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1 **Original research article (Full paper)**

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

3 **Short title:** Floral uniformity in species-rich lineages.

4

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22

23 SUMMARY

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

42

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

44

45

46 INTRODUCTION

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

53 The evolution of the flower and the relationship between plant and pollinator is
54 considered one of the major key innovations in angiosperm evolutionary history (Vamosi and
55 Vamosi, 2010; Van der Niet and Johnson, 2012; Barrett, 2013; Sauquet and Magallón, 2018).
56 Characters of the floral phenotype are tightly linked with pollination efficiency and
57 consequently with overall plant reproductive success (Rosas-Guerrero et al., 2014). As
58 pollinators positively select specific floral traits across evolutionary time (Gervasi and
59 Schiestl, 2017), flowers are under constant and strong selective pressure to change. In this
60 sense, shifts in floral strategy are often observed over an evolutionary time scale (Stebbins,
61 1970; e.g. O'Meara et al., 2016). In the context of a single lineage, these shifts are frequently
62 linked to changes in species diversification dynamics, accelerating speciation rates if the new
63 floral features increase fitness in a given environmental context (e.g. O'Meara et al., 2016;
64 see Armbruster, 2014, for a review). This principle has been key to argue that changes in
65 floral strategy are among the most important drivers in bursts of angiosperm species
66 diversification (Vamosi et al., 2018).

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

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

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

99 *Myrcia* consistently features among the most species-rich tree genera in biodiversity
100 hotspots of South America (e.g. Cerrado savanna biome: Françoso et al., 2016; and Atlantic
101 Rainforest: Oliveira-Filho & Fontes, 2000). After ca. 30 million years of evolution in these
102 species-rich environments (Mannion et al., 2014; Santos et al., 2017) and assuming that
103 morphological changes arise as lineages diverge in ecological niches (Pfennig & Pfennig,
104 2010), *Myrcia* would be expected to have developed several specialised floral strategies (e.g.
105 Junker et al., 2013, but see Tobias et al., 2014) and changes in macroevolutionary dynamics.
106 To better understand the absence of these expected evolutionary patterns in the genus, we
107 analyse floral disparity for over 160 species in the macroevolutionary context of *Myrcia*.

108

109 MATERIALS AND METHODS

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

112 **Study group**

113 We select *Myrcia* as study group because: (1) it has a central ecological role in the
114 biomes in which it is most diverse (Neotropical rainforests and savannas), presupposing high
115 levels of interspecific interactions (e.g. it is one of the richest pollen sources for pollinators
116 (Wilms et al., 1996) and fruit sources for vertebrates (Staggemeier et al., 2017) in these
117 biomes), (2) the availability of a series of recent systematic revisions that have significantly
118 increased taxonomic stability (e.g. Santos et al., 2016; Lucas et al. 2016, 2018), and (3)
119 *Myrcia* has diversified into one of the most species-rich areas on the globe, most probably
120 after the establishment of the modern latitudinal gradient of species diversity (Mannion et al.,
121 2014, Santos et al., 2017).

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

128 **Sampling strategy**

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

140 **Trait measurements and environmental data**

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

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

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

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

174 **Phylogeny reconstruction**

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

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

186 **Phylomorphospace and morphological diversity (disparity)**

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

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

207 To be able to include all traits into the disparity analysis (measurements A – P, plus
208 anther gland and inflorescence categorical traits) a second distance matrix was calculated
209 using the *mean character difference* index (Foote, 1997), following Chartier et al. (2017).
210 Disparity was calculated for each clade on this matrix as the mean pairwise morphological
211 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

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

246 RESULTS

247 **Phylomorphospace and phylogenetic signal of floral traits**

248 The phylomorphospace reconstructed on the PCA based on 16 floral traits, shows no
249 visible trend of morphological diversification, with phylogenetic trajectories of the nine
250 subgeneric clades of *Myrcia* overlapping each other (Fig.2b; see Supporting Information
251 Notes S1). In addition, all (n=36) but four pairwise comparisons (post hoc tests) among the
252 nine clades were non-significant (overall npMANOVA, $p = 0.014$; post hoc tests: see Tab.1),
253 meaning that no group was significantly morphologically different from all the others. The
254 handful of species falling in the edge of the morphospace (highlighted in Fig.2c) increase the
255 overall disparity of the genus, but belong to different clades in the phylogeny. There is, thus,
256 no clade that presents any distinct new combinations of features; new combinations of
257 features are present in a few species scattered throughout the phylogeny. This is confirmed by
258 phylogenetic signal measurements, which is low for most floral trait measurements (all but
259 four traits score Pagel's $\lambda < 0.6$; traits F, I, L, and O score Pagel's $\lambda < 0.8$; see
260 Supporting Information Notes S1), and a lack of correlation between pairwise morphological
261 dissimilarities and phylogenetic dissimilarities (Mantel statistic, $r = 0.01496$; Significance =
262 0.3249). Floral morphological diversity is, therefore, not correlated with phylogenetic
263 distance, which further underlines the lack of a phylogenetic pattern in the evolution of floral
264 shape.

