Original research article (Full paper)

Title: Floral uniformity through evolutionary time in a species-rich tree lineage.

Short title: Floral uniformity in species-rich lineages.

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SUMMARY

- Changes in floral morphology are expected across evolutionary time and are often promoted as important drivers in angiosperm diversification. Such a statement, however, is in contrast to empirical observations of species-rich lineages that show apparent conservative floral morphologies even under strong selective pressure to change from their environments.
- Here, we provide quantitative evidence for prolific speciation despite uniform floral morphology in a tropical species-rich tree lineage. We analyse floral disparity in the environmental and phylogenetic context of *Myrcia* (Myrtaceae), one of the most diverse and abundant tree genera in Neotropical biomes.
- Variation in floral morphology among *Myrcia* clades is exceptionally low, even among distantly related species. Discreet floral specializations do occur, but these are few, present low phylogenetic signal, have no strong correlation with abiotic factors, and do not affect overall macroevolutionary dynamics in the lineage.
- Results show that floral form and function may be conserved over large evolutionary time-scales even in environments full of opportunities for ecological interactions and niche specialization. Species accumulation in diverse lineages with uniform flowers apparently does not result from shifts in pollination strategies, but from speciation mechanisms that involve other, non-floral plant traits.

Keywords: diversification, extinction, macroevolution, morphospace, *Myrcia,* Myrtaceae.

INTRODUCTION

Tropical forests harbour the most species-rich biomes on earth (Brown, 2014). These lush environments provide endless opportunities for interspecific relationships, powerful sources of selective pressure enhancing species and phenotypic diversity into different ecological niches (Schemske et al., 2009). In angiosperms, a constant cycle of ecological niche opening and filling has resulted in the evolution and diversification of floral strategies (Endress, 1994).

The evolution of the flower and the relationship between plant and pollinator is considered one of the major key innovations in angiosperm evolutionary history (Vamosi and Vamosi, 2010; Van der Niet and Johnson, 2012; Barrett, 2013; Sauquet and Magallón, 2018). Characters of the floral phenotype are tightly linked with pollination efficiency and consequently with overall plant reproductive success (Rosas-Guerrero et al., 2014). As pollinators positively select specific floral traits across evolutionary time (Gervasi and Schiestl, 2017), flowers are under constant and strong selective pressure to change. In this sense, shifts in floral strategy are often observed over an evolutionary time scale (Stebbins, 1970; e.g. O'Meara et al., 2016). In the context of a single lineage, these shifts are frequently linked to changes in species diversification dynamics, accelerating speciation rates if the new floral features increase fitness in a given environmental context (e.g. O'Meara et al., 2016; see Armbruster, 2014, for a review). This principle has been key to argue that changes in floral strategy are among the most important drivers in bursts of angiosperm species diversification (Vamosi et al., 2018).

The appearance of these novel traits, in addition to environmental changes, promotes or demotes lineages in macroevolutionary adaptive landscapes, affecting rates of species diversification and extinction (Sanderson and Donoghue, 1994). The identification of novel traits in the flower that lead to such changes has therefore been central to many plant evolutionary studies in the last decade (e.g. de Vos et al., 2014; Silvestro et al., 2014; Sauquet et al. 2017; Vamosi et al., 2018). Changes in floral strategy and their effect on species turnover (i.e. cycles of species diversification and extinction) can be inferred by examining extant floral morphological diversity (i.e. disparity) relative to molecular-based phylogenetic trees (e.g. Lagomarsino et al., 2016). Consequently, the link between morphological changes of the flower and accelerated species diversification rates is frequently presented in the literature, with numerous studies emphasizing this connection (e.g. Van der Niet et al., 2014; Serrano-Serrano et al., 2017; Lagomarsino et al., 2017).

Contrary to expectation, however, it is notable that a large number of angiosperm lineages present apparent uniform floral morphologies. This trend includes species-rich lineages of woody plants such as *Myrcia*, *Eugenia* (Myrtaceae; Vasconcelos et al., 2018), *Croton* (Euphorbiaceae; Webster, 1993), *Mimosa* (Fabaceae; Barneby, 1991), *Solanum* (Solanaceae; Symon, 1979), some Malpighiaceae (Anderson, 1979), Sapotaceae (Chartier et al., 2017) and *Miconia* (Melastomataceae; Renner, 1989), to cite a few. These groups are crucial components of the woody tropical flora in both abundance and diversity of species (e.g. Bernacci et al., 2004; Murray-Smith et al., 2009) and the uniformity of their floral morphologies, despite considerable species diversification, may be more common than previously thought. Nevertheless, studies have neglected these cases and there is a lack of quantitative studies that investigate floral uniformity over long evolutionary time in species-rich lineages.

In this study, we present quantitative evidence for considerable species diversification in a tropical tree genus without radical changes in flower morphology. We contrast multivariate and macroevolutionary dynamics analyses to demonstrate floral uniformity through evolutionary time in one of the most speciose and abundant Neotropical genera. *Myrcia* (Myrtaceae, Myrtales) is an angiosperm genus of ca. 700 species (WCSP, 2017) and is characterized by inconspicuous and fairly unspecialized flowers that are mostly self-incompatible, pollinator dependent, and do not offer nectar relying only on pollen as pollinator reward (Fig.1; Nic Lughadha and Proença, 1996; Gressler et al., 2006).

Myrcia consistently features among the most species-rich tree genera in biodiversity hotspots of South America (e.g. Cerrado savanna biome: Françoso et al., 2016; and Atlantic Rainforest: Oliveira-Filho & Fontes, 2000). After ca. 30 million years of evolution in these species-rich environments (Mannion et al., 2014; Santos et al., 2017) and assuming that morphological changes arise as lineages diverge in ecological niches (Pfennig & Pfennig, 2010), *Myrcia* would be expected to have developed several specialised floral strategies (e.g. Junker et al., 2013, but see Tobias et al., 2014) and changes in macroevolutionary dynamics. To better understand the absence of these expected evolutionary patterns in the genus, we analyse floral disparity for over 160 species in the macroevolutionary context of *Myrcia*.

MATERIALS AND METHODS

Unless otherwise stated, all analyses were performed using the software R v.3.4.0 (R Core Team, 2017). Functions are referred to as follows: *function name*{*package name*}.

Study group

We select *Myrcia* as study group because: (1) it has a central ecological role in the biomes in which it is most diverse (Neotropical rainforests and savannas), presupposing high levels of interspecific interactions (e.g. it is one of the richest pollen sources for pollinators (Wilms et al., 1996) and fruit sources for vertebrates (Staggemeier et al., 2017) in these biomes), (2) the availability of a series of recent systematic revisions that have significantly increased taxonomic stability (e.g. Santos et al., 2016; Lucas et al. 2016, 2018), and (3) *Myrcia* has diversified into one of the most species-rich areas on the globe, most probably after the establishment of the modern latitudinal gradient of species diversity (Mannion et al., 2014, Santos et al., 2017).

