1 **From tree tops to the ground: reversals to terrestrial habit in *Galeandra* orchids**
2 **(Epidendroideae: Catasetinae)**
3
4 **Authors:** Aline C. Martins[1*], Thuane Bochorny[2], Oscar A. Pérez-Escobar[3], Guillaume
5 Chomicki[4,5], Silvana H. N. Monteiro[6]& Eric de Camargo Smidt[1]
6

7 *1. Universidade Federal do Paraná, Setor de Ciências Biológicas, Centro Politécnico,*

8 *Jardim da Américas, Curitiba - PR, 81531-980. C. P. 19031, Brazil.*

9 *2. Universidade Estadual de Campinas, Programa de Pós-Graduação em Biologia*

10 *Vegetal, Campinas - SP, 13083-970. C.P. 6109, Brazil.*

11 *3. Identification and Naming Department, Royal Botanic Gardens Kew, Richmond,*

12 *TW9 3AB, UK*

13 *4. Department of Plant Sciences, University of Oxford, South Park Road, Oxford OX1*

14 *3RB, UK.*

15 *5. The Queen's college, High St, Oxford OX1 4AW, UK.*

16 *6. Universidade Estadual de Feira de Santana, Programa de Pós-Graduação em*

17 *Botânica, Departamento de Ciências Biológicas, BR 116N km 3, 44031-460 Feira de*

18 *Santana, BA, Brazil*

19

20 **Correspondence author:* martinsalinec@gmail.com
21

22
23

24

Abstract

The colonization of the epiphytic niche of tropical forest canopies played an important role in orchid's extraordinary diversification in the Neotropics. However, reversals to the terrestrial habit occurred sparsely in species of Epidendroideae. To better understand which factors might have been involved in reversals to terrestrial habits in the predominantly epiphytic Epidendroideae, we investigate *Galeandra* diversification in time and space. We hypothesized that the reversal to the terrestrial habitat is linked to the origin of open vegetation habitats in South America. We generated a time-calibrated phylogeny based on a matrix of 17 out of 20 species of *Galeandra* plus outgroups and seven DNA markers. We found that *Galeandra* originated towards end of the Miocene, about 10 Ma in Amazonia (including the Guiana Shield). The terrestrial clade originated synchronously with the rise of dry vegetation biomes, suggesting that aridification during the last 5 million years dramatically impacted the diversification of epiphytic lineages in the Neotropics. Habit is correlated with floral spur lengths and geographic range size. The longer spurs and narrower ranges are found in epiphytic species: probably adapted to a specialized pollination mode, associated to the long-tongued Euglossini bees, which also prefer forested habits. The terrestrial species presents variable floral spurs and wide distribution ranges, with evidence of self-pollination, suggesting the loss of specialized pollination system and concomitant range expansion.

Key-words: Orchids, Amazonia, ancestral area estimation, molecular dating, South American arid diagonal

51
52 **Introduction**
53
54   The colonization of the epiphytic niche in tropical forest canopies played an

55 important role in the extraordinary diversification of orchids in the Neotropics (Benzing,

56 1987; Gentry and Dodson, 1987; Givnish et al. 2015). Epiphyte microsite specialization

57 together with biotic and abiotic variables including pollinator shifts, CAM

58 photosynthesis, and presence in cordilleras have been proposed as the main drivers of

59 orchid (Givnish et al., 2015; Pérez-Escobar et al., 2017a). The origin of epiphytism in

60 land plants, including orchids, ferns, and leafy liverworts, followed the establishment of

61 angiosperm-dominated canopies in the Cenozoic, possibly facilitated by climate change

62 in the Paleocene/Eocene border (Chomicki et al., 2015a; Feldberg et al., 2014;

63 Schuettpelz and Pryer, 2009)

64   Orchids are the most diverse group among epiphytes, accounting for 68%

65 (19,000) of the 27,600 vascular epiphyte species (Gentry and Dodson, 1987; Zotz,

66 2013). The evolution of epiphytism may have enhanced orchid diversification by

67 allowing the conquest of new, largely unoccupied niches (Givnish et al., 2015), and

68 often terrestrial orchid lineages are less diverse than the epiphytic (Gravendeel et al.,

69 2004). The epiphytic habit offers the option of colonizing a larger heterogeneity of

70 habitats: all the surface of branches, twigs and bark. However it requires adaptation to

71 low substrate stability, limited nutrient and water supplies, and to colonize the

72 patchiness biotope (Laube and Zotz, 2003). Thus, the canopy is difficult to colonize and

73 only a plant with a complex suite of adaptations could survive as an epiphyte (Benzing,

74 1987). Orchid adaptations to tree bark includes root with layer(s) of dead cells known as

75 velamen, which enhance water and nutrient absorption and protects photosynthetic roots

76 against UV-B radiation (Chomicki et al., 2015a), and thick succulent leaves and stems

77 that store water (Freudenstein and Chase, 2015).

78   In Orchidaceae, epiphytism evolved at least four to seven times over the

79 past 43 million yr and was possibly lost about seven to ten times (Chomicki et al.,

80 2015). In the species-richest subfamily Epidendroideae the epiphytic habit

81 predominates, yet the ancestral condition in orchid is clearly terrestrial (Chomicki et al.,

82 2015; Freudenstein and Chase, 2015). Reversals to the terrestrial habit occurred many

83 times in Epidendroideae, including the Collabieae (Xiang et al., 2014), *Dendrobium*

84 (Xiang et al., 2016), most *Eulophia* (Martos et al., 2014), *Cyanaeorchis* (Batista et al.,

85 2014), *Bletia+Hexalectris+Basiphyllaea* (Sosa et al., 2016), many Pleurothallidinae

86 (Freudenstein and Chase, 2015), Malaxideae (Cameron, 2005) and *Galeandra*

87 (Catasetinae). Although most reversals are associated to species-poor lineages, some are

88 associated to speciose clades, potentially resulting from rapid diversification (Cameron,

89 2005). The reversals to terrestrial habit are still poorly understood, but they might

90 involve deep changes in morphological adaptations (Zhang et al., 2017) such as the loss

91 or reduction of the velamen (Chomicki et al., 2015a) or occupation of new habitats

92 (Sosa et al., 2016). Some of these adaptations are possibly linked to the presence of

93 AGL12 gene, which are otherwise lost in epiphytic orchids (Zhang et al., 2017).

94   To better understand the factors that might have influenced the reversal to

95 the terrestrial habits in a linage within the predominantly epiphytic Epidendroideae, we

96 investigate *Galeandra* diversification dynamics, climatic preferences, flower

97 morphology and area of occurrence, using phylogenetic comparative methods.

98 *Galeandra* is a widely distributed genus in the Neotropical region, ranging from

99 southern Florida to northern Argentina, and five of its *ca* 20 species are terrestrial

100 (Monteiro et al., 2010). *Galeandra* species occur across a wide range of biogeographic

101 regions, mainly Amazonia, Cerrado savannahs and Atlantic Forest. Epiphytic

102 *Galeandra* usually occupy more restricted distribution ranges, and occurs in forested

103 areas (e.g. Amazonia), while the terrestrial species occupy open vegetation ecosystems

104 (e.g. Cerrado, excep *G. beyrichii* which inhabits in forest areas, especially altitude

105 forests). The origin of the savannas worldwide, including the south American Cerrado,

106 coincides with the gradual cooling that started at the Miocene climatic optimum

107 (~15Ma) (Zachos et al., 2001), and they fully established about 5 Ma (Simon et al.,

108 2009). Terrestrial *Galeandra* might have occupied these novel environments only

109 recently. This raises the question whether terrestrial *Galeandra* are adapted to cooler

110 and dryer habitats than epiphytic species occurring in rainforests. Further, it is not clear

111 whether the origin of terrestrial *Galeandra*, and potential biome shifts (from forested to

112 open savannas) occurred synchronously to the origin of the Cerrado.

