeds in the absence of pollinators by both autogamous self-pollination or apomixis, as in *Miconia angelana* R.Romero & R.Goldenb., *Microlicia fasciculata* Mart. ex Naudin, and *Leandra melastomoides* Raddi (Santos et al., 2010, 2012; Goldenberg and Varassin, 2001).

We then tested seven variants of two main trait evolution models: Brownian Motion (BM) and Ornstein-Ühlenbeck (OU) (following an approach based on de Alencar et al., 2017). BM models assume that traits evolve randomly (non-adaptively) or by stabilizing selection towards a moving optimum (O'Meara et al., 2006). OU models assume that a trait is evolving under a selection regime, with parameters estimating differences in the phenotypic optima (θ), phenotypic rate (σ^2) or selective strength (α) between species dependent or independent from pollinators. The θ describes hypothetical phenotypes for which populations are evolving in an OU process. The σ^2 measures the intensity of stochastic fluctuations in the evolutionary process and α indicates the attraction to the θ and measures the speed for which a trait evolves to the optimum (Hansen, 1997; Butler and King, 2004; Beaulieu et al., 2012).

We separately fitted seven models to the SDI dataset over 1000 simmap trees where the two-state reproductive system (dependent and independent on pollinators) were mapped using the R package *OUwie* (Beaulieu et al., 2012). The seven models were: (1) BM1, a BM model with a single evolutionary rate, (2) BMS, a multi-rate BM model that allows σ^2 values to vary between states, (3) OU1, a OU model with a single optimum, (4) OUM, a model that assumes distinct θ values for each reproductive system, but a single σ^2 value, (5) OUMV, a model that allows distinct σ^2 values as well as distinct θ values for each reproductive system, (6) OUMA, a model that allows distinct α and θ values for each reproductive system, and (7) OUMVA a model that allows distinct σ^2, α, and θ values for each reproductive system. If OU models fit better than BM models, we expect both θ and σ^2 values to be higher for species that depend on pollinators contrasting to those that do not depend on pollinators, suggesting that the first can be evolving under an adaptive regime.

After running all analyses, we verified whether the values in the resulting matrices were positive and whether the estimated values of θ were biologically reasonable (i.e. within the range of possible SDI values). We calculated the ΔAICc (the difference in AICc values of each model relative to the model with the lowest AICc values) to investigate which model best explained trait evolution in each *simmap* tree (1000 simulations). A model was considered the best fit when the ΔAICc between the best and the second-best models was greater than two (Burnham and Anderson, 2002). All statistical analyses were performed in the R software version 3.6.1 (R Core Team, 2019).

3. Results

3.1. Stamen dimetrism in Melastomataceae flowers

The highest SDI value was verified in *Siphanthera cordifolia* (SDI = 0.45), followed by *Acisanthera limnobios* (SDI = 0.43), *Siphanthera fasciculata* (SDI = 0.43) and two species of the genus *Fordiophyton Fordiophyton huizhouense* (SDI = 0.43) and *Fordiophyton chenii* (SDI = 0.42). In most species that show some degree of stamen dimetrism (i.e. those that are either subdimetric or dimetric), the stamens in the antesepalous whorl are longer than those in antepetalous whorl, resulting in positive SDI values. Exceptions were found in *Meriania brevipedunculata*,

M. parvifolia, M. squamulosa and *Siphanthera subtilis*, where the stamens in the antepetalous whorl were long and the SDI values were negative (Table S1). When the SDI values were categorized, 190 species (56.5 %) were considered isometric, 129 species (38.3 %) were considered subdimetric and only 17 species (5.2 %) were considered dimetric. This shows that nearly half of the species of Melastomataceae analysed in this study has some degree of stamen dimetrism (Fig. 2).