Myrcia is subdivided into nine sections corresponding to clades that have received strong support in independent phylogenetic analyses (e.g. Lucas et al., 2011; Staggemeier et al., 2015; Wilson et al., 2016; Santos et al., 2017; see Fig.1). Reliable estimates of species numbers are available for these nine clades (Lucas et al., 2011; 2018), which is necessary for the evaluation of diversification rates in incomplete phylogenetic datasets (e.g. Rabosky et al., 2014).

Sampling strategy

Species were selected according to the most recent phylogeny for the genus (Santos et al., 2017), based on systematic revisions (Lucas et al., 2018) to represent the broadest possible phylogenetic diversity and geographical distribution. Each clade was represented by at least 10% of its species diversity in the morphological disparity analysis. We included additional samples of some widespread species complexes (e.g. *Myrcia tomentosa*, *M. splendens*) in the morphological disparity analysis; these were not considered pseudo-replicates for the question addressed as high phenotypic plasticity in these complexes suggests that species delimitation is not clear (e.g. Lima et al., 2015). In total, 161 species were sampled (120 of which were also represented in the phylogenetic analyses, see below), corresponding to 22% of *Myrcia* species diversity (Table 1, SI: Methods). For a full list of vouchers see Supporting Information Dataset S1.

Trait measurements and environmental data

After a preliminary survey, we chose a series of floral traits based on the following criteria: (1) the selected traits clearly vary among species, (2) it is possible to record the trait in question for every species, (3) traits can be measured with a dissecting microscope, and (4) they have or may have relevance in reproductive strategy, based on reproductive biology surveys (such as NicLughadha & Proença, 1996; Gressler et al., 2006).

Morphological trait measurements were made on specimens from the Royal Botanic Gardens Kew Herbarium, using, where possible, the same vouchers as used in the phylogenetic reconstruction. Specimens used in the phylogenetic analysis that did not bear buds or flowers were substituted by flowering specimens from similar geographical locations and identifications confirmed by specialists. We selected an average of three buds and three recently opened flowers from each specimen. Buds and flowers were boiled in water for 10 minutes, left to cool overnight and then fixed in 70% ethanol for longer preservation. Each bud and flower was cut longitudinally (Fig.2ai) and structures were measured using a Nikon ShuttlePix model P-400R digital microscope. Sixteen floral traits were measured (A – P, Fig.2aii) and the final measurement for each trait corresponds to the mean measurement of that structure among all measured buds and flowers per specimen. We chose this approach to ease the effect of post-anthetic distortions in floral structures. We also recorded three inflorescence traits (estimated number of flowers; length of main axis; flowers clustered or scattered) and the presence/absence of oil glands on the anthers (a proxy for flower specialization; Armbruster, 2012) from herbarium specimens.

From specimen labels, we extracted information on plant height and two environmental variables: altitude and vegetation type (rainforest vs. savanna). Relative investment in inflorescences was estimated by dividing the mean length of the main inflorescence axis by the plant's mean height. This may be considered a coarse estimate, but the consistent paniculate pattern of the inflorescence in addition to observations in the field show that this approximation makes empirical sense. Additional label data and inflorescence traits were recovered directly from herbarium material. See Supporting Information Methods S1 for details about data collection.

Five percent of the dataset (236 entries) was scored as "missing data" (NA), corresponding to the few cases where no suitable material was available. Because most continuous trait analyses do not allow missing data, missing data was substituted by the corresponding mean trait values for the whole dataset. This imputation method is considered impartial for datasets with NA values below 10% (Shrive et al., 2006).

Phylogeny reconstruction

Our phylogenetic reconstruction is based on one nuclear (ITS) and four chloroplast (psbA-trnH, trnQ-rps16, trnL-trnF, ndhF) markers from the study of Santos et al. (2017; see original publication for GenBank accession numbers). This molecular matrix was used to reconstruct a dated phylogeny in BEAST (Drummond et al., 2012). Substitution models were based on Santos et al. (2017) and calibration parameters followed those of the pollen-fossil

approach and secondary calibration points of Vasconcelos et al. (2017). The final topology is similar to those found in previous studies (Staggemeier et al., 2015; Wilson et al., 2016; Santos et al.; 2017). The resulting tree contains 146 taxa, including 133 ingroup and 13 outgroup taxa, and is available in Supporting Information Methods S1. For phylogenetic signal analysis, the final tree was pruned (using function *drop.tip*{ape}; Paradis et al., 2004) to exclude outgroups.

Phylomorphospace and morphological diversity (disparity)

A representation of the floral morphospace for 161 species of *Myrcia* was built with a principal component analysis (PCA) on the 16 continuous floral measurements in millimetres using the function *PCA*{*FactoMineR*} (Lê et al., 2008). This analysis allowed scoring the effect of each trait on the morphospace distribution. To visualize phylogenetic relationships over the PCA plot, we used the function *phylomorphospace*{*phytools*} (Revell, 2012). This function is based on Sidlauskas (2008) and creates a projection of the phylogenetic tree into a morphospace. In this way, it is possible to visualize how phylogeny tips diverge and converge from ancestral nodes in the morphospace along evolution. Morphological differences among clades were tested with a npMANOVA using the function *adonis*{*vegan*} (Oksanen et al., 2007). This allowed us to show which clades are significantly different from others in the phylomorphospace.

To test for dependence between measurements and phylogenetic relationships, values of Pagel's *lambda* were estimated for each continuous trait with the function *fitContinuous*{geiger} (Harmon et al., 2008). Values of *lambda* closer to 1 indicate stronger phylogenetic signal (Pagel, 1999), i.e. a strong dependence between trait and phylogeny. Finally, a Mantel test (function *mantel*{*vegan*}) was used to compare morphological and phylogenetic distances to identify patterns of phylogenetic signal in our floral data set. For this test, a Euclidean distance matrix was built from the continuous morphological traits and a phylogenetic dissimilarity matrix was estimated using the function *cophenetic.phylo*{*ape*} (Paradis et al., 2004).

To be able to include all traits into the disparity analysis (measurements A – P, plus anther gland and inflorescence categorical traits) a second distance matrix was calculated using the *mean character difference* index (Foote, 1997), following Chartier et al. (2017). Disparity was calculated for each clade on this matrix as the mean pairwise morphological distance between pairs of species belonging to a given clade. For each clade, disparity was

212 further tested for correlation against age and species number using Spearman's rank
213 correlations in function *cor.test*{*stats*}.

214 **Correlations between traits and environmental variables**

215 Morphological variation was investigated in relation to altitude and vegetation type,
216 according to herbarium label (see Supporting Information Methods S1). Traits tested
217 included floral shape (i.e. the "filling" of the morphospace, represented as the PCA of 16
218 floral measurements in millimetres, as seen above), presence of oil glands on anthers, relative
219 investment in inflorescence (i.e. inflorescence length divided by plant height) and
220 inflorescence display (flower number and arrangement on panicle [clustered or scattered])
221 and plant height (in meters). npMANOVA (function *adonis*{*vegan*}; Oksanen et al., 2007)
222 was used to test whether species of similar altitude/vegetation occupy significantly different
223 areas in the morphospace. Kruskal-Wallis rank sum tests (function *kruskal.test*{stats}) were
224 used to test for the correlation between vegetation/altitude, relative investment in
225 inflorescence and number of flowers per inflorescence and plant height. Correlation between
226 vegetation/altitude and presence/absence of anther glands and flowers organised in clusters
227 on the inflorescence was tested with chi-squared tests.