113   Morphological differences of floral characters can be also observed in

114 terrestrial and epiphytic *Galeandra*. Flowers of epiphytic *Galeandra* possess a long and

115 filiform spur, while terrestrial taxa exhibit flowers with short and saclike spurs

116 (Monteiro et al., 2010). Long floral spurs usually produce nectar or oil, and are accessed

117 by an enlarged part of pollinator's body (e.g tongues), but also legs (Steiner and

118 Whitehead, 1990; Whittall and Hodges, 2007). Floral spur enlargement is usually linked

119 to pollinators shifts, and it has been hypothesized as an adaptive response to predefined

4

120   pollinator morphology (Whittall and Hodges, 2007). The extent to which different floral

121   morphologies are associated to distinct habitats is unknown, but habitat preference

122   might have driven the evolution of particular pollination syndromes.

123   To investigate *Galeandra* diversification in time and space, and potential

124   associated floral trait shifts, we used DNA sequences from Monteiro *et al.* (2010) plus

125   newly generated sequence data of *Galeandra,* including a denser outgroup sampling.

126   We aim to answer the following questions: (*i*) when and where did *Galeandra*

127   originate? (*ii*) When did reversals to terrestrial habits occur, and where and under which

128   habitat? (*iii*) Was habitat shift correlated with shifts in floral morphology, range size or

129   climatic niche?

130

131   **Material and methods**

132

133   *Phylogenetics and dating analysis*

134   Our study builds upon the sampling of Monteiro et al. (2010) for *Galeandra*

135   plus newly generated sequences for *G. leptoceras* and *G. macroplectra*, totalizing 17

136   (out of 20) species in the genus. The outgroup sampling was also enlarged to better

137   accommodate molecular dating calibrations (see below), comprising representatives of

138   all Catasetinae genera, including *Cyrtopodium* and *Eulophia,* and newly generated

139   sequences of *Catasetum, Clowesia, Cyanaeorchis* and *Cycnoches*. Voucher information

140   and GenBank numbers are presented in Table S1. DNA extraction, PCR conditions and

141   sequencing methods are described in Monteiro *et al.* (2010).

142   The final matrix consisted of 31 taxa and 6014 nucleotides for five plastid

143   (*ycf1, psb*A-*trn*H, *rpo*B-*trn*C and *trn*S-*trn*G), and two nuclear (xdh, ITS and ETS)

144   markers. Alignments were performed in MAFFT v. 7 (Katoh and Standley, 2013), with

145   default parameters except for the plastid markers which were aligned using the G-INS-i

146   strategy following recommendations for sequences with global homology. ITS and ETS

147   which were aligned using the Q-INS-i strategy, which considers the secondary RNA

148   structure (Katoh and Standley, 2013). The alignments were manually edited in Geneious

149   6.0 (Biomatters, 2013) to correct obvious alignment errors. In the absence of supported

150   (Maximum Likelihood Bootstrap Support [MLBS] > 75%) phylogenetic incongruence

151   between plastid and nuclear markers, the matrices were concatenated, also in Genious.

152   Prior to molecular dating analysis, we performed Maximum Likelihood

153   searches and compared our results with a previously published phylogeny of *Galeandra*

154   (Monteiro et al., 2010) and Catasetinae (Pérez-Escobar et al., 2017a). Maximum

155   likelihood tree searches and bootstrapping of the combined dataset using 1000 replicates

156   were performed in RAxML v. 8 (Stamatakis, 2006) using the graphical user interface

157   raxmlGUI 1.3.1 (Silvestro and Michalak, 2012), under the GTR + G model of DNA

158   evolution.

159   We subsequently time-calibrated our phylogeny, relying on the same matrix

160   of four plastid and three nuclear markers, comprising 31 taxa and 6014 nucleotides,

161   using a Bayesian relaxed-clock approach implemented in BEAST 1.8.3 (Drummond et

162   al., 2012). Absolute divergence times were estimated under the GTR+ G substitution

163   model, and Yule tree speciation model. The Markov Chain Monte Carlo (MCMC) chain

164   was run for 50 million generations, sampling every 10000 generations. Orchids appear

165   to have diverged from the common ancestor of all other members of Asparagales in the

166   Cretaceous around 110 Ma and the crown group ca. 90 Ma, and upper Epidendroids

167   diverged in the Paleogene, around 50 Ma (Chomicki et al., 2015). There are four

168   unambiguous Orchidaceae macrofossils, but none of them is assigned to taxa closely

169   related to Catasetinae (Ramírez et al., 2007). Therefore, the phylogeny of *Galeandra*

170   was secondarily calibrated based on the age obtained by Chomicki *et al.* (2015) for

171   Catasetinae's crown group of 19.8 (95% Highest Posterior Density Interval [95% HPD]:

172   14.68-25.73 Ma). These ages are perfectly congruent with (Givnish et al., 2015). A

173   normal distribution prior was applied on the Most Recent Common Ancestor (MRCA)

174   of Catasetinae, with Mean=19.8 and Stdev=3 (95% HPD 14.87 – 24.73). A maximum

175   clade credibility tree was summarized in TreeAnnotator v. 1.8.0 (part of BEAST

176   package) with a 25% burn-in, when effective sample size (ESS) for all parameters were

177   superior to 200, as assessed in Tracer 1.5. Trees were visualized and initially edited in

178   FigTree 1.4.0 (Rambaut, 2009).

179

180   *Ancestral area estimation*

181   Species ranges were coded from the literature and from herbarium specimens

182   (ALCB, AMES, AMO, B, BM, BR, F, INPA, K, K-L, NY, P, PORT, RB, S, US and W

183   - abbreviations according to Index Herbariorum (http://sweetgum.nybg.org/science/ih/).

184   Biogeographical areas were derived from literature, as well as from distribution patterns

185   observed in other plant lineages (e.g. Rubiaceae: Antonelli *et al.*, 2009; Bromeliaceae:

186   Givnish *et al.*, 2011; *Cycnoches*: (Pérez-Escobar et al., 2017c). We coded the

6

187   geographical range of *Galeandra* as: A = Central America, B = Chocó, C = Amazonia,

188   D = Guiana Shield, E = Dry Diagonal and F = Atlantic Forest; Fig. S1 shows coded

189   biogeographical regions. Specimens without reliable geographic locality or with

190   dubious identification were excluded.

191   For Ancestral Area Estimation (AAE), we relied on the R package

192   BioGeoBEARS (Matzke, 2013), which evaluates several biogeographic models

193   altogether to test for the contribution of evolutionary processes (i.e., range expansion,

194   range extinctions, vicariance, founder-event speciation, within-area speciation) to

195   explain the distribution of modern species. We analyzed independently the models DEC

196   (Ree and Smith, 2008), a modified version of DIVA (Ronquist, 1997), named DIVA-

197   like, and a modified version of BayArea (Landis et al., 2013) or BayArea-like, adding

198   separately the founder-speciation parameter *j*. We assessed the overall fitness of the

199   models conducting likelihood ratio tests based on AICc scores. *Galeandra macroplectra*

200   was excluded from biogeographic analysis due to negative branch lengths.