3.2. Ancestral state reconstruction

For the ancestral state reconstruction, the model Ordered, in which transitions between extremes are forbidden, was the best fit for the data and used to perform ancestral state reconstruction using discrete data (Table S4). In this analysis, there was no support to infer what was the

ancestral state of the family, but most of the backbone (i.e. oldest nodes) was recovered as subdimetric with higher probability. The same result was observed in ancestral state reconstructions using SDI as a continuous trait (Fig. 2A).

Stamen dimetrism is not a conserved trait in the evolution of the family, given that at least 165 changes between states were estimated. However, it is noticeable that the Ordered model was the best fit for the data and so no jump transitions between morphological extremes were estimated, showing that the evolution of stamen dimetrism, even though labile, is gradual. The subdimetric state is an unconditional transition between the isometric and dimetric states. Although changes between dimetric and subdimetric stamens occur, the majority of changes were observed between the two most common states, isometric and subdimetric (Fig. 2B; Table S4).
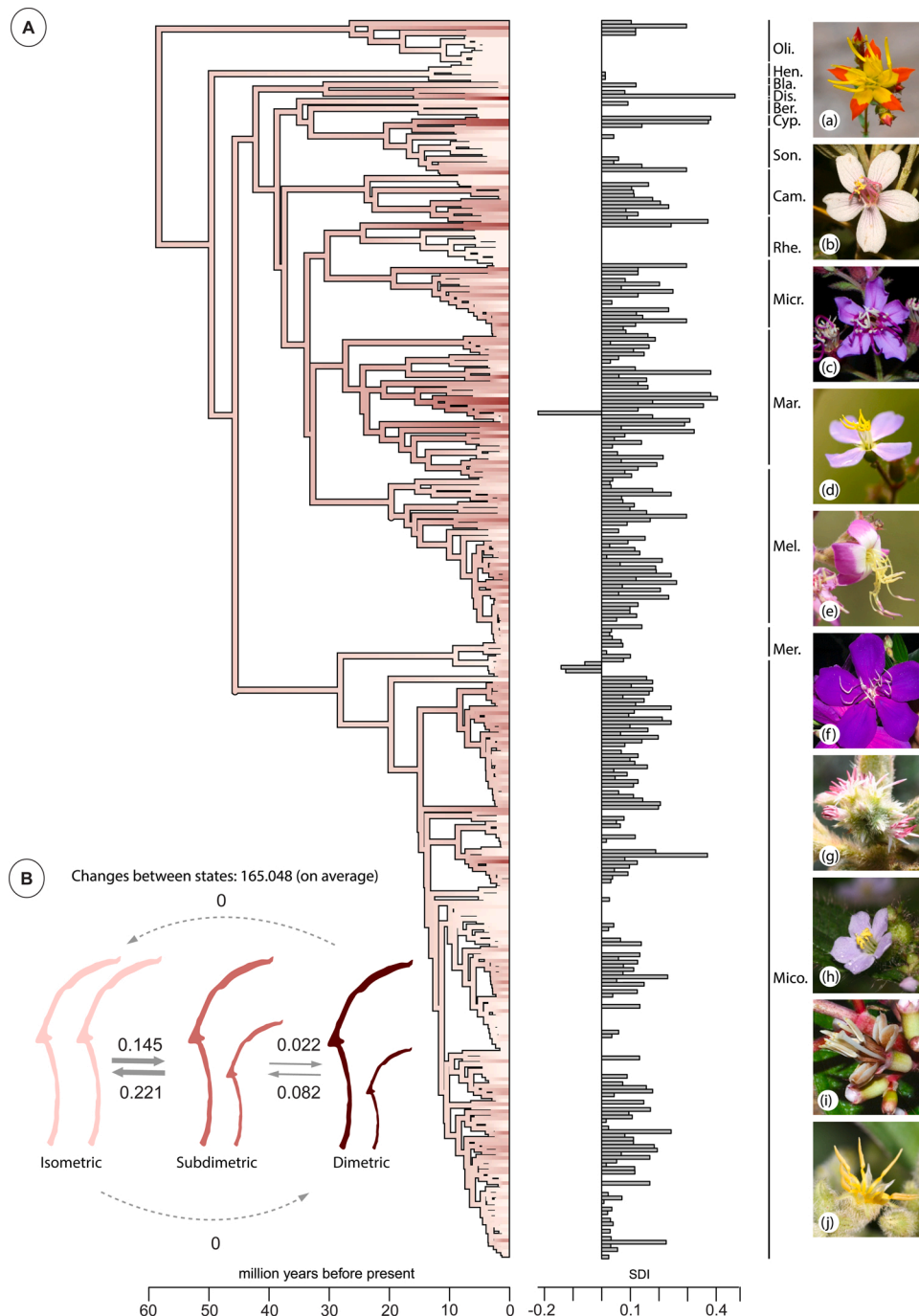
Fig. 2. (A) Ancestral state reconstruction of stamen dimetrism, considering 336 species and 14 tribes of Melastomataceae and SDI as a continuous trait. To the right, a barplot showing SDI values for each species. Tribes are abbreviated as: Olisbeoideae (Oli.), Henrietteeae (Hen.), Blakeeae (Bla.), Dissochaetaea (Dis.), Bertolonieae (Ber.), Cyphostyleae (Cyp.), Sonerileae (Son.), Cambessedesieae (Cam.), Rhexieae (Rhe.), Microlicieae (Micr.), Marcetieae (Mar.), Melastomateae (Mel.), Merianieae (Mer.), Miconieae (Mico.). (B) Transition rates among the three states using the "Ordered model". The thickness of the arrows indicates the frequency of change between states. Examples of some Melastomataceae flowers: (a) *Cambessedesia hilariana* (Kunth) Cambessedesieae, (b) *Trembleya parviflora* (D. Don) Microlicieae, (c) *Pleroma oleifolium* (Triana) Melastomateae, (d) *Pterolepis* sp. Melastomateae, (e) *Macairea radula* (Bonpl.) DC. Marcetieae, (f) *Pleroma stenocarpum* (Schrank et Mart. ex DC.) Triana Melastomateae, (g) *Leandra dasythrica* (A.Gray) Cogn. Miconieae, (h) *Pleroma* sp. Melastomateae, (i) *Miconia* sp. Miconieae, (j) *Miconia paniculata* (Naudim) Miconieae. Colours: (light pink) isometric, (pink) subdimetric, (dark pink) dimetric.