228 **Interpretation of phylogenetic heterogeneity**

229 Analysis of phylogenetic branching patterns allows for the estimation of areas of the
230 phylogenetic tree that show significant variation in diversification or extinction rates
231 (Rabosky, 2006). Increased availability of phylogenetic tree data has been accompanied by
232 increased statistical power to analyze such rate heterogeneity in ultrametric trees (see
233 summary in TESS vignette, Höhna et al., 2015), although not without controversy (e.g.
234 Moore et al., 2016). To infer patterns of phylogenetic heterogeneity, two methods were
235 contrasted; a BAMM analysis (v2.5; Rabosky et al., 2014; 2017) was used to identify
236 significant rate shifts that could be associated with cryptic key innovative phenotypic
237 characters highlighted by morphological analyses. Empirical priors were generated based on
238 the *Myrcia* phylogeny pruned for outgroups and an estimated total diversity of 700 species
239 (WCSP, 2017). Sampling estimates per clade are based on Lucas et al. (2011) and can be
240 accessed in Supporting Information Methods S1. TESS (Höhna et al., 2015) was used to
241 estimate changes in speciation and extinction rates over time and to calculate number of rate
242 shifts based on marginal likelihood and Bayes factors. For TESS, the original phylogeny was
243 rescaled to minimize the effects of clade overrepresentation; tips were randomly pruned from

over-sampled clades prior to analysis (8 from clade *Sympodiomyrcia*, 5 from clade *Guianensis* and 4 from clade *Eugeniopsis*).

RESULTS

Phylomorphospace and phylogenetic signal of floral traits

The phylomorphospace reconstructed on the PCA based on 16 floral traits, shows no visible trend of morphological diversification, with phylogenetic trajectories of the nine subgeneric clades of *Myrcia* overlapping each other (Fig.2b; see Supporting Information Notes S1). In addition, all (n=36) but four pairwise comparisons (post hoc tests) among the nine clades were non-significant (overall npMANOVA, p= 0.014; post hoc tests: see Tab.1), meaning that no group was significantly morphologically different from all the others. The handful of species falling in the edge of the morphospace (highlighted in Fig.2c) increase the overall disparity of the genus, but belong to different clades in the phylogeny. There is, thus, no clade that presents any distinct new combinations of features; new combinations of features are present in a few species scattered throughout the phylogeny. This is confirmed by phylogenetic signal measurements, which is low for most floral trait measurements (all but four traits score Pagel's *lambda* < 0.6; traits F, I, L, and O score Pagel's *lambda* < 0.8; see Supporting Information Notes S1), and a lack of correlation between pairwise morphological dissimilarities and phylogenetic dissimilarities (Mantel statistic, r = 0.01496; Significance = 0.3249). Floral morphological diversity is, therefore, not correlated with phylogenetic distance, which further underlines the lack of a phylogenetic pattern in the evolution of floral shape.

Effects of environmental variables on the evolution of floral traits

Given the strong conservation of floral form, null hypothesis significance tests were performed to uncover possible effects of environmental variables (altitude and vegetation) on floral and inflorescence traits. Almost all results receive no statistical support (Fig.3) and highlight a lack of floral trait variation linked to environmental conditions in *Myrcia*. The only significant correlation shows that the mean relative investment in inflorescence is three times greater in plants occurring in savannas than in rainforests (Fig.3b, Kruskal-Wallis test: p<0.001). *Myrcia* species growing in savanna environments are shorter, consisting mainly of subshrubs and shrubs (Kruskal Wallis ANOVA: p<0.001). This shows constraints to change between distinct biomes, i.e. *Myrcia* shrubs and subshrubs from savanna vegetation present similar inflorescence displays as trees in rainforests (Fig.4).

Correlations among disparity, clade age, and number of species per clade

Floral morphological disparity correlates neither with species number (Fig.5a; Spearman's rank correlation, rho=0.32, p=0.41) nor clade age (Fig.5b; Spearman's rank correlation, rho=0.45, p=0.23). However, when excluding the two outliers (and oldest clades) *Aulomyrcia* and *Calyptranthes* from the analyses, disparity significantly increased with clade age (Fig.5b, Spearman's rank correlation, rho=0.82, $p<0.05$). This suggests eventual stabilization in morphological disparity through time reinforcing a trend to conserve floral morphology in a lineage. Furthermore, the significant increase of species number per clade relative to clade age (Fig.5c, Spearman's rank correlation, rho=0.87, $p<0.01$) indicates that species richness depends on time for species accumulation rather than accelerated species diversification rates. This is also corroborated by macroevolutionary dynamics analyses (see below).

Macroevolutionary dynamics constancy

Our analyses of macroevolutionary dynamics in *Myrcia* indicate a general lack of phylogenetic heterogeneity and support a slow process of species turnover in the genus resulting from low extinction rates. BAMM estimates of diversification rate shifts show no shift in diversification rates and all parts of the tree sharing a similar macroevolutionary dynamic (Fig.6a). TESS results also support constant moderate speciation rate of 0.3 species per million years and, additionally, low extinction rates of less than 0.1 species per million year through time (Fig.6b); these results in addition to the strong correlation between age and total species diversity per clade (Fig.5c) suggest constant and homogeneous accumulation of species diversity throughout the genus over time, without clear increases in rates of diversification or extinction. Despite apparent disparity in species number between clades, variation in species diversity is likely due to the relative older age of some clades. Additional results regarding macroevolutionary analyses can be found in Supporting Information Notes S1.

DISCUSSION

Innovation is not (always) the key: Moving in circles on a long lasting adaptive peak

The species-rich lineage analysed here presents a highly homogeneous floral morphology, with overlapping clades in the phylomorphospace and no obvious floral specializations towards different ecological niches. This trend is unexpected after c. 30 million years of evolution (Santos et al., 2017) in the Neotropics, one of the most biodiverse

environments on earth, full of opportunities for interactions with different pollinators (Brockhurst et al., 2014). If a structure crucial for lineage fitness is constrained and does not change over long periods of evolutionary time, as *Myrcia* flowers are, this is interpreted as an adaptive plateau, or a long-lasting peak in an adaptive landscape (Svensson and Calsbeek, 2012). A similar adaptive plateau has been also considered for floral evolution in other diverse Neotropical groups such as Melastomataceae (Renner, 1989) and Malpighiaceae, where Davis et al. (2014) call the trend a "long-term morphological stasis". Adaptive plateaus in reproductive structures may be crucial to our understanding of why rates of morphological evolution may slow down in certain lineages. This is currently one of the key-questions in studies of angiosperm macroevolution (Sauquet and Magallon, 2018).