201

202   *Range size*

203   To determine if range size is associated to plant habit, we evaluate

204   distribution ranges of all *Galeandra* species sampled in our phylogeny. Distribution

205   ranges were obtained by literature and herbarium specimens' examination, as stated in

206   the previous section. We calculated the Extent of Occurrence (EOO) and Area of

207   Occupancy (AOO) for each species using GeoCAT (Bachman et al. 2011,

208   http://geocat.kew.org). EOO represents the defined area contained within the shortest

209   imaginary boundary drawn to encompass all the known sites of occurrence of a taxon,

210   often measured by a minimum convex polygon; AOO represents the area within its

211   "extent of occurrence" which is occupied by a taxon, usually calculated by the sum of

212   all square grids in which the species were registered (IUCN, 2013). Because EOO

213   extrapolates the area of occurrence of a species, we choose AOO for our analysis. The

214   Table S5 shows the values of AOO measured and used in the analysis.

215

216   *Ancestral state estimation of spur length*

217   Fifteen out of the 20 know species of *Galeandra* (17 included in our DNA

218   sequence matrix) were included in the analysis. We obtained minimum, mean and

219 maximum values of spur length and width (Figure S1) from herbarium specimens and
220 literature (Monteiro et al., 2010). Whenever possible, we gathered measures from at
221 least five individuals per species. Table S6 provides a list with all measurements of
222 species studied and herbarium specimens sourced. Maximum Likelihood Ancestral
223 State Estimation (ASE) of mean spur length values was conducted using an ultrametric
224 tree derived from dating analysis (see above) and the function *contMap* of the R
225 package 'phytools' (Revell, 2012). In addition, to investigated the evolution of spur
226 length through time, we produced a traitgram (Evans et al., 2009) by plotting our
227 ultrametric tree as function of time (from root age to present) and phenotype (i.e. spur
228 length) using the function *phenogram* of the package 'phytools'. Uncertainties of the
229 ASEs were explored by plotting the probability density of the ancestral estimation in the
230 traitgram.
231
232 *Correlation tests*
233 We further test for the correlated evolution between plant habit (0=terrestrial,
234 1=epiphytic), and spur length under a quantitative genetic threshold model (Wright,
235 1934; Felsenstein, 2012). This model is applied to discrete variables (e.g. viviparity:
236 Lambert & Wiens, 2013; feeding mode in fishes: Revell, 2013), whose probability of
237 state change is associated to an underlying continuous variable. Correlation analysis
238 was implemented on a Bayesian framework for 1,000,000 generations, with a sampling
239 fraction of 100 generations using the function *threshBayes* in the R package 'Phytools'
240 (Revell, 2012).
241
242 *Phylomorphospace*
243
244 To visualize relationship between the spur length, range size and plant habit,
245 while simultaneously accounting for phylogenetic relationship, we generated a
246 morphospace using spur length and range size as continuous variables. To this end, we
247 relied on the function *phylomorphospace* of the R package phytools (Revell, 2012).
248
249 *Climatic variables*
250 We mapped 639 georeferenced collection records obtained from floras, GBIF
251 database and herbarium specimens (mean 37, maximum number of record per species
252 157), and they represent the known distribution of *Galeandra* and extant species

8

253   included in our taxon sampling. To query GBIF database, we relied on the function *occ*

254   of the R package SPOCC (Chamberlain, 2016). We further extracted corresponding

255   values of elevation and 19 climatic variables (30 seconds resolution) reflecting

256   temperature and precipitation regimes from the WorldClim database (available at:

257   http://www.worldclim.org/current; Hijmans et al., 2005), using the function *extract* of

258   the R package RASTER (Hijmans, 2016).

259

260   *Non-metric dimensional scaling analyses*

261   To avoid spurious results arising from inclusion of correlated variable, we

262   determined the Pearson's correlation coefficients between the bioclim variables and

263   altitude and then included only variables with a Pearson's correlation coefficient <0.5,

264   taking a single variable in correlated clusters. This way, we selected the bioclimatic

265   variables 1 (Annual mean temperature), 2 (Mean diurnal temperature range), 12

266   (Annual precipitation), 13 (Precipitation of wettest week), 14 (Precipitation of driest

267   week), and 18 (Precipitation of warmest quarter). We analysed these variables using

268   the R-package VEGAN (Oksanen et al., 2007) to perform non-metric dimensional

269   scaling analyses (NMDS) using the dataset of 657 georeferenced herbarium specimens.

270   To ask whether (i) epiphytic versus terrestrial *Galeandra*, and (ii) short-spurred versus

271   long-spurred *Galeandra* had different niches, we computed the 95% confidence

272   intervals for each group. Overlap between confidence intervals suggests the absence of

273   significant niche differentiation among groups.

274

275   **Results**

276

277   *Phylogeny* of Galeandra *and time of origin of terrestrial and epiphytic clades*

278

279   Our matrix of 31 taxa and 6014 nucleotides for four plastid and three

280   nuclear genes yielded a tree with the same relationships found by Monteiro *et al.*,

281   (2010) analysis for *Galeandra* species (Fig. S1). Our enlarged outgroup sampling

282   scheme represents the genus level relationships in the tribe Catasetinae with high

283   support for the core Catasetinae *sensu* (Dressler, 1983)*.* In our phylogeny, *Galeandra* is

284   sister group to the core Catasetinae (*Catasetum*, *Clowesia*, *Cycnoches*, *Mormodes*,

285   *Dressleria*) plus *Grobya+Cyanaeorchis* (but larger datasets indicate

9

286 *Grobya*+*Cynaeorchis* as sister to *Galeandra*+coreCatasetinae (Batista et al., 2014;

287 Pérez-Escobar et al., 2017a, 2017b, 2016, 2015). *Galeandra devoniana* was recovered

288 as sister to the remaining *Galeandra* species, which is split into a terrestrial and a

289 epiphytic clades, both well supported (ML bootstrap support = 100). Absolute age

290 estimation (Fig. 1, Fig. S2) yielded a root age (i.e. the split between *Eulophia* and

291 *Cyrtopodium*+Catasetina) of 29 Ma (95% HPD: 17-44). The MRCA of *Galeandra* plus

292 the core Catasetinae age is 17 Ma (95% HPD = 11-23), and the crown group of

293 *Galeandra* originated in the Miocene ca. 9 Ma (95% HPD: 5-13). The terrestrial clade

294 and epiphytic clade diverged from each other in the late Miocene about 7 Ma (95%

295 HPD: 4-11) (Fig 1). The epiphyte clade included species from Amazonia, Mexico,

296 Venezuela, and Guianas, while the terrestrial clade encompassed species occurring in

297 the Dry Diagonal of South America, e.g. Cerrado, and open environments in Colombia

298 and Venezuela. The terrestrial and epiphytic clades crown ages are approximately 4 Ma

299 (95% HPD: 2-6). Most of the adaptive radiation in *Galeandra* occurred in the Pliocene-

300 Pleistocene border (>3 Ma). The Mexican clade of epiphytes diverged 2 (1-5) Ma.

301

302 *Geographic origin of terrestrial habit and biogeographic history of* Galeandra

303

304   BioGeoBEARS multi-model approach yielded DEC+j as the best fitting

305 model for the *Galeandra* phylogeny (Tab. S3 provides AICc values of all

306 biogeographical models tested). The MRCA of *Galeandra* is inferred to have lived in an

307 area encompassing Amazonia and Guiana Shield (Fig. 1). The ancestral area for the

308 terrestrial clade, which has at least one widespread species, *G. beyrichii*, included

309 Amazonia, Dry Diagonal and Atlantic Forest. On the other hand, the MRCA of the

310 epiphytic clade is restricted to Northern South America, i.e. Amazonia and Guiana

311 Shield. Among the epiphytic *Galeandra,* only *G. blanchetii* occurs in (and is restricted

312 to) open vegetation of the Dry Diagonal.

313

314 *Terrestrial habit origin and correlates with spur length evolution, range size and*

315 *climatic niche*

316   Figure 2 shows the continuous trait map of floral spur evolution in *Galeandra,*

317 highlighting the marked differences in spur length between the terrestrial and the

318 epiphytic clades. Short spur length was the ancestral condition in *Galeandra* flowers

10

319 (Fig. 2A), being the shortest in *G. devoniana*. The terrestrial clade shows an

320 intermediate pattern of floral spur length. *Galeandra beyrichi* is the species that presents

321 the shortest spur in this clade. The MRCA of the epiphytic clade had longer floral spurs,

322 and *G. magnicolumna* is the species with the largest floral spurs. The traitgram in Fig.

323 2B (and density traitgram in Fig. S4) shows the floral spur length mapping in time,

324 highlighting the different rate of evolution of the considered states (short and long spur).