3.3. Correlated evolution among floral traits

In the analysed species, the evolution of the antesepalous stamen length is correlated with both petal ($r = 0.18; p < 0.05$) and style lengths ($r = 0.13; p < 0.05$). The antepetalous stamen length is correlated with style length ($r = 0.11; p < 0.05$) and strongly correlated with antesepalous stamen length ($r = 0.98; p < 0.01$), but it is not correlated with the petal length ($r = 0.12; p = 0.12$). The length of the style also shows significant correlation with petal length ($r = 0.46; p < 0.01$; Fig. 3).

3.4. Morphological evolution

During the evolutionary history of Melastomataceae, an increased difference in length between stamens of distinct whorls (higher SDI) is present in plants that depend on pollinators for fruit set ($F = 3.89; p < 0.05$) (Fig. 4). By contrasting the fit of each one of the seven evolutionary models separately (Table S5), we observed that the model OUMV was best fitted in the majority of the stochastic mappings, with 34 % of the total fits. This model allows distinct values of σ^2 and θ for each reproductive system and, in this case, higher values of θ and σ^2 associated with the evolution of species that depend on pollinators (Fig. 5). This suggests that stamen dimetrism in species that depend on pollinators may be higher and with higher variation around the optima (Fig. 5b). In these lineages, optimum SDI values can be higher than the double of the SDI values of plants that do not depend on bee pollinators to set fruits.