Examples of morphological stasis such as *Myrcia* flowers are important to showcase, as in contemporary evolutionary studies, there has been a constant focus on key innovations and shifts between trait states that change macroevolutionary dynamics (e.g. Hunter, 1998; Silvestro et al., 2014, Lagomarsino et al., 2016; Serrano-Serrano et al., 2017). Focussing only on the high frequency of trait shifts during evolution may lead to the assumption that homogeneous phenotypes such as *Myrcia* flowers do not persist across evolutionary time when a lineage is under strong selection (Schluter, 2000). For that reason, highly diverse groups with homogeneous flowers are sometimes thought to result from recent explosive speciation events where there has not been time for the appearance of clear phenotypic disparity (Stebbins, 1974). Our results reinforce that such groups can instead result from a tendency to maintain certain combinations of traits over long periods of time. In the case of *Myrcia*, this evolutionary pattern seems associated to a particularly successful eco-evolutionary relationship (i.e. pollen gathering bee pollination; see discussion below).

Species with distinct combinations of floral traits exist also in *Myrcia* (i.e. the few points scattered around the periphery of the morphospace), but are rare and not related to any particular lineage. These distinct combinations of traits can possibly be associated with evolutionary dead-ends, conferring a short-term adaptive advantage but leading those lineages to extinction before further speciation events could take place (Barrett, 2013). That is, if floral shape changes radically, the adaptive peak is lost and lineages with distinct morphologies tend to disappear (Schluter, 2000; Barrett, 2013).

The presence of macroevolutionary stability (i.e. no significant shifts in diversification rates) also corroborates large-scale stability of overall fitness in these lineages. In this sense, the success of some of the largest tropical angiosperm lineages may be related

to keeping an optimum reproductive strategy over long periods of evolutionary time while being flexible to change in other aspects (see discussion below).

The optimum 'unspecialised' floral strategy of *Myrcia*

The adaptive plateau in the floral morphology of *Myrcia* may be related to a particular pollination system that confers reproductive success in multiple geographical and temporal contexts. Distinct clusterings in a floral morphospace are traditionally interpreted as distinct display strategies (e.g. Chartier et al., 2014; Lagomarsino et al., 2017) and a single cluster of species in the phylomorphospace, as observed in *Myrcia*, indicates that a stable mode of floral display is shared among most species. In this case, these are small, polystemonous, white, open flowers distributed in paniculate inflorescences.

Strong selective pressure to maintain this phenotype appears linked to a generalist melittophilous system that relies on pollen-collecting bees as main functional pollinator. Evidence from reproductive biology studies shows that pollinator guilds and pollination mode are similar throughout the geographic and phylogenetic range of *Myrcia* (see information for 17 species in Supporting Information Notes S1). Bee lineages responsible for successful pollination of *Myrcia* include corbiculates (bumblebees and stingless bees) and, less frequently, the distantly related *Xylocopa* and Halictidae (e.g. Danforth et al., 2006; Fidalgo and Kleinert, 2009; Martins et al., 2014). Stingless bees (Meliponini), the most important pollinators of *Myrcia* flowers, are abundant and conspicuous in the environments where the latter occur. They present social behaviour, requiring large amounts of pollen, frequently collected by buzz behaviour, for maintaining their colony (Wilms et al., 1996; Michener, 2007). The polystemonous, mass-flowering and unspecialized flowers of Myrtaceae (including *Myrcia*) are among the most important pollen sources for these bee lineages in the Neotropics (Wilms et al., 1996; Fidalgo and Kleinert, 2009; Obregon and Nates-Parra, 2014).

This mutualistic bee-flower interaction may have existed since the origin of *Myrcia,* as relevant pollinator groups were already present on South American plateaus (e.g. Brazilian and Guiana shields) during the Oligocene (Rasmussen and Cameron, 2010; Camargo, 2013), potential areas of early-diversification in *Myrcia* (Santos et al., 2017). The abundance of these bees throughout the distribution range of *Myrcia* and the success of this relationship may have been the main reason for the maintenance of the relatively unspecialised floral shape over evolutionary time.

Alternatives to plant-pollinator interaction as driving force for plant speciation

The optimum floral strategy in *Myrcia* and its association with widespread generalist bees probably allows reproductive success of these plants to be maintained in a multitude of different conditions across geography and time. The remarkable species richness may then have resulted from keeping a constant successful floral strategy that confers lineage growth continuity, corroborated by estimated low extinction rates.

Pollination ecology may explain low extinction rates in *Myrcia* but does not alone explain high species diversity. *Myrcia* presents a net-diversification rate of ca. 0.28 species per million years, with an absolute speciation rate of ca. 0.3 (Fig.6). Such numbers are below those estimated for lineages that have undergone recent explosion in speciation rates, such as the Andean Centropogonids (Lagomarsino et al., 2016) and *Lupinus* (Hughes et al., 2006), but are comparable to those of Asterales, which have the highest speciation rates among angiosperms orders (Magallón and Sanderson, 2001). Since changes in pollination strategy do not appear to be driving diversification in this group, other selective pressures must be examined to explain high speciation rates and species accumulation through time in *Myrcia*. Assuming species estimates are correct (i.e. there is no taxonomic inflation), the elevated number of *Myrcia* species must be explained by flexibility to change in other traits of the plant that allow adaptation to distinct environmental factors (e.g. see Webster, 1993, and Arévalo et al., 2017, for *Croton*); it is likely that speciation mechanisms will be explained by factors unrelated to pollination, as sympatric species of *Myrcia* all share similar pollinators and floral morphological disparity is low.

Reproductive isolation and speciation may be achieved by other means in *Myrcia*. Fruits in *Myrcia* are always fleshy berries and are also not highly variable in shape (Lucas et al., 2011), but changes in epidermal and anatomical composition (Galan et al., 2016) promote variation in colour and texture, subtly changing display and dispersal mode. These fruits are dispersed by a diversity of animals, mainly birds and mammals (Gressler et al., 2006; Staggemeier et al., 2017). Dispersal by vertebrates frequently moves seed germination far from the parental plant, promoting colonization of new habitats and causing geographical isolation between populations, leading to allopatric speciation (Coyne and Orr, 2004). This mode of pre-zygotic reproductive isolation, in addition to the apparent lack in niche specificity (as *Myrcia* species are present in most South American biomes, Santos et al., 2017), may be a key-driver in steady speciation rates of *Myrcia*.