325 We observed a shift in morphological rates at the base of the long-spurred clade of

326 *Galeandra* (epiphytic species) (Fig. 2B). The terrestrial and short-spurred clade presents

327 a higher rate of morphological change.

328   The figure 3 presents the results of analysis of phylomorphospace

329 correlating floral spur length, range size and habit. Here, epiphytic species formed a

330 cluster of lineages with narrow range sizes (up to 200 km^2) and longer floral spur (2-2.5

331 cm). Only one species, *Galeandra devoniana,* does not integrate this group, but presents

332 an isolated pattern of correlation between a short spur length and moderately small

333 range size. The terrestrial species, on the other hand, do not form a cohesive group.

334 They present no pattern of range size or floral spur length.

335   The NDMS analysis found only the bioclimatic variables 1, 2, 12, 13, 14

336 and 18 to be non-correlated (Fig. S5). We detected no niche differentiation between

337 terrestrial and epiphytic *Galeandra* neither between long and short-spurred *Galeandra*

338 (Fig. S6 and Fig. S7).

339

340 **Discussion**

341

342 *Miocene origin of* Galeandra *in Amazonia*

343

344   *Galeandra* arose towards the Late Miocene, about 10 Ma, close to the climatic

345 optimum when several plant clades in the Neotropical Region originated (Antonelli et

346 al., 2009; Gustafsson et al., 2010; Hoorn et al., 2010; Pérez-Escobar et al., 2017c). At

347 that time, the Guiana and the Brazilian Shields became large islands. A period of

348 intensified mountain uplift started at the same time in Northern, promoting the origin

349 and extinction of mega wetlands in Amazonia, and shifting the drainage of the Amazon

350 Basin eastwards (Antonelli et al., 2009; Hoorn et al., 2010).

351   Our ancestral area estimation analyses suggest the MRCA of *Galeandra*

352 inhabited in Amazonia (including the Guiana Shield) towards the Late Miocene, at a

353 period where the region occupied a very large part of South America, extending as far

11

354   as South Parana region (Hoorn et al., 2010). Early diversification of *Galeandra*

355   occurred in forested areas, while diversification of terrestrial lineages in *Galeandra*

356   might have taken place in both forested areas and the Dry Diagonal, influenced by the

357   rise of dry vegetation biomes. The extend of tropical forests in South America changed

358   with the Andean uplift and its consequences on regional climate, as well as with global

359   climate changes, which favored the establishment of the Dry vegetation areas in South

360   America (Antonelli et al., 2009; Posadas and Ortiz-Jaureguizar, 2016).

361   The expansion of open vegetation biomes started by the end of Miocene,

362   resulting in the formation of large biomes in the Pliocene such as the Cerrado (Simon et

363   al., 2009), and the establishment of complex system of dry vegetation biomes. Such dry

364   areas are mostly arranged in the dry diagonal of South America, but with accessory

365   patches occurring in all Neotropical Region (Werneck, 2011). In the case of Cerrado,

366   the boundaries appear to have been porous to the migration and recruitment of lineages

367   from a range of wet and dry forest vegetation types. Adjacent biomes including the

368   Amazonia and Atlantic wet forests, tropical and subtropical dry thorn scrub (Caatinga

369   and Chaco), subtropical grasslands, and wetlands have all contributed to the recruitment

370   of Cerrado lineages. The migration of lineages from surrounding ecosystems was

371   facilitated by its nested distribution and enhanced the striking Cerrado's species

372   richness (Simon et al., 2009). The MRCA of the terrestrial *Galeandra* (4 Ma: HDP 2-6)

373   could have originated on the tropical forests or on the recently formed open vegetation

374   areas. In spite of beginning the diversification about 10 Ma, most plant lineages

375   characteristic of Cerrado diversified only recently, 4 Ma or less (Simon et al., 2009).

376   Therefore, the diversification of both clades (epiphytes and terrestrial) is congruent with

377   this time of drastic transformation in the distribution of forest and open vegetation.

378   However, only species with terrestrial habitat were able to occupy the recently formed

379   biomes, at the same time expanding their distribution. Today, terrestrial *Galeandra*

380   occupy several different open vegetation areas, not only the core Cerrado vegetation,

381   but also costal dunes in Brazil, savannas of Venezuela, islands of savanna in Amazon

382   and even shaded humid forests, in the case of *G. beyrichii*, which probably possess

383   secondary adaptation to forests (Monteiro et al., 2010).

384   Tropical broadleaf forests provide a plethora of niches for epiphytic plants, and

385   both origins of epiphytism and these forests are profoundly connected (Schuettpelz and

386   Pryer, 2009). Therefore, it is not surprising that the early diversification of the epiphytic

387   clade of *Galeandra* occurred on the forested areas (Amazonia and Guiana Shield), until

12

388 the Pliocene. In the Quaternary, the Dry Diagonal area became important for this group
389 too. These epiphytes do not occupy the open vegetation, but the gallery forests, which
390 are riverine forests forming narrow strips along the river valleys in the Cerrado biome
391 (Oliveira-Filho and Ratter, 1995). Many plants and animal species from Amazonian or
392 Atlantic Forest domains crosses the Cerrado through those gallery forests, some
393 expanding their distribution within Cerrado (Costa, 2003) and several authors have
394 pointed to affinities between the woody flora of the Cerrado and the Atlantic and
395 Amazon rain forests (Gottsberger and Silberbauer-Gottsberger, 2006; Oliveira-Filho
396 and Fontes, 2000). In the case of *Galeandra*, the gallery forests are occupied by
397 Amazonian species, reinforcing the relatedness of Amazon and gallery forests (Oliveira-
398 Filho and Ratter, 1995; Pennington et al., 2006).

399 In the epiphytic clade, a dispersal to Central America occurred about 3 Ma (1-5),
400 possibly following the final closure of the Isthmus of Panama, finally formed only 2.8
401 Ma (O'Dea et al., 2016, but see Jaramillo et al., 2017). The union of both land masses
402 started an exchange of fauna and flora (called GABI or Great American Biotic
403 Interchange – see (Cody et al., 2010)), but for plants there are several evidences of
404 previous dispersals between South and Central/North America (Bacon et al., 2013;
405 Olmstead, 2013). *Galeandra* species might have dispersed overseas as it happened for
406 other orchid species (Renner, 2004), by land, facilitated by the recently formed
407 connection of the Isthmus of Panama or by long distance dispersal across the Andes.

408
409 *Habit, floral spur length and range size*
410
411 Changes from epiphytic to terrestrial habitat might have played a role in
412 *Galeandra* ecological requirements far beyond light and water levels, and may have
413 also affected interactions with bee pollinators. Epiphytic species presents longer floral
414 spurs, which can be associated to the evolution with long tongued pollinators, such as
415 Euglossini bees, the primary pollinators of Catasetinae orchids (Dressler, 1982; Ramírez
416 et al., 2011).

417 Pollination observations in *Galeandra* flowers are scarce, limited to some
418 punctual observations of pollinaria attached to male orchid bee's body (i.e. fragrance
419 seeking) (Pearson and Dressler, 1985; Romero-Gonzalez and Warford, 1995) or
420 "Anthophoridae" bees (possibly *Xylocopa*) (nectar or pollen seeking) (Chase and Hills,
421 1992; Romero-Gonzalez and Warford, 1995). However, how *Galeandra* attract their
422 pollinators remains a mystery. Floral spurs in epiphytic *Galeandra* apparently lack any

13

423   nutritional reward to pollinators, indicating a possible deceptive attraction, very
424   common in orchids (Ackerman, 1986; Jersáková et al., 2006; Nilsson, 1998; Pansarin
425   and Maciel, 2017). Fragrance-seeking bees can find rewards at least in *G. devoniana*, *G.*
426   *magnicolumna* and *G. stangeana* (SHNM pers. obs.) Observations on cultivated
427   epiphytic species of *Galeandra (G. cristata*, *G. santarenensis*, *G. stangeana)* shows that
428   they are self-compatible, but not able to self-pollinate, therefore requiring cross-
429   pollination (SHNM pers. obs.).