4. Discussion

Our results show that stamen dimetrism is not a conserved trait in the
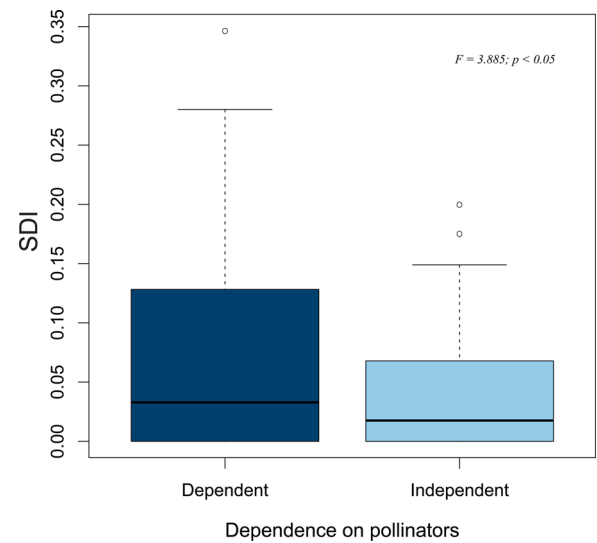
Fig. 4. Evolutionary relationship between stamen dimetrism index (SDI) and dependence of pollinators to set fruits in Melastomataceae. Boxplots are composed of boxes showing 25 % and 75 % quartiles and the median is represented by the large black line within the box. Whiskers show the highest values within the box range defined as 1.5x interquartile range. Outliers are plotted as empty dots.