Once populations are found in allopatry, other selective forces may act, leading to changes in vegetative traits that make these distinct evolutionary units recognised as different

411 species of *Myrcia*. Vegetative structures, such as leaves, are indeed extremely variable in
412 size, texture and thickness (e.g. Silva Moraes et al., 2017). Growth habit varies from small
413 subshrubs of c. 10 cm to trees of 40 m, sometimes even in closely related species (e.g. Santos
414 et al., 2016; Silva Moraes et al., 2017). Furthermore, there is evidence for high levels of
415 diversity of chemical compounds in *Myrcia* leaves (e.g. Stefanello et al., 2011), reflecting
416 selective pressure from herbivores and natural enemies that is very strong in tropical areas
417 (Schemske et al., 2009). Pressures from herbivores as drivers of speciation have been
418 suggested for *Inga* (Fabaceae), a genus of similar floral homogeneity (Kursar et al., 2009) but
419 much younger age (Richardson et al., 2001). This flexibility in habit and vegetative traits may
420 have been also critical for *Myrcia* species to diversify and colonize even the least hospitable
421 Neotropical biomes (e.g. the "Dry Diagonal" of South America, Simon et al., 2009). As these
422 newly formed species secondarily expand their distribution and are occasionally found in
423 sympatry again, it is possible that their genetic differences are high enough to prevent gene
424 flow even when occasional cross-pollination happens between closely related species (i.e.
425 post-zygotic isolation; see similar case in Cozzolino and Widmer, 2005).
426 Allopatric speciation seems to be a reasonable explanation when closely related
427 species share pollinators, especially when they also present similar flowering phenology, as
428 many *Myrcia* species do (Staggemeier et al. 2010). However, sympatric speciation via subtle
429 changes in reproductive phenology (including both anthesis time and flowering season)
430 cannot be discarded until thorough studies aiming to test these hypotheses are performed (e.g.
431 see Savolainen et al., 2006). Furthermore, actual pollinator observations in the field are
432 indispensible to confirm the speciation mechanisms suggested here.
433
434 **Conclusion**
435 Previous studies may have placed too much emphasis on the consequences of floral
436 morphological changes for high rates of angiosperm diversification. These changes appear
437 not to be the strongest driver of plant speciation in many species-rich tropical tree lineages.
438 Species diversification in *Myrcia* and other species-rich lineages with homogeneous flowers
439 seems to be unrelated to shifts in pollination strategy. A generalized but highly efficient
440 pollination system has apparently reached an adaptive plateau early during the evolution of
441 the genus, thereby forming the basis for the long-lasting stable diversification process
442 involving various non-floral traits. The origins of high species diversity in the absence of
443 floral change are important when considering evolution of tropical plant diversity. The key to
444 the success of some of the largest Neotropical angiosperm lineages may have been building

445 remarkable species richness via simple variations within a theme on top of an advantageous
446 adaptive plateau.
447
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458 T.N.C.V. and E.L. designed the research and generated the dataset. T.N.C.V and M.C.
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461
462 REFERENCES
463 **Anderson WR. 1979.** Floral conservatism in neotropical Malpighiaceae. *Biotropica* **11**: 219–
464 223.

465 **Arévalo R, van Ee BW, Riina R, Berry PE, Wiedenhoeft AC. 2017.** Force of habit:
466 shrubs, trees and contingent evolution of wood anatomical diversity using *Croton*
467 (Euphorbiaceae) as a model system. *Annals of Botany* **119**: 563–579.

468 **Armbruster WS. 2012.** Evolution and ecological implications of "specialized" pollinator
469 rewards. In: Patiny S, eds. *Evolution of plant-pollinator relationships.* Cambridge, UK:
470 Cambridge University Press, 44–67.

471 **Armbruster WS. 2014.** Floral specialization and angiosperm diversity: phenotypic
472 divergence, fitness trade-offs and realized pollination accuracy. *AoB Plants* **6:** pii: plu003

473 **Barrett SCH. 2013.** The evolution of plant reproductive systems: how often are transitions
474 irreversible? *Proceedings of the Royal Society of London B* **280:** 20130913.

Barneby RC. 1991. Sensitivae censitae: a *description of the genus* Mimosa *Linnaeus (Mimosaceae) in the New World. Memoirs of The New York Botanical Garden Volume 65.* New York, NY, USA: The New York Botanical Gardens Press.

Bernacci LC, Durigan G, Correia G, Arbocz G, Catharino E, Metzger JPM. 2004. Composição florística e estrutura da vegetação em fragmentos florestais do Planalto de Ibiúna. Anexo 7. In: Metzger JP, eds, *Conservação da Biodiversidade em Paisagens Fragmentadas no Planalto Atlântico de São Paulo.* São Paulo, Brazil: FAPESP 99/05123-4 technical report.

Brockhurst MA, Chapman T, King KC, Mank JE, Paterson S, Hurst GD. 2014. Running with the Red Queen: the role of biotic conflicts in evolution. *Proceedings of the Royal Society of London B* **281**: 20141382.

Brown JH. 2014. Why are there so many species in the tropics? *Journal of Biogeography* **41:** 8–22.

Camargo JMF. 2013. Historical biogeography of the Meliponini (Hymenoptera, Apidae, Apinae) of the Neotropical region. In: Vit P, Pedro S, Roubik D, eds. *Pot-Honey.* Berlin, Germany: Springer, 19–34.

Cardoso D, Särkinen T, Alexander S, Amorim AM, Bittrich V, Celis M, Daly DC, Fiaschi P, Funk VA, Giacomin LL et al. 2017. Amazon plant diversity revealed by a taxonomically verified species list. *Proceedings of the National Academy of Sciences of the USA* **114**: 10695–10700.

Chartier M, Jabbour F, Gerber S, Mitteroecker P, Sauquet H, von Balthazar M, Staedler Y, Crane PR, Schönenberger J. 2014. The floral morphospace – a modern comparative approach to study angiosperm evolution. *New Phytologist* **204:** 841–853.

Chartier M, Löfstrand S, von Balthazar M, Gerber S, Jabbour F, Sauquet H, Schönenberger J. 2017. How (much) do flowers vary? Unbalanced disparity among flower functional modules and a mosaic pattern of morphospace occupation in the order Ericales. *Proceedings of the Royal Society of London B* **284:** 20170066.

Coyne JA, Orr HA. 2004. *Speciation*. Sunderland, MA: Sinauer Associates.

Cozzolino S, Widmer A. 2005. Orchid diversity: an evolutionary consequence of deception? *Trends in Ecology and Evolution* **20:** 487–494.

505 **Danforth BN, Sipes S, Fang J, Brady SG. 2006.** The history of early bee diversification
506 based on five genes plus morphology. *Proceedings of the National Academy of Sciences of*
507 *the USA* **103:** 15118–23

508 **Davis CC, Schaefer H, Zhenxiang X, Baum DA, Donoghue MA, Harmon LJ. 2014.** Long
509 term morphological stasis maintained by a plant-pollinator mutualism. *Proceedings of the*
510 *National Academy of Sciences of the USA* **111**: 5914–5919

511 **Endress PK. 1994.** *Diversity and evolutionary biology of tropical flowers.* Cambridge, UK:
512 Cambridge University Press.

513 **Fidalgo ADO, Kleinert ADM. 2009.** Reproductive biology of six Brazilian Myrtaceae: is
514 there a syndrome associated with buzz-pollination? *New Zealand Journal of Botany* **47**: 355–
515 365.

516 **Foote M. 1997.** The evolution of morphological diversity. *Annual Review of Ecology,*
517 *Evolution and Systematics* **28:** 129–152.

518 **Françoso RD, Haidar RF, Machado RB. 2016.** Tree species of South America central
519 savanna: endemism, marginal areas and the relationship with other biomes. *Acta Botanica*
520 *Brasilica* **30**: 78–86.