430   Terrestrial species have a variable spur length, but in general shorter than
431   epiphytic. The occupation of terrestrial habits might have led to shift in pollinator's
432   requirements or independence of pollination by animals. Euglossini bees are diverse and
433   widespread in forested habitats, mostly on cloud or lowland forests (Cameron, 2004;
434   Dressler, 1982), presenting low diversity in open vegetation habitats like Cerrado (Faria
435   and Silveira, 2011). The orchid bee fauna occurring in open vegetation biomes are
436   frequently associated to patches of forests occurring along the rivers and there is no
437   species endemic to these biomes, but shared with adjacent large forested biomes like
438   Amazon or Atlantic Forest (Faria and Silveira, 2011). Because flowers of terrestrial
439   *Galeandra* appear to be rewardless, pollination by deceit might also occur in this clade.
440   Also, terrestrial *G. beyrichii* and *G. montana* present very wide distribution ranges (i.e.
441   so and so) and high levels of fruit production in herbaria material, suggesting self-
442   pollination is common among these taxa. However, evidences for self-compatibility, but
443   not spontaneous self-pollination, were observed on cultivated plants of the terrestrial *G.*
444   *styllomisantha*.

445   The distribution of orchids might be limited by the joint effect of habitat
446   availability and pollination limitation (Gravendeel et al., 2004; McCormick and
447   Jacquemyn, 2014). Epiphytic *Galeandra* usually presents a narrow geographic range
448   size compared to the terrestrial species. It might be linked to the habit occupied *per se*
449   or to different mechanisms of pollination (dependent of pollinators or not). It could also
450   reflect a higher dependency on particular pollinators. Smaller range sizes are typically
451   found in more specialized mutualisms, as compared to their generalist relatives
452   (Chomicki et al., 2015b). Some evidences of epiphytic orchids with a restricted
453   distribution when compared to the geographic range size of terrestrial species were
454   indicated by Zhang et al. (2015) which related this difference to environmental
455   variables. The low availability of substrates in epiphytic habitats results in restricted and
456   irregular moisture supplies, making water shortages a limiting factor for the

457 establishment and growth of epiphytes (Benzing, 1987; Laube and Zotz, 2003; Zhang et

458 al., 2015). On the other hand, the terrestrial species do not form a cohesive group

459 regarding range size or floral spur length.

460 Slow morphological change in interaction-related traits is a feature of highly

461 specialized mutualisms, and suggests stabilizing selection (Chomicki and Renner, 2017;

462 Davis et al., 2014). Our morphological analysis shows terrestrial *Galeandra* has a high

463 spur morphorate (Fig. 2), potentially resulting from disruption of bee pollination. As

464 mentioned earlier, long-spurred taxa are likely to have more specialized pollination

465 syndromes than short-spurred ones, which are deemed to be more generalist.

466
467 **Conclusions**
468

469 *Galeandra*, a primarily epiphytic orchid lineage, arose in Miocene about 10 Ma

470 in South America, and most probably the epiphytic clade diversified in Amazon.

471 Terrestrial habit in *Galeandra* arose synchronously with the expansion of open

472 vegetation savannas around 5 Ma. Surprisingly the change from epiphytic to terrestrial

473 habitat does not involve significant changes in climatic niche, perhaps explaining the

474 frequency of such transition in Epidendroid orchids. However, terrestrial species tend to

475 occupy larger geographical ranges probably facilitated by their ecological requirements,

476 but also pollination mode. Floral morphology suggests a shift from pollination by long

477 tongued bees to pollination by morphologically distinct bees or independence from

478 animal pollination.

479
480 **Acknowledgements**
481

490
491

References

Ackerman, J.D., 1986. Mechanisms and evolution of food-deceptive pollination system in orchids. Lindleyana 1, 108–113. doi:10.1017/S1464793105006986

Antonelli, A., Nylander, J.A.A., Persson, C., Sanmartín, I., 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. Proc. Natl. Acad. Sci. U. S. A. 106, 9749–54. doi:10.1073/pnas.0811421106

Bachman, S., Moat, J., Hill, A.W., de laTorre, J., Scott, B., 2011. Supporting red list threat assessments with GeoCAT: Geospatial conservation assessment tool. Zookeys 150, 117–126. doi:10.3897/zookeys.150.2109

Bacon, C.D., Mora, A., Wagner, W.L., Jaramillo, C.A., 2013. Testing geological models of evolution of the Isthmus of Panama in a phylogenetic framework. Bot. J. Linn. Soc. 171, 287–300. doi:10.1111/j.1095-8339.2012.01281.x

Batista, J.A.N., Mota, A.C.M., Proite, K., Bianchetti, L. de B., Romero-González, G.A., Espinoza, H.M.H., Salazar, G.A., 2014. Molecular phylogenetics of Neotropical *Cyanaeorchis* (Cymbidieae, Epidendroideae, Orchidaceae): Geographical rather than morphological similarities plus a new specie. Phytotaxa 156, 251–272. doi:10.11646/phytotaxa.156.5.1

Benzing, D.H., 1987. Vascular epiphytism: taxonomic participation and adaptive diversity. Ann. Missouri Bot. Gard. 74, 183–204.

Biomatters, 2013. Geneious 6.1.6.

Cameron, K.M., 2005. Leave it to the leaves: A molecular phylogenetic study of Malaxideae (Epidendroideae, Orchidaceae). Am. J. Bot. 92, 1025–1032. doi:10.3732/ajb.92.6.1025

Cameron, S.A., 2004. Phylogeny and biology of Neotropical orchid bees (Euglossini). Annu. Rev. Entomol. 49, 377–404. doi:10.1146/annurev.physchem.52.1.71

Chamberlain, S., 2016. spocc: Interface to Species Occurrence Data Sources.

Chase, M.W., Hills, H.G., 1992. Orchid Phylogeny, flower sexuality, and fragrance-seeking. Bioscience 42, 43–49.

Chomicki, G., Bidel, L.P.R., Ming, F., Coiro, M., Zhang, X., Wang, Y., Baissac, Y., Jay-Allemand, C., Renner, S.S., 2015a. The velamen protects photosynthetic orchid roots against UV-B damage, and a large dated phylogeny implies multiple gains and losses of this function during the Cenozoic. New Phytol. 205, 1330–1341.

Chomicki, G., Renner, S.S., 2017. Partner abundance controls mutualism stability and the pace of morphological change over geologic time. Proc. Natl. Acad. Sci. 114, 3951–3956. doi:10.1073/pnas.1616837114

Chomicki, G., Ward, P.S., Renner, S.S., 2015b. Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics. Proc. R. Soc. B Biol. Sci. 282.

Cody, S., Richardson, J.E., Rull, V., Ellis, C., Pennington, R.T., 2010. The great American biotic interchange revisited. Ecography 33, 326–332. doi:10.1111/j.1600-0587.2010.06327.x

Costa, L.P., 2003. The historical bridge between the Amazon and the Atlantic forest of Brazil a study of molecular phylogeography with small mammals. J. Biogeogr. 30, 71–86. doi:10.1046/j.1365-2699.2003.00792.x

Davis, C.C., Schaefer, H., Xi, Z., Baum, D.A., Donoghue, M.J., Harmon, L.J., 2014. Long-term morphological stasis maintained by a plant-pollinator mutualism. Proc. Natl. Acad. Sci. U. S. A. 111, 5914–9. doi:10.1073/pnas.1403157111

16

Dressler, R.L., 1983. Classification of the Orchidaceae and their probable origin. Telopea 2, 413–424.