265 **Effects of environmental variables on the evolution of floral traits**

266 Given the strong conservation of floral form, null hypothesis significance tests were
267 performed to uncover possible effects of environmental variables (altitude and vegetation) on
268 floral and inflorescence traits. Almost all results receive no statistical support (Fig.3) and
269 highlight a lack of floral trait variation linked to environmental conditions in *Myrcia*. The
270 only significant correlation shows that the mean relative investment in inflorescence is three
271 times greater in plants occurring in savannas than in rainforests (Fig.3b, Kruskal-Wallis test:
272 $p < 0.001$). *Myrcia* species growing in savanna environments are shorter, consisting mainly of
273 subshrubs and shrubs (Kruskal Wallis ANOVA: $p < 0.001$). This shows constraints to change
274 between distinct biomes, i.e. *Myrcia* shrubs and subshrubs from savanna vegetation present
275 similar inflorescence displays as trees in rainforests (Fig.4).

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

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

288

289 **Macroevolutionary dynamics constancy**

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

303

304 **DISCUSSION**

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

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

310 environments on earth, full of opportunities for interactions with different pollinators
311 (Brockhurst et al., 2014). If a structure crucial for lineage fitness is constrained and does not
312 change over long periods of evolutionary time, as *Myrcia* flowers are, this is interpreted as an
313 adaptive plateau, or a long-lasting peak in an adaptive landscape (Svensson and Calsbeek,
314 2012). A similar adaptive plateau has been also considered for floral evolution in other
315 diverse Neotropical groups such as Melastomataceae (Renner, 1989) and Malpighiaceae,
316 where Davis et al. (2014) call the trend a “long-term morphological stasis”. Adaptive plateaus
317 in reproductive structures may be crucial to our understanding of why rates of morphological
318 evolution may slow down in certain lineages. This is currently one of the key-questions in
319 studies of angiosperm macroevolution (Sauquet and Magallon, 2018).

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

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

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

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

345

346 **The optimum ‘unspecialised’ floral strategy of *Myrcia***

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

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

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

376

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

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

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

398 Reproductive isolation and speciation may be achieved by other means in *Myrcia*.
399 Fruits in *Myrcia* are always fleshy berries and are also not highly variable in shape (Lucas et
400 al., 2011), but changes in epidermal and anatomical composition (Galan et al., 2016) promote
401 variation in colour and texture, subtly changing display and dispersal mode. These fruits are
402 dispersed by a diversity of animals, mainly birds and mammals (Gressler et al., 2006;
403 Staggemeier et al., 2017). Dispersal by vertebrates frequently moves seed germination far
404 from the parental plant, promoting colonization of new habitats and causing geographical
405 isolation between populations, leading to allopatric speciation (Coyne and Orr, 2004). This
406 mode of pre-zygotic reproductive isolation, in addition to the apparent lack in niche
407 specificity (as *Myrcia* species are present in most South American biomes, Santos et al.,
408 2017), may be a key-driver in steady speciation rates of *Myrcia*.

409 Once populations are found in allopatry, other selective forces may act, leading to
410 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 indispensable 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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456

457 AUTHOR CONTRIBUTION

458 T.N.C.V. and E.L. designed the research and generated the dataset. T.N.C.V and M.C.
459 analysed the data. T.N.C.V and E.L. wrote the paper. M.C., A.M., G.P., J.S., and A.W.
460 contributed with further discussion and writing of the manuscript.

461

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708

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

715

	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

716

717 **FIGURE LEGENDS**

718 **Figure 1:** Floral similarity across the *Myrcia* phylogeny (phylogeny based on Santos et al.,
719 2017). Section names are given for the nine clades with consistent bootstrap and posterior
720 probability support (crown nodes marked with black dots). (a) *Myrcia rubella* (section
721 *Aulomyrcia*); (b) *M. linearifolia* (section *Myrcia*); (c) *M. nivea* (clade *Aguava*); (d) *M.*
722 *multipunctata* (section *Eugeniopsis*); (e) *M. mutabilis* (clade *Sympodiomyrcia*); (f)
723 *Calyptranthes brasiliensis* (clade *Calyptranthes*). Scale bar in (a) to (f) = 5mm. Species name
724 abbreviations: “M.” – *Myrcia*, “Ma.” – *Marlierea*, “C.” – *Calyptranthes*.

725 **Figure 2:** Change of floral form through evolutionary time in *Myrcia*. (a) Floral
726 measurements; (a-i) flower of *Myrcia rubella* in longitudinal section, (a-ii) schematic
727 drawing of flower showing the 16 (A – P) traits measured. (b) Floral phylomorphospace
728 showing distribution of species in multivariate space according to flower structure and
729 phylogenetic relationships; (c) twelve species placed at the periphery of the morphospace are
730 shown in red. Scale bar in (a) = 5 mm. The nine infrageneric sections are color-coded in (b)
731 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 = *Calyptanthes*, 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 = *Calyptanthes*, myr = *Myrcia*, tom = *Tomentosa*, ret =
760 *Reticulosae*, gom = *Gomidesia*, sym = *Sympodiomyrcia*, agu = *Aguava*, eug = *Eugeniopsis*.

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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.