521 **Galan ATOF, Martos L, Machado NC, Mourão KSM. 2016.** A survey of ontogeny of
522 pericarp features as contribution to the infratribal characterization of Myrteae (Myrtaceae).
523 *Nordic Journal of Botany* **34:** 596–604.

524 **Gervasi DD, Schiestl FP. 2017.** Real-time divergent evolution in plants driven by
525 pollinators. *Nature Communications* **8:** 14691.

526 **Gressler E, Pizo MA, Morellato LPC. 2006.** Polinização e dispersão de sementes em
527 Myrtaceae do Brasil. *Brazilian Journal of Botany* **29:** 509–530.

528 **Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W. 2008.** GEIGER: investigating
529 evolutionary radiations. *Bioinformatics* **24**: 129–131.

530 **Höhna S, May MR, Moore BR. 2015.** TESS: an R package for efficiently simulating
531 phylogenetic trees and performing Bayesian inference of lineage diversification rates.
532 *Bioinformatics* **32**: 789–791.

533 **Hunter JP. 1998.** Key innovations and the ecology of macroevolution. *Trends in Ecology*
534 *and Evolution* **13**: 31–36.

Junker RR, Blüthgen N, Brehm T, Binkenstein J, Paulus J, Martin Schaefer H, Stang, M. 2013. Specialization on traits as basis for the niche-breadth of flower visitors and as structuring mechanism of ecological networks. *Functional Ecology* **27:** 329–341.

Kursar TA, Dexter KG, Lokvam J, Pennington RT, Richardson JE, Weber MG, Murakami ET, Drake C, McGregor R, Coley PD. 2009. The evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus Inga. *Proceedings of the National Academy of Sciences of the USA* **106:** 18073–18078.

Lagomarsino LP, Condamine FL, Antonelli A, Mulch A, Davis CC. 2016. The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). *New Phytologist* **210**: 1430–1442.

Lagomarsino LP, Forrestel EJ, Muchhala N., Davis CC. 2017. Repeated evolution of vertebrate pollination syndromes in a recently diverged Andean plant clade. *Evolution* **71**: 1970–1985.

Lê S, Josse J, Husson F. 2008. FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software* **25:** 1–18.

Lima DF, Mauad AVS, da Silva-Pereira V, Smidt EC, Goldenberg R. 2015. Species boundaries inferred from ISSR markers in the *Myrcia laruotteana* complex (Myrtaceae). *Plant Systematics and Evolution* **301:** 353–363.

Lucas EJ, Matsumoto K, Harris SA, Nic Lughadha EM, Bernardini B, Chase MW. 2011. Phylogenetics, morphology, and evolution of the large genus *Myrcia* s.l. (Myrtaceae). *International Journal of Plant Sciences* **172:** 915–934.

Lucas E, Wilson CE, Lima DF, Sobral M, Matsumoto K. 2016. A conspectus of *Myrcia* sect. *Aulomyrcia* (Myrtaceae). *Annals of the Missouri Botanical Garden* **101:** 648–698.

Lucas EJ, Amorim BS, Lima DF, Lima-Lourenço AR, Nic Lughadha EM, Proença CEB, Rosa PO, Rosário AS, Santos LL, Santos MF. et al. 2018. A new infra-generic classification of the species-rich Neotropical genus *Myrcia* s.l. *Kew Bulletin* **73:** 9.

Magallón S, Sanderson MJ. 2001. Absolute diversification rates in angiosperm clades. *Evolution* **55:** 1762–1780.

Mannion PD, Upchurch P, Benson RB, Goswami A. 2014. The latitudinal biodiversity gradient through deep time. *Trends in Ecology and Evolution* **29:** 42–50.

565 **Martin CH, Wainwright PC. 2013.** Multiple fitness peaks on the adaptive landscape drive
566 adaptive radiation in the wild. *Science* **339**: 208–211.

567 **Martins AC, Melo GA, Renner SS. 2014.** The corbiculate bees arose from New World oil-
568 collecting bees: Implications for the origin of pollen baskets. *Molecular Phylogenetics and*
569 *Evolution* **80:** 88–94.

570 **Michener CD. 2007.** *The Bees of the World*, 2nd ed. Baltimore, MD, USA: The John
571 Hopkins University Press.

572 **Moore BR, Höhna S, May MR, Rannala B, Huelsenbeck JP. 2016.** Critically evaluating
573 the theory and performance of Bayesian analysis of macroevolutionary mixtures.
574 *Proceedings of the National Academy of Sciences of the USA* **113:** 9569–9574.

575 **Murray-Smith C, Brummitt NA, Oliveira-Filho AT, Bachman S, Moat J, NicLughadha**
576 **E, Lucas EJ. 2009.** Plant diversity hotspots in the Atlantic coastal forests of Brazil.
577 *Conservation Biology* **23:** 151–163.

578 **NicLughadha E, Proença C. 1996.** A survey of the reproductive biology of the Myrtoideae
579 (Myrtaceae). *Annals of the Missouri Botanical Garden* **83:** 480–503.

580 **Obregon D, Nates-Parra G. 2014.** Floral preference of *Melipona eburnea* Friese
581 (Hymenoptera: Apidae) in a Colombian Andean region. *Neotropical Entomology* **43:** 53–60.

582 **Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson GL, Solymos P, Stevens MHH,**
583 **Wagner H. 2007.** The vegan package. *Community ecology package* **10:** 631–637.

584 **Oliveira-Filho AT, Fontes MAL. 2000.** Patterns of floristic differentiation among Atlantic
585 forests in southeastern Brazil and the influence of climate. *Biotropica* **32:** 793–810.

586 **O'Meara BC, Smith SD, Armbruster WS, Harder LD, Hardy CR, Hileman LC,**
587 **Hufford L, Litt A, Magallón S, Smith SA, et al. 2016.** Non-equilibrium dynamics and
588 floral trait interactions shape extant angiosperm diversity *Proceedings of the Royal Society of*
589 *London B* **283:** 20152304

590 **Pagel M. 1999.** Inferring the historical patterns of biological evolution. *Nature* **401:** 877–
591 884.

592 **Paradis E, Claude J, Strimmer K. 2004.** APE: analyses of phylogenetics and evolution in R
593 language. *Bioinformatics* **20:** 289–290.

Pfennig K, Pfennig D. 2009. Character displacement: ecological and reproductive responses to a common evolutionary problem. *The Quarterly Review of Biology* **84:** 253–276.

R Core Team. 2017 (version 3.4.0). A Language and Environment for Statistical Computing R Foundation for Statistical Computing, Vienna, Austria.

Rabosky DL. 2006. Likelihood methods for detecting temporal shifts in diversification rates. *Evolution* **60:** 1152–1164.

Rabosky DL, Grundler M, Anderson C, Shi JJ, Brown JW, Huang H, Larson JG. 2014. BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods in Ecology and Evolution* **5:** 701–707.