Dressler, R.L., 1982. Biology of the orchid bees (Euglossini). Annu. Rev. Ecol. Syst. 13, 373–394.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–73. doi:10.1093/molbev/mss075

Evans, M.E.K., Smith, S.A., Flynn, R.S., Donoghue, M.J., 2009. Climate, niche evolution, and diversification of the "Bird Cage" evening primroses (Oenothera, Sections Anogra and Kleinia). Am. Nat. 173, 225–240. doi:10.1086/595757

Faria, L.R.R., Silveira, F.A., 2011. The orchid bee fauna (Hymenoptera, Apidae) of a core area of the Cerrado, Brazil: The role of riparian forests as corridors for forest-associated bees. Biota Neotrop. 11, 87–94.

Feldberg, K., Schneider, H., Stadler, T., Schäfer-Verwimp, A., Schmidt, A.R., Heinrichs, J., 2014. Epiphytic leafy liverworts diversified in angiosperm-dominated forests. Sci. Rep. 4, 5974.

Felsenstein, J., 2012. A Comparative method for both discrete and continuous characters using the threshold model. Am. Nat. 179, 145–156.

Freudenstein, J. V., Chase, M.W., 2015. Phylogenetic relationships in Epidendroideae (Orchidaceae), one of the great flowering plant radiations: Progressive specialization and diversification. Ann. Bot. 115, 665–681. doi:10.1093/aob/mcu253

Gentry, A.H., Dodson, C.H., 1987. Diversity and biogeography of Neotropical vascular epiphytes. Ann. Missouri Bot. Gard. 74, 205–233.

Givnish, T.J., Barfuss, M.H.J., van Ee, B., Riina, R., Schulte, K., Horres, R., Gonsiska, P.A., Jabaily, R.S., Crayn, D.M., Smith, J.A.C., Winter, K., Brown, G.K., Evans, T.M., Holst, B.K., Luther, H., Till, W., Zizka, G., Berry, P.E., Sytsma, K.J., 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny. Am. J. Bot. 98, 872–895. doi:10.3732/ajb.1000059

Givnish, T.J., Spalink, D., Ames, M., Lyon, S.P., JUnter, S.J., Zuluaga, A., Iles, W.J.D., Clements, M.A., Arroyo, M.T.K., Leebens-Mack, J., Endara, L., Kriebel, R., Neubig, K.M., Whitten, W.M., Williams, N.H., Cameron, K.M., 2015. Orchid phylogenomics and multiple drivers of their extraordinary diversification. Proc. R. Soc. B 282.

Gottsberger, G., Silberbauer-Gottsberger, I., 2006. Life in the Cerrado, a south American tropical seasonal ecosystem. origin, structure, dynamics and plant use (vol. 1). Reta Verlag, Ulm.

Gravendeel, B., Smithson, A., Slik, F.J.W., Schuiteman, A., 2004. Epiphytism and pollinator specialization: drivers for orchid diversity? Philos. Trans. R. Soc. Lond. B. Biol. Sci. 359, 1523–35. doi:10.1098/rstb.2004.1529

Gustafsson, A.L.S., Verola, C.F., Antonelli, A., 2010. Reassessing the temporal evolution of orchids with new fossils and a Bayesian relaxed clock, with implications for the diversification of the rare South American genus *Hoffmannseggella* (Orchidaceae: Epidendroideae). BMC Evol. Biol. 10, 177. doi:10.1186/1471-2148-10-177

Hijmans, R.J., 2016. raster: Geographic Data Analysis and Modeling.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25, 1965–1978. doi:10.1002/joc.1276

17

592   Hoorn, C., Wesselingh, F.P., ter Steege, H., Bermudez, M.A., Mora, A., Sevink, J.,
593   Sanmartin, I., Sanchez-Meseguer, A., Anderson, C.L., Figueiredo, J.P., Jaramillo,
594   C., Riff, D., Negri, F.R., Hooghiemstra, H., Lundberg, J., Stadler, T., Sarkinen, T.,
595   Antonelli, A., 2010. Amazonia through time: Andean uplift, climate change,
596   landscape evolution, and biodiversity. Sci. 330, 927–931.
597   doi:10.1126/science.1194585
598   IUCN, 2013. Guidelines for Using the IUCN Red List Categories and Criteria. Version
599   10.1. Prepared by the Standards and Petitions Subcommittee. [WWW Document].
600   URL http://www.iucnredlist.org/documents/RedListGuidelines.pdf
601   Jaramillo, C., Montes, C., Cardona, A., Silvestro, D., Antonelli, A., Bacon, C.D., 2017.
602   Comment (1) on "Formation of the Isthmus of Panama" by O'Dea & al. Sci. Adv.
603   3.
604   Jersáková, J., Johnson, S.D., Kindlmann, P., 2006. Mechanisms and evolution of
605   deceptive pollination in orchids. Biol. Rev. 81, 219–235.
606   doi:10.1017/S1464793105006986
607   Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software
608   version 7: Improvements in performance and usability. Mol. Biol. Evol. 30, 772–
609   780. doi:10.1093/molbev/mst010
610   Lambert, S.M., Wiens, J.J., 2013. Evolution of viviparity: A phylogenetic test of the
611   cold-climate hypothesis in phrynosomatid lizards. Evol. 67, 2614–2630.
612   doi:10.1111/evo.12130
613   Landis, M.J., Matzke, N.J., Moore, B.R., Huelsenbeck, J.P., 2013. Bayesian analysis of
614   biogeography when the number of areas is large. Syst. Biol. 62, 789–804.
615   doi:10.1093/sysbio/syt040
616   Laube, S., Zotz, G., 2003. Which abiotic factors limit vegetative growth in a vascular
617   epiphyte? Funct. Ecol. 17, 598–604. doi:10.1046/j.1365-2435.2003.00760.x
618   Martos, F., Johnson, S.D., Peter, C.I., Bytebier, B., 2014. A molecular phylogeny
619   reveals paraphyly of the large genus Eulophia (Orchidaceae): A case for the
620   reinstatement of *Orthochilus*. Taxon 63, 9–23. doi:10.12705/631.6
621   Matzke, N.J., 2013. BioGeoBEARS: BioGeography with Bayesian (and Likelihood)
622   Evolutionary Analysis in R Scripts.
623   McCormick, M.K., Jacquemyn, H., 2014. What constrains the distribution of orchid
624   populations? New Phytol. 202, 392–400. doi:10.1111/nph.12639
625   Monteiro, S.H.N., Selbach-Schnadelbach, A., de Oliveira, R.P., van den Berg, C., 2010.
626   Molecular Phylogenetics of *Galeandra* (Orchidaceae: Catasetinae) based on
627   Plastid and Nuclear DNA Sequences. Syst. Bot. 35, 476–486.
628   doi:10.1600/036364410792495944
629   Nilsson, L.A., 1998. Deep flowers for long tongues. Trends Ecol. Evol. 5347, 259–260.
630   O'Dea, A., Lessios, H.A., Coates, A.G., Eytan, R.I., Restrepo-moreno, S.A., Cione,
631   A.L., Collins, L.S., Queiroz, A. De, Farris, D.W., Norris, R.D., Stallard, R.F.,
632   Woodburne, M.O., Aguilera, O., Aubry, M., Berggren, W.A., Budd, A.F., Cozzuol,
633   M.A., Coppard, S.E., Duque-caro, H., Finnegan, S., Gasparini, G.M., Grossman,
634   E.L., Johnson, K.G., Keigwin, L.D., Knowlton, N., Leigh, E.G., Leonard-pingel,
635   J.S., Marko, P.B., Pyenson, N.D., Rachello-dolmen, P.G., Soibelzon, E.,
636   Soibelzon, L., Todd, J.A., Vermeij, G.J., Jackson, J.B.C., 2016. Formation of the
637   Isthmus of Panama. Sci. Adv. 2, 1–12.
638   Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, H., 2007. The vegan package.
639   Community Ecol. Packag. 10, 631–637.
640   Oliveira-Filho, A.T., Fontes, M.A.L., 2000. Patterns of floristic differentiation among
641   Atlantic forests in southeastern Brazil and the influence of climate. Biotropica 32,

642   793–810.