evolutionary history of this large Pantropical clade - Melastomataceae. In fact, stamen dimetrism is evolutionarily labile, with gradual transitions between states and no shifts between the extremes of the gradient
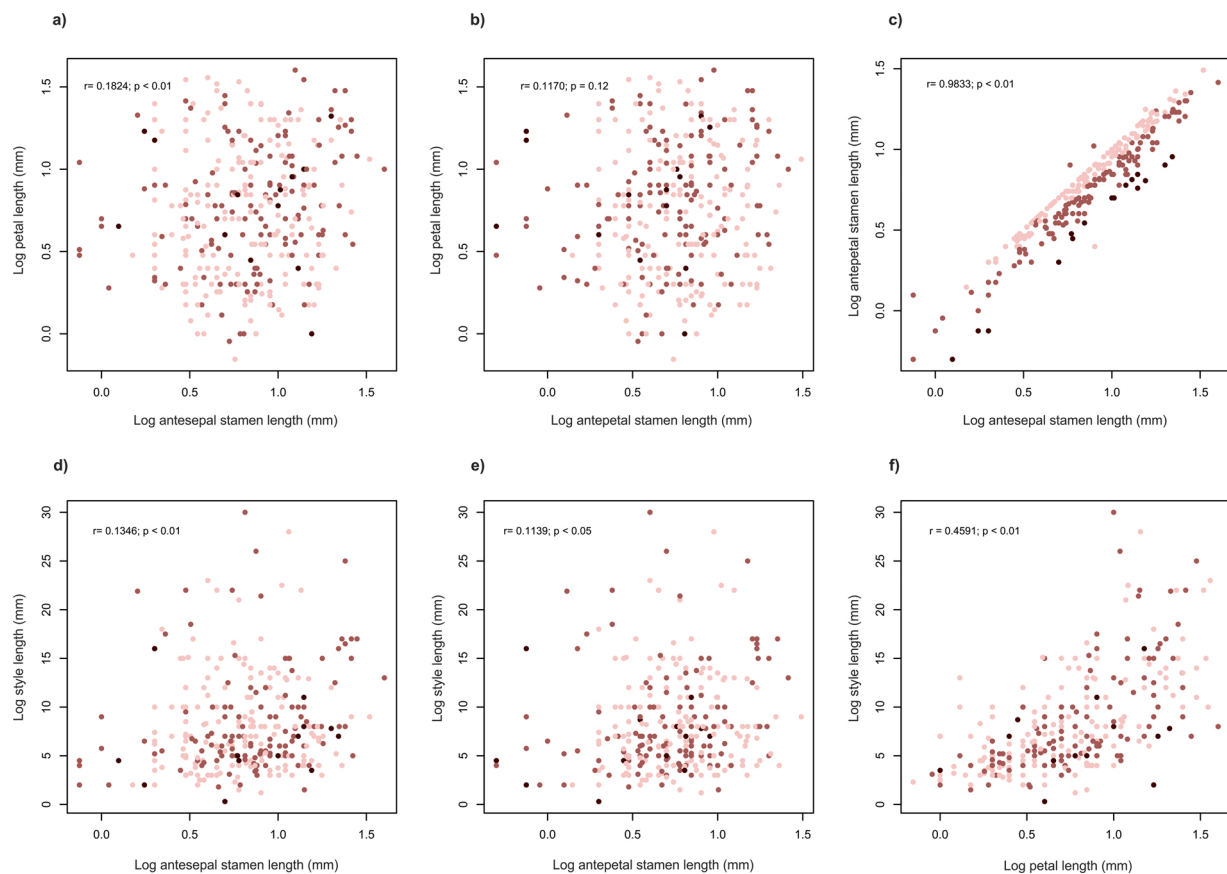
Fig. 3. Evolutionary correlations between floral traits in Melastomataceae. a) Petals and antesepalous stamen length; b) Petal and antepetalous stamen length; c) Antepetalous stamen and antesepalous stamen length; d) Style and antesepalous stamen length; e) Style and antepetalous stamen length; f) Style and petal length. Colours: (light pink) isometric, (pink) subdimetric, (dark pink) dimetric.
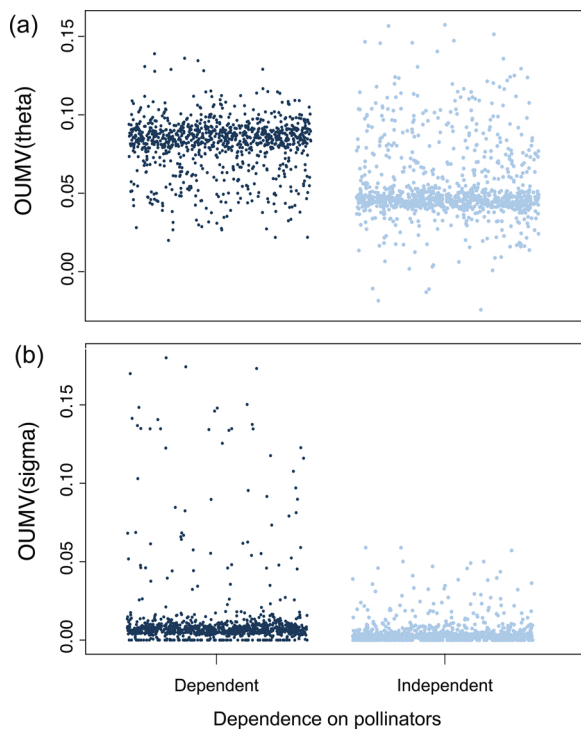
Fig. 5. Parameter estimates when OUMV was chosen as the best model in analyses of evolution of stamen dimetrism in Melastomataceae. (a) Adaptive optima for each category showing that optimum values of SDI are higher in species that depend on pollinators; (b) Phenotypic fluctuations around the optima of each category. Species that are independent on pollinators (light blue) have lower fluctuation when compared with species that depend on pollinators (dark blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

(i.e. isometrism to dimetrism, or vice versa). The subdimetric state is functional in relation to the division of labour and is essential in the transition between the other two extreme states (Fig. 2B). During the floral evolution of Melastomataceae, only the length of the antesepalous stamens was correlated with the length of petals and styles. Finally, we observed that stamen dimetrism converged to two distinct adaptive optima, one for each state of reproductive system, and with a higher phenotypic fluctuation around the optima in species that depend on bees for reproduction.