Rabosky DL, Mitchell JS, Chang J. 2017. Is BAMM flawed? Theoretical and practical concerns in the analysis of multi-rate diversification models. *Systematic Biology* **66:** 477–498.

Rasmussen C, Cameron SA. 2010. Global stingless bee phylogeny supports ancient divergence, vicariance and long distance dispersal. *Biological Journal of the Linnean Society* **99:** 206–232.

Renner SS. 1989. A survey of reproductive biology in Neotropical Melastomataceae and Memecylaceae. *Annals of the Missouri Boanical Gardens* **76:** 496–518.

Revell LJ. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3:** 217–223.

Rosas-Guerrero V, Aguilar R, Martén-Rodríguez S, Ashworth L, Lopezaraiza-Mikel M, Bastida JM, Quesada M. 2014. A quantitative review of pollination syndromes: do floral traits predict effective pollinators? *Ecology Letters* **17:** 388–400.

Richardson JE, Pennington RT, Pennington TD, Hollingsworth PM. 2001. Rapid diversification of a species-rich genus of Neotropical rain forest trees. *Science* **293:** 2242–2245.

Sanderson MJ, Donoghue MJ. 1994. Shifts in diversification rate with the origin of angiosperms. *Science* **264:** 1590–1593.

Santos MF, Sano PT, Forest F, Lucas E. 2016. Phylogeny, morphology and circumscription of *Myrcia* sect. *Sympodiomyrcia* (*Myrcia* s.l., Myrtaceae). *Taxon* **65:** 759–774.

Santos MF, Lucas E, Sano PT, Buerki S, Staggemeier VG, Forest F. 2017. Biogeographical patterns of *Myrcia* s.l. (Myrtaceae) and their correlation with geological and climatic history in the Neotropics. *Molecular Phylogenetics and Evolution* **108:** 34–48.

Sauquet H, von Balthazar M, Magallón S, Doyle JA, Endress PK, Bailes EJ, Barroso de Morais E, Bull-Hereñu K, Carrive L, Chartier M, et al. 2017. The ancestral flower of angiosperms and its early diversification. *Nature Communications* **8:** 16047.

Sauquet H, Magallón S. 2018. Key questions and challenges in angiosperm macroevolution. *New Phytologist*. (in press)

Savolainen V, Anstett MC, Lexer C, Hutton I, Clarkson JJ, Norup MV, Powell MP, Springate D, Salamin N, Baker WJ. 2006. Sympatric speciation in palms on an oceanic island. *Nature* **441:** 210.

Schluter D. 2000. *The ecology* of adaptive radiation. Oxford, UK: Oxford University Press.

Shrive FM, Stuart H, Quan H, Ghali WA. 2006. Dealing with missing data in a multi-question depression scale: a comparison of imputation methods. *BMC Medical Research Methodology* **6:** 57.

Serrano-Serrano ML, Rolland J, Clark JL, Salamin N, Perret M. 2017. Hummingbird pollination and the diversification of angiosperms: an old and successful association in Gesneriaceae. *Proceedings of the Royal Society of London B* **284:** 20162816.

Sidlauskas B. 2008. Continuous and arrested morphological diversification in sister clades of characiform fishes: a phylomorphospace approach. *Evolution* **62:** 3135–3156.

Silva Moraes AC, Vitória AP, Rossatto DR, de Miranda LDAP, Funch LS. 2017. Leaf phenology and morphofunctional variation in *Myrcia amazonica* DC.(Myrtaceae) in gallery forest and "campo rupestre" vegetation in the Chapada Diamantina. *Brazilian Journal of Botany* **40:** 439–450.

Silvestro D, Zizka G, Schulte K. 2014. Disentangling the effects of key innovations on the diversification of Bromelioideae (Bromeliaceae). *Evolution* **68:** 163–175.

Simon MF, Grether R, de Queiroz LP, Skema C, Pennington RT, Hughes CE. 2009. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proceedings of the National Academy of Sciences of the USA* **106:** 20359–20364.

Schemske DW, Mittelbach GG, Cornell HV, Sobel JM, Roy K. 2009. Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution and Systematics* **40:** 245–269.

Staggemeier VG, Diniz-Filho JAF, Forest F, Lucas E. 2015. Phylogenetic analysis in *Myrcia* section *Aulomyrcia* and inferences on plant diversity in the Atlantic rainforest. *Annals of Botany* **115:** 747–761.

Staggemeier VG, Cazetta E, Morellato LPC. 2017. Hyperdominance in fruit production in the Brazilian Atlantic rain forest: the functional role of plants in sustaining frugivores. *Biotropica* **49:** 71–82.

Stebbins GL. 1970. Adaptive radiation of reproductive characteristics in angiosperms, I: pollination mechanisms. *Annual Review in Ecology, Evolution and Systematics* **1**: 307–326.

Stebbins GL. 1974. *Plant species. Evolution above the species level.* Cambridge, MA, USA: Harvard University Press.

Stefanello MEA, Pascoal AC, Salvador MJ. 2011. Essential oils from neotropical Myrtaceae: chemical diversity and biological properties. *Chemistry & Biodiversity* **8:** 73–94.

Svensson E, Calsbeek R. 2012. *The adaptive landscape in evolutionary biology*. Oxford, UK: Oxford University Press.

Symon DE. 1979. Sex forms in *Solanum* (Solanaceae) and the role of pollen collecting insects. In Hawkes JG, Lester RN, Skelding AD, eds. *The Biology and Taxonomy of the Solanaceae.* London, UK: Academic Press, London. 385–397.

Tobias JA, Cornwallis CK, Derryberry EP, Claramunt S, Brumfield RT, Seddon N. 2014. Species coexistence and the dynamics of phenotypic evolution in adaptive radiation. *Nature* **506:** 359–363.

Vamosi JC, Vamosi SM. 2010. Key innovations within a geographical context in flowering plants: towards resolving Darwin's abominable mystery. *Ecology Letters* **13:** 1270–1279.

Vamosi JC, Magallón S, Mayrose I, Otto SP, Sauquet H. 2018. Macroevolutionary patterns of flowering plant speciation and extinction. *Annual Review of Plant Biology* **69**: 685–706.

Van der Niet T, Johnson SD. 2012. Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology & Evolution* **27:** 353–361.

Van der Niet T, Peakall R, Johnson SD. 2014. Pollinator-driven ecological speciation in plants: new evidence and future perspectives. *Annals of Botany* **113:** 199–212.

Vasconcelos TNC, Proença CEB, Ahmad B, Aguilar DS, Aguilar R, Amorim BS, Campbell K, Costa IR, De-Carvalho PS, Faria JEQ, et al. 2017. Myrteae phylogeny, calibration, biogeography and diversification patterns: Increased understanding in the most species rich tribe of Myrtaceae. *Molecular Phylogenetics and Evolution* **109:** 113–137.

Vasconcelos TNC, Lucas EJ, Faria JEQ, Prenner G. 2018. Floral heterochrony promotes flexibility of reproductive strategies in the morphologically homogeneous genus *Eugenia* (Myrtaceae). *Annals of Botany* **121:** 161–174.

de Vos JM, Hughes CE, Schneeweiss GM, Moore BR, Conti E. 2014. Heterostyly accelerates diversification via reduced extinction in primroses. *Proceedings of the Royal Society of London B* **281:** 20140075.