643   Oliveira-Filho, A.T., Ratter, J.A., 1995. A study of the origin of central Brazilian forests
644   by the analysis of plant species distribution patterns. Edinburgh J. Bot. 52, 141–
645   194. doi:10.1017/S0960428600000949

646   Olmstead, R.G., 2013. Phylogeny and biogeography in Solanaceae, Verbenaceae and
647   Bignoniaceae: A comparison of continental and intercontinental diversification
648   patterns. Bot. J. Linn. Soc. 171, 80–102. doi:10.1111/j.1095-8339.2012.01306.x

649   Pansarin, E.R., Maciel, A.A., 2017. Evolution of pollination systems involving edible
650   trichomes in orchids. AoB Plants 9, 1–13. doi:10.1093/aobpla/plx033

651   Pearson, D.L., Dressler, R.L., 1985. Two-year study of male orchid bee (Hymenoptera:
652   Apidae: Euglossini) attraction to chemical baits in lowland south-eastern Perú. J.
653   Trop. Ecol. 1, 37. doi:10.1017/S0266467400000067

654   Pennington, R.T., Lewis, G., Ratter, J.A., 2006. Neotropical savannas and dry forests:
655   plant diversity, biogeography and conservation. CRC Press Florida.

656   Pérez-Escobar, O.A., Balbuena, J.A., Gottschling, M., 2016. Rumbling Orchids: How to
657   Assess Divergent Evolution between Chloroplast Endosymbionts and the Nuclear
658   Host. Syst. Biol. 65, 51–65. doi:10.1093/sysbio/syv070

659   Pérez-Escobar, O.A., Chomicki, G., Condamine, F.L., de Vos, J.M., Martins, A.C.,
660   Smidt, E.C., Klitgård, B., Gerlach, G., Heinrichs, J., 2017a. Multiple Geographical
661   Origins of Environmental Sex Determination enhanced the diversification of
662   Darwin's Favourite Orchids. Sci. Rep. 7, 12878. doi:10.1038/s41598-017-12300-y

663   Pérez-Escobar, O.A., Chomicki, G., Condamine, F.L., Karremans, A.P., Bogarín, D.,
664   Matzke, N.J., Silvestro, D., Antonelli, A., 2017b. Recent origin of Neotropical
665   orchids in the world's richest plant biodiversity hotspot. New Phytol. 215, 891–
666   905.

667   Pérez-Escobar, O.A., Gottschling, M., Chomicki, G., Condamine, F.L., Klitgård, B.,
668   Pansarin, E.R., Gerlach, G., 2017c. Andean mountain building did not preclude
669   dispersal of lowland epiphytic orchids in the Neotorpics. Sci. Rep. 7, 4919.
670   doi:http://dx.doi.org/10.1101/106393

671   Pérez-Escobar, O.A., Gottschling, M., Whitten, W.M., Salazar, G., Gerlach, G., 2015.
672   Sex and the Catasetinae (Darwin's favourite orchids). Mol. Phylogenet. Evol. 97,
673   1–10. doi:10.1016/j.ympev.2015.11.019

674   Posadas, P., Ortiz-Jaureguizar, E., 2016. Evolução da região andina da América do Sul,
675   in: Carvalho, C.J.B., Almeida, E.A.B. (Eds.), Biogeografia Da América Do Sul:
676   Análise de Tempo, Espaço E Forma. Editora Roca.

677   Rambaut, A., 2009. FigTree: Tree figure drawing tool.

678   Ramírez, S.R., Eltz, T., Fujiwara, M.K., Gerlach, G., Goldman-Huertas, B., Tsutsui,
679   N.D., Pierce, N.E., 2011. Asynchronous diversification in a specialized plant-
680   pollinator mutualism. Sci. 333, 1742–1746. doi:10.1126/science.1209175

681   Ramírez, S.R., Gravendeel, B., Singer, R.B., Marshall, C.R., Pierce, N.E., 2007. Dating
682   the origin of the Orchidaceae from a fossil orchid with its pollinator. Nature 448,
683   1042–5. doi:10.1038/nature06039

684   Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range
685   evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57, 4–14.
686   doi:10.1080/10635150701883881

687   Renner, S., 2004. Plant dispersal across the tropical atlantic by wind and sea currents.
688   Int. J. Plant Sci. 165, S23–S33. doi:10.1086/383334

689   Revell, L.J., 2013. Ancestral character estimation under the threshold model from
690   quantitative genetics. Evol. 68, 743–759. doi:10.1111/evo.12300

691   Revell, L.J., 2012. phytools: An R package for phylogenetic comparative biology (and

692   other things). Methods Ecol. Evol. 3, 217–223. doi:10.1111/j.2041-
693   210X.2011.00169.x

694 Romero-Gonzalez, G.A., Warford, N., 1995. Three new *Galeandra* (Orchidaceae:
695   Cyrtopodiniinae). Lindleyana 10, 75–91.

696 Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification
697   of historical biogeography. Syst. Biol. 46, 195. doi:10.2307/2413643

698 Schuettpelz, E., Pryer, K.M., 2009. Evidence for a Cenozoic radiation of ferns in an
699   angiosperm-dominated canopy. Proc. Natl. Acad. Sci. 106, 11200–11205.
700   doi:10.1073/pnas.0811136106

701 Silvestro, D., Michalak, I., 2012. raxmlGUI: a graphical front-end for RAxML. Org.
702   Divers. Evol. 12, 335–337. doi:DOI: 10.1007/s13127-011-0056-0

703 Simon, M.F., Grether, R., de Queiroz, L.P., Skema, C., Pennington, R.T., Hughes, C.E.,
704   2009. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in
705   situ evolution of adaptations to fire. Proc. Natl. Acad. Sci. 106, 20359–20364.
706   doi:10.1073/pnas.0903410106

707 Sosa, V., Cameron, K.M., Angulo, D.F., Hernández-Hernández, T., 2016. Life form
708   evolution in epidendroid orchids: ecological consequences of the shift from
709   epiphytism to terrestrial habit in Hexalectris. Taxon 65, 235–248.
710   doi:10.12705/652.2

711 Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic
712   analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–90.
713   doi:10.1093/bioinformatics/btl446

714 Steiner, K.E., Whitehead, V.B., 1990. Pollinator adaptation to oil-secreting flowers -
715   *Rediviva* and *Diascia*. Evol. 44, 1701–1707.

716 Werneck, F.P., 2011. The diversification of eastern South American open vegetation
717   biomes: Historical biogeography and perspectives. Quat. Sci. Rev. 30, 1630–1648.
718   doi:10.1016/j.quascirev.2011.03.009

719 Whittall, J.B., Hodges, S.A., 2007. Pollinator shifts drive increasingly long nectar spurs
720   in columbine flowers. Nature 447, 706–9. doi:10.1038/nature05857

721 Wright, S., 1934. An analysis of variability in the number of digits in an inbred strain of
722   guinea pigs. Genetics 19, 506–536.