4.1. Evolution of stamen dimetrism is labile but gradual in Melastomataceae

Among bees, the ability to vibrate for pollen collection may have first evolved during the Early Cretaceous in their common ancestor (100–145 Myr) and it is currently present in seven unrelated families and in more than 70 genera (Buchmann, 1983; Cardinal et al., 2018). Similarly, the evolution of the buzz pollination syndrome is observed in several unrelated lineages of angiosperms (Vallejo-Marín et al., 2010). In Melastomataceae, flowers with poricidal anthers have probably emerged early in the evolution of the family (Clausing and Renner, 2001) and this trait is conserved within the family. Thus, even species that do not depend on pollinators or that are not pollinated by vibrating bees, such as apomictic and some more generalists, still show this trait because they have inherited it (Caetano et al., 2018). Buchmann (1983), for instance, shows that some species with poricidal anthers are not buzz pollinated. Although no study has explicitly placed a time-frame on the evolution of poricidal anthers, the family crown is estimated to be 63 Myr (77–56 Myr, Reginato et al., 2020), and almost all of their extant representatives present this trait, a remarkable case of long-lasting adaptive plateau

(Renner, 1989; but see Dellinger et al., 2019). Therefore, most of the floral diversity of Melastomataceae can be attributed to exploring a bee behavioural niche that already existed before the main diversification of the family (Phillips et al., 2020).

Besides poricidal anthers, plants from unrelated families have developed additional specific floral adaptations, such as stamen dimetrism, in response to the selective pressures from pollen collecting bees (Vogel, 1978; Vallejo-Marín et al., 2010). Stamen dimetrism likely evolves from the need of nectarless plants to restrict access to their pollen grains, reducing male gametophyte loss to inefficient pollinators and pollen thieves, that is, visitors that remove pollen with little or no contribution to pollen deposition on stigmas (Harder and Wilson, 1997; Hargreaves et al., 2009). Therefore, the close interaction between buzz pollinated plants and their pollinators has given rise to remarkable examples of convergent evolution and co-adaptations (Vogel, 1978; Thorp, 2000; Barrett, 2010; De Luca and Vallejo-Marín, 2013; Vallejo-Marín, 2019). However, stamen dimetrism is not a conserved trait and appeared at distinct moments and clades along the evolutionary history of Melastomataceae.

This floral trait, as well as the other traits analysed in this study, is highly labile during the evolution of this lineage (a well-documented pattern also found in several other clades of angiosperms; e.g. Stebbins, 1970a, b; Knight et al., 2005; Johnson, 2006; Taylor, 2009; Vasconcelos and Proenca, 2015; Vasconcelos et al., 2019). This demonstrates that, even though there is a relative stability of morphological and pollination strategies within the family, there is also an evolutionary flexibility that may have been important for species of Melastomataceae to explore distinct reproductive strategies throughout their evolution. This scenario corroborates the idea that most Melastomataceae species have reached an 'adaptive plateau' on which most of the linages is wandering, despite some departures from the buzz pollination to other pollination systems or even apomixis (Dellinger et al., 2018; Varassin et al., 2008; Brito et al., 2016, 2017b; Caetano et al., 2018).

Species within Melastomataceae comprise shrubs, woody climbers, herbs, or trees and are one of the most abundant and diversified plant group in the tropics (Clausing and Renner, 2001). Different degrees of stamen dimetrism evolved at different moments and possibly in different environments since the early diversification of this family. This suggests that selective pressures imposed over these structures have been present in all habitats that these species occupied, corroborating the key role of buzzing bees that also have a widespread distribution in the globe, in this process (Cardinal et al., 2018; Dellinger et al., 2019). However, it is also noticeable that, even though flexibility exists, shifts between morphological extremes were not verified, demonstrating that the evolution of dimetrism is flexible but also gradual in Melastomataceae. This suggests that the evolution of stamen dimetrism may be constrained and evolutionarily correlated to other floral traits.