WCSP. 2017. World Checklist of Selected Plant Families. Facilitated by the Royal Botanic Gardens, Kew. Published on http://apps.kew.org/wcsp/.<apps.kew.org/wcsp/>. [Accessed 1 August 2017]

Webster GL. 1993. A provisional synopsis of the sections of the genus *Croton* (Euphorbiaceae). *Taxon* **42:** 793–823

Wilms W, Imperatriz-Fonseca VL, Engels W. 1996. Resource partitioning between highly eusocial bees and possible impat of the introduced Africanized honey bee on native stingless bees in the Brazilian atlantic rainforest. *Studies on Neotropical Fauna and Environment* **31:** 137–151.

Wilson CE, Forest F, Devey DS, Lucas EJ. 2016. Phylogenetic Relationships in *Calyptranthes* (Myrtaceae) with particular emphasis on its monophyly relative to *Myrcia* sl. *Systematic Botany* **41**: 378–386.

Table 1. Results from NPANOVA showing degree of dissimilarity between clades (sections) based on morphospace analyses. Values above the diagonal represent F values and those below the diagonal show relationships that are not significantly different (ns); asterisks mark those with p<0.01 (significantly distinct clades). aul = *Aulomyrcia*, cal = *Calyptranthes*, myr = *Myrcia*, tom = *Tomentosa*, ret = *Reticulosae*, gom = *Gomidesia*, sym= *Sympodiomyrcia*, agu = *Aguava*, eug = *Eugeniopsis*.

	ret	cal	sym	myr	gom	tom	agu	eug	aul
ret	<NA>	3.542	2.115	0.86	1.572	4.376	2.433	1.577	4.957
cal	Ns	<NA>	0.208	4.269	6.632	8.978	1.316	0.276	3.829
sym	Ns	ns	<NA>	3.029	3.986	5.597	0.987	-0.072	1.223
myr	Ns	ns	ns	<NA>	3.132	8.879	2.322	2.447	9.151
gom	Ns	*	ns	ns	<NA>	18.253	5.485	3.615	8.627
tom	Ns	ns	ns	ns	ns	<NA>	4.779	4.569	10.294
agu	ns	ns	ns	ns	ns	ns	<NA>	0.306	5.101
eug	ns	ns	ns	ns	ns	ns	ns	<NA>	1.767
aul	ns	ns	ns	*	*	*	ns	ns	NA

FIGURE LEGENDS

Figure 1: Floral similarity across the *Myrcia* phylogeny (phylogeny based on Santos et al., 2017). Section names are given for the nine clades with consistent bootstrap and posterior probability support (crown nodes marked with black dots). (a) *Myrcia rubella* (section *Aulomyrcia*); (b) *M. linearifolia* (section *Myrcia*); (c) *M. nivea* (clade *Aguava*); (d) *M. multipunctata* (section *Eugeniopsis*); (e) *M. mutabilis* (clade *Sympodiomyrcia*); (f) *Calyptranthes brasiliensis* (clade *Calyptranthes*). Scale bar in (a) to (f) = 5mm. Species name abbreviations: "M." – *Myrcia*, "Ma." – *Marlierea*, "C." – *Calyptranthes*.

Figure 2: Change of floral form through evolutionary time in *Myrcia*. (a) Floral measurements; (a-i) flower of *Myrcia rubella* in longitudinal section, (a-ii) schematic drawing of flower showing the 16 (A – P) traits measured. (b) Floral phylomorphospace showing distribution of species in multivariate space according to flower structure and phylogenetic relationships; (c) twelve species placed at the periphery of the morphospace are shown in red. Scale bar in (a) = 5 mm. The nine infrageneric sections are color-coded in (b) as follows: yellow = *Aulomyrcia*, blue = *Calyptranthes*, gray = *Myrcia*, pink = *Tomentosa*,

732 black = *Reticulosae*, green = *Gomidesia*, orange = *Sympodiomyrcia*, purple = *Aguava*, red =
733 *Eugeniopsis*.
734 **Figure 3:** Correlation between floral traits and environmental variables in *Myrcia*. (a)
735 Species distribution in the morphospace is not correlated with either altitude or the type of
736 vegetation (NA represents missing data for vegetation type); (b) relative inflorescence
737 investment is not correlated with altitude, but significantly increases in savanna vegetation
738 (boxplots represents: thick bars – median, error bars – range of observations excluding
739 outliers, transparent dots – outliers); (c) estimated number of flowers per inflorescence, (d)
740 flower clustering in the inflorescence and (e) presence/absence of anther oil gland are not
741 correlated with either altitude or the environment. Analyses of significance value in "a" are
742 based on a perMANOVA, in "b" are based on Kruskal-Wallis ANOVA; and in "c", "d" and
743 "e" are based on chi-squared test. Non-significance ("ns") was considered for $p>0.05$.
744 **Figure 4:** Biome transition from rainforest (i) to savanna (ii) does not significantly affect
745 floral traits, but plant habit decreases substantially in savanna biomes (Kruskal-Wallis
746 ANOVA; $p<0.001$) increasing investment in inflorescence relative to plant size (see also
747 Fig.3b).
748 **Figure 5:** Spearman's rank correlation contrasting: (a) Disparity and species diversity
749 (rho=0.32, p=0.54), (b) Disparity and crown age (rho=0.45, p= 0.21, for all datasets;
750 rho=0.82, $p<0.001$, when the two oldest clades (in gray) are excluded); and (c) clade crown
751 age and species diversity (rho=0.87, $p<0.01$). Abbreviations for the nine sections in *Myrcia*
752 are as follows: aul = *Aulomyrcia*, cal = *Calyptranthes*, myr = *Myrcia*, tom = *Tomentosa*, ret
753 = *Reticulosae*, gom = *Gomidesia*, sym= *Sympodiomyrcia*, agu = *Aguava*, eug = *Eugeniopsis*.
754 **Figure 6:** Speciation rates in *Myrcia*: (a) BAMM phylorate showing no evidence for shifts in
755 diversification rates. Clade crown-nodes are marked by a black dot. (b) Oscillation in
756 speciation and extinction rates during the last 25 million years in *Myrcia* (inferred by TESS)
757 showing (i) an intermediate rate of speciation with no significant acceleration over time and
758 (ii) a continuous low extinction rate. Abbreviations for the nine sections in *Myrcia* are as
759 follows: aul = *Aulomyrcia*, cal = *Calyptranthes*, myr = *Myrcia*, tom = *Tomentosa*, ret =
760 *Reticulosae*, gom = *Gomidesia*, sym= *Sympodiomyrcia*, agu = *Aguava*, eug = *Eugeniopsis*.
761
762 SUPPORTING INFORMATION
763 Supporting Information Dataset S1 – Trait Dataset.
764 Supporting Information Methods S1 – Additional details on methods.
765 Supporting Information Notes S1 – Additional data analyses information.