723 Xiang, X.G., Jin, W.T., Li, D.Z., Schuiteman, A., Huang, W.C., Li, J.W., Jin, X.H., Li,
724   Z.Y., 2014. Phylogenetics of tribe Collabieae (Orchidaceae, Epidendroideae) based
725   on four chloroplast genes with morphological appraisal. PLoS One 9.
726   doi:10.1371/journal.pone.0087625

727 Xiang, X.G., Mi, X.C., Zhou, H.L., Li, J.W., Chung, S.W., Li, D.Z., Huang, W.C., Jin,
728   W.T., Li, Z.Y., Huang, L.Q., Jin, X.H., 2016. Biogeographical diversification of
729   mainland Asian *Dendrobium* (Orchidaceae) and its implications for the historical
730   dynamics of evergreen broad-leaved forests. J. Biogeogr. 43, 1310–1323.
731   doi:10.1111/jbi.12726

732 Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, Global
733   Rhythms, Aberrations in Global Climate 65Ma to Present. Sci. 292, 686–693.
734   doi:10.1126/science.1059412

735 Zhang, G.-Q., Liu, K.-W., Li, Z., Lohaus, R., Hsiao, Y.-Y., Niu, S.-C., Wang, J.-Y.,
736   Lin, Y.-C., Xu, Q., Chen, L.-J., Yoshida, K., Fujiwara, S., Wang, Z.-W., Zhang,
737   Y.-Q., Mitsuda, N., Wang, M., Liu, G.-H., Pecoraro, L., Huang, H.-X., Xiao, X.-J.,
738   Lin, M., Wu, X.-Y., Wu, W.-L., Chen, Y.-Y., Chang, S.-B., Sakamoto, S., Ohme-
739   Takagi, M., Yagi, M., Zeng, S.-J., Shen, C.-Y., Yeh, C.-M., Luo, Y.-B., Tsai, W.-
740   C., Van de Peer, Y., Liu, Z.-J., 2017. The *Apostasia* genome and the evolution of
741   orchids. Nature 549, 379.

742   Zhang, Z., Yan, Y., Tian, Y., Li, J., He, J.S., Tang, Z., 2015. Distribution and
743   conservation of orchid species richness in China. Biol. Conserv. 181, 64–72.
744   doi:10.1016/j.biocon.2014.10.026
745   Zhang, Dai, Y., Hao, G.-Y., Li, J.-W., Fu, X.-W., Zhang, J.-L., 2015. Differentiation of
746   water-related traits in terrestrial and epiphytic *Cymbidium* species. Front. Plant Sci.
747   6, 1–10. doi:10.3389/fpls.2015.00260
748   Zotz, G., 2013. The systematic distribution of vascular epiphytes – a critical update.
749   Bot. J. Linn. So 171, 453–481.
750
751
752
753
754
755
756

757
758   **Figure legends**

759

760   **Fig. 1**. Time-calibrated phylogeny and biogeographic results, showing area coding, and
761   distribution map. Pie charts on nodes represents ancestral area resulted from the
762   BioGeoBEARS analysis and color code follows the legend on the map: A = Central
763   America, B = Choco, C = Amazonia, D = Guiana Shield, E = Dry Diagonal and F =
764   Atlantic Forest. Grey bars represent 95% highest posterior density interval for age
765   estimates. Colored circles on tips represent the occurrence of that species in the
766   delimited geographical areas. *Galeandra macroplectra* presents a grey circle, because it
767   was excluded from the BioGeoBEARS analysis because of negative branch lengths.
768   Photographs in circles represent floral morphological variation in the genus. Colors
769   encircling orchid pictures represent the distribution range of the species according to
770   delimited areas on the map. Photo credits: Adarilda Petini-Benelli (*G. stangeana*, *G.*
771   *blanchetii*), Günter Gerlach (*G. devoniana*), Silvana H. N. Monteiro (*G. cristata*, *G.*
772   *montana*).

773

774   **Fig. 2.** Spur length evolution in *Galeandra* species. A. Ancestral state estimation of spur
775   length in *Galeandra* species; B. Phenogram showing spur length evolution in time.

776

777   **Fig. 4**. Phylomorphospace analysis result, considering simultaneously spur length and
778   range size as continuous variables, and phylogenetic relationships. Each dot in the
779   graphics represent one *Galeandra* species and the colours distinguish them by habit,
780   terrestrial (yellow) and epiphytic (green). *X* and *Y* axes represent respectively, range size
781   (in km^2) and floral spur length (in cm).

782

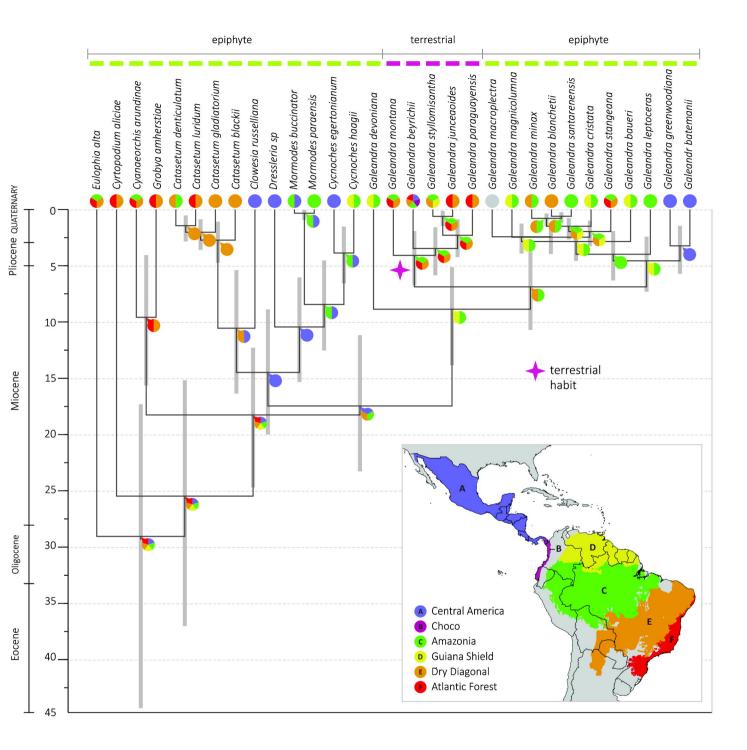783   **Online Supplementary materials**

784

785   **Fig. S1.** Measurements of *Galeandra* floral spur shown in *G. santarenesis* flower. A.
786   spur width, B. spur length (illustration by A. E. Rocha)
787   **Fig. S2.** Maximum likelihood phylogeny for the matrix of 31 taxa and 6014 nucleotides
788   for *Galeandra* and outgroups, analysed in RaxML. Values above branches show the ML
789   bootstrap for 1000 replicates.

790   **Fig. S3.** Time-calibrated tree for the matrix of 31 taxa and 6014 nucleotides for

791   *Galeandra*and outgroups analyzed in BEAST 1.8.3

792   **Fig. S4**. Traitgram for spur length evolution in time, showing uncertainty about

793   character state using transparent probability density.

794   **Fig. S5**. Cluster dendogram showing bioclimatic variables relatedness. Bioclimatic

795   variables highlighted in yellow are non-correlated.

796   **Fig. S6**. Non-metric multidimensional scaling results: epiphytic x terrestrial species.

797   **Fig. S7**. Non-metric multidimensional scaling results: small x long spurred species.

798

799   **Table S1.** List of species used in this study with voucher information and GenBank

800   accession numbers. Newly generated sequences are written in bold. Herbarium

801   acronyms followed the *Index Hebariorum* http://sweetgum.nybg.org/science/ih/

802   **Table S2.** Botanical collections revised for geographical distribution data of *Galeandra.*

803   Herbarium acronyms followed the *Index Hebariorum*

804   http://sweetgum.nybg.org/science/ih/

805   **Table S3**. Statistical results from BioGeoBEARS multimodel approach. In bold the

806   best-fit model.

807   **Table S4.** *Galeandra* species and their Area of Occupancy (AOO) and Extent of

808   Occurrence (EOO), in square meters (km^2) calculated in GeoCAT (Bachman et al.

809   2011; http://geocat.kew.org)

810   **Table S5**. Measurements of floral spur length and width. Herbarium acronyms followed

811   the *Index Hebariorum* http://sweetgum.nybg.org/science/ih/.

812

Figure caption species (left to right): *Galeandra stangeana*, *Galeandra cristata*, *Galeandra blanchetii*, *Galeandra beyrichii*, *Galeandra montana*, *Galeandra devoniana*

Map legend:
- A — Central America
- B — Choco
- C — Amazonia
- D — Guiana Shield
- E — Dry Diagonal
- F — Atlantic Forest

★ terrestrial habit