4.2. Evolutionary correlation among floral traits

The lengths of most floral structures considered are evolutionarily correlated, which is expected since allometric, developmental and selective constraints are a common feature among floral parts (Murren, 2012; Armbruster et al., 2014; Pélabon et al., 2014). Following allometry, larger flowers (i.e. those with larger petals) also tend to evolve longer antesepalous stamens and styles. The length of both antesepalous stamen and style in such flowers is strategic in guaranteeing the process of pollination because it allows the contact of "safe sites" on the body of bees, areas where pollen grains cannot be easily groomed by them (Westerkamp, 1996; Lunau et al., 2014; Koch et al., 2017). In turn, petal size fulfils a different role in pollination since it affects the floral display (Ollerton et al., 2007; Makino and Ohashi, 2016). Larger petals presumably produce more intense displays that are required in systems that depend on pollinators (Ohashi and Yahara, 2009). However, it is remarkable that the length of the antepetalous stamens does not

correlate with petal length, showing that it opposes allometric expectations. The absolute majority of Melastomataceae species with stamen dimetrism present an arrangement of stamens in which the antepetalous stamens are smaller than the antesepalous ones. The length of antepetalous stamens is also probably related to the positioning of anther pores on the ventral region of the bee body, guaranteeing their bee-feeding function (Luo et al., 2008; Vallejo-Marín et al., 2009).

Regardless the feeding and pollinating stamen position, smaller stamens could reduce the production of pollen that is used as a reward for bee pollinators, given that this is an energetically expensive resource (Luo et al., 2008). This suggests that stamen dimetrism (as the solution for the pollen dilemma when reproduction is pollinator dependent) can be achieved following the principle of "evolution along the lines of least resistance" (Stebbins, 1974, p: 31). If the antepetalous stamens remain small when flowers enlarge in pollinator dependent systems, stamen dimetrism is achieved without radical changes in floral organization. We also found that some species of tribe Merianieae have larger antesepalous stamens than antepetalous ones, which results in negative SDI values (Michelangeli et al., 2015; Almeda and Robinson, 2011). In addition to that, in the genus *Rhynchanthera*, stamen dimorphism occurs as a result from different sizes in stamens of the same whorl (Renner, 1990). Therefore, our data confirms that selective pressures that favour stamen dimetrism can be strong enough to lead to the evolution of this trait through different ontogenetic pathways in a few cases (Basso-Alves, 2016; Konzmann et al., 2020).

4.3. Evidence for the influence of reproductive strategies in the evolution of stamen dimetrism

Regardless of the developmental mechanisms that lead to stamen dimetrism in pollen flowers, our results suggest that higher values of SDI are associated with species that depend on pollinators to reproduce in Melastomataceae. In these species, the absolute reliance on pollinators for fruit set and reproduction probably imposes a stronger selective pressure for evolution of morphologies that are more efficient for pollination (De Luca and Vallejo-Marín, 2013; Solís-Montero and Vallejo-Marín, 2017). Given that the vast majority of Melastomataceae species are buzz-pollinated, including the ones analysed here, this result presents some support for the hypothesis that dependence on bees that simultaneously harvest pollen and pollinate flowers influence the evolution of stamen dimetrism in pollen flowers.

Even though this conclusion sounds intuitive, the limited availability of reproductive biology data for the group (i.e. 81 species out of c. 5000) impose some restrictions to the extent in which this trend can be generalized for the whole family. Other ecological interactions that were not taken into consideration in our study, such as competition for pollinators in sympatric species (Moreira-Hernández and Muchhala, 2019), could be also driving the evolution of stamen dimetrism in Melastomataceae. In that sense, more data from field experiments are crucial to expand this dataset and fully support these conclusions.

Non-strict dependence on pollinators for reproduction is common across Melastomataceae and may alleviate the selective pressures associated with the evolutionary dilemma of pollen production as a resource for both bees and plants. Apomixis, for instance, is common in many species of the Miconieae tribe (Renner, 1989; Goldenberg and Shepherd, 1998; Santos et al., 2012; Caetano et al., 2013; Brito et al., 2017a; Caetano et al., 2018). Similarly, the selective pressure for stamen dimetrism should be low in species that present alternative floral resources. For example, *Axinaea* (Merianieae), where flowers offer the tissue of anther appendices as rewards to birds (Dellinger et al., 2019). It is worth mentioning that the two species in our study with this condition *Axinaea macrophylla* (SDI = 0.08) and *A. costaricensis* (SDI = 0) had low SDI values as well as other Miconieae species that offer nectar *Charianthus alpinus* (SDI = 0.13); *C. nodosus* (SDI = 0.10); *C. dominicensis* (SDI = 0.04); *Miconia hyemalis* (SDI = 0.04) (Varassin et al., 2008). Therefore, future studies can shed light on other aspects of stamen

dimetrism, that possibly are subject to other types of selective pressures.

Interestingly, reduced levels of stamen dimetrism and pollinators dependency are also correlated to smaller flowers, perhaps because only a small proportion of pollen suffices to guarantee female success. It is known that there is an evolutionary correlation among smaller flowers, fruits with few seeds, and more generalized pollination systems (e.g. Bawa, 2016; Bawa et al., 2019). Furthermore, in these flowers there is also a greater proximity between anther and stigma, increasing chances of self-fertilization, even in pollen flowers (Goldenberg and Shepherd, 1998; Santos et al., 2010; Vallejo-Marín et al., 2014; Brito et al., 2017b). In this sense, the reduction in the selective pressure that drives the stamen's dimetrism may also be related to the loss of more complex and more specialized floral phenotypes in these smaller flowers (Vallejo-Marín et al., 2014). The convergent correlated evolution of such floral traits in plants that do not depend on pollinators for reproduction would optimize the use of resources (e.g. smaller flower size and/or a reduction in pollen production) and could be treated as another case of the "selfing syndrome" (Jain, 1976; Sicard and Lenhard, 2011).

A further difference between systems that depend or not on pollinators is observed in the evolution of stamen dimetrism. The model that best explained the evolution of stamen dimetrism, the OUMV, presents variation in σ^2, with greater fluctuations of SDI values in the evolution of species that are dependent on pollinators when compared to species that do not depend on pollinators. Although again we recognize that our sample size in this analysis is small and that more field data are necessary to draw any stronger conclusions, such higher fluctuation of SDI values makes sense when we consider the "matches" between flowers and vibrating bees with different body sizes. The morphological match between pollen flowers and vibrating bees is directly related to the flower reproductive success (Solís-Montero and Vallejo-Marín, 2017). Vibrating bees present a large variation in body size ranging from very small (e.g., *Andrena* species < 4 mm in length) to very large species (e.g., *Xylocopa* species >20 mm) (Cardinal et al., 2018). This may have driven the evolution of stamen dimetrism according to the local guild of most effective pollinators (Stebbins, 1970a, b; Johnson and Steiner, 1997; Cardinal et al., 2018).

4.4. Conclusions

Our study showed that stamen dimetrism is highly labile and that its evolution and shifting between adaptive zones is probably greater in species with large flowers that depend on pollinators for reproduction. The interchange between relying on pollinators for reproduction or not has consequences for floral specialization throughout the evolution of a lineage. Gradual and flexible changes in the stamen dimetrism may have allowed Melastomataceae to thrive with a single prevalent pollination strategy, becoming the largest radiation of pollen flowers in angiosperms.

Author contribution

L.R.F.M., A.P.S.C and V.L.G.B. planned the study. L.R.F.M. collected the data. L.R.F.M., M.R., T.V. and V.L.G.B. performed the analyses. L.R. F.M., A.P.S.C., T.V. and V.L.G.B. wrote the first draft of the manuscript. All authors substantially contributed to revision. We have no competing interests.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ppees.2021.125589.

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