

1 The interplay of biogeographic history, floral morphology, and climatic niche in
2 *Palicourea* (Rubiaceae), an ecologically important group of Neotropical plants

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12 ABSTRACT

13 Investigating how biotic and abiotic factors interact to shape species distributions is critical to
14 understanding current biodiversity patterns. This is particularly relevant in the Neotropics, a species-rich
15 region home to several biodiversity hotspots, where the interplay of factors promoting diversification
16 resulted in the assembly of the world's richest flora. Using *Palicourea*, a species-rich and ecologically
17 and morphologically diverse group of Neotropical plants in the coffee family, we aimed to identify
18 patterns resulting from the interaction of biogeographic history, evolution of floral morphology, and
19 climatic niche evolution. We used genome-wide target enrichment data using a family-specific probe set
20 for hundreds of loci, to infer the most completely sampled multi-locus phylogeny of *Palicourea* to date.
21 To explore abiotic factors driving evolution, we modeled species historical ranges and the probability of
22 climatic niche overlap. We integrated our results with natural history knowledge on inflorescence
23 morphology characters linked to pollination to examine the interplay of biogeographic, floral
24 morphology, and realized climatic niche on the evolution of this remarkably diverse group of Neotropical
25 plants. Our results demonstrate that *Palicourea* has a highly structured biogeographic history, with
26 limited long distance dispersal events generally occurring between climatically similar regions. Further,
27 we pinpoint an important role of climatic niche conservatism and convergent evolution of characters
28 related to pollination in the evolutionary history of *Palicourea*. An interaction of biotic and abiotic factors
29 has led to a continental radiation of plants that form an important ecological cornerstone of the
30 Neotropics.

31

32 Keywords: *Biogeography, climatic niche, floral morphology, Neotropics, niche conservatism,*
33 *phylogenetics, species coexistence*

34 **Background**

35 Lineage diversification is shaped by the ability of species to move across the landscape and to adapt to
36 changing conditions (Donoghue, 2008; Smith *et al.*, 2014). As speciation and evolution proceed, closely
37 related species are generally expected to fill similar ecological niches, and share phenotypic
38 characteristics (Donoghue, 2008; Wiens *et al.*, 2010). However, according to coexistence theory, the
39 long-term sympatry of species (either through secondary contact or *in situ* speciation) requires that
40 lineages diverge in one or more niche dimensions (Elton, 1946; Macarthur & Levins, 1967; Godoy *et al.*,
41 2014; Quintero & Landis, 2020). What determines macroevolutionary patterns across groups is thus a
42 combination of biogeographic range evolution and ecological processes including niche conservatism
43 (i.e., the retention of niche requirements of the common ancestor in closely related descendant taxa),
44 niche evolution (e.g., the dispersal of species to new habitats and climatic regimes), dispersal ability, and
45 competition (Wiens & Donoghue, 2004). Investigating the interplay of historical and ecological processes
46 is paramount to understanding the forces shaping current biodiversity patterns.

47 The Neotropics is a species-rich region encompassing several of the most biodiverse places on Earth
48 including the Amazon, the Atlantic Forest, and the Andean mountains (Gentry, 1982; Kreft & Jetz, 2007;
49 Barthlott *et al.*, 2007; Ribeiro *et al.*, 2009). Notably, Neotropical plants constitute the world's richest flora
50 (Gentry, 1982). Previous studies have indicated that Neotropical plant diversity was generated by the
51 interplay of abiotic and biotic factors like biome heterogeneity, the formation of new habitats that
52 triggered ecological opportunity, and pollinator shifts (Hughes & Eastwood, 2006; Madriñán *et al.*, 2013;
53 Lagomarsino *et al.*, 2016; Tripp & Tsai, 2017; Antonelli *et al.*, 2018). The interaction of these factors
54 resulted in adaptive radiations and replicated radiations in cloud forests and sky islands in the Andes,
55 where lineages rapidly diversified by filling up empty niches in recently formed habitats (Hughes &
56 Eastwood, 2006; Simon *et al.*, 2009; Contreras-Ortiz *et al.*, 2018; Donoghue *et al.*, 2022). Other processes
57 leading to Neotropical plant diversification include the steady accumulation of niche-conserved species
58 through time in climatically stable, isolated habitats like the seasonally dry tropical forests (Särkinen *et*
59 *al.*, 2012).

60 Past studies suggest that plant distributions are, in general, the result of retention of ancestral niche and
61 dispersal into areas with climatic conditions for which species are pre-adapted (Sedio *et al.*, 2013; Frost *et*
62 *al.*, 2017; Wang *et al.*, 2018; Sanín *et al.*, 2022a). However, growing evidence also indicates that biome
63 shifts played an important role in the evolution of several plant taxa (Frost *et al.*, 2022) and that those
64 shifts, particularly across elevation gradients in tropical biomes, are accompanied by pollinator shifts
65 (Dellinger *et al.*, 2023). Combining species-level phylogenies with spatial and ecological data to
66 investigate how niche evolution, shifts in traits related to pollination biology, and biogeographic

67 distribution play out at a broad ecogeographic scale in Neotropical plant taxa is paramount to
68 understanding the patterns and processes resulting in the assembly of the world's richest flora (Vargas *et*
69 *al.*, 2020).

70 Amongst Neotropical plants, *Palicourea* (Rubiaceae) is a species-rich genus (~650 spp.), striking in their
71 diversity of distribution patterns, climatic niche breadth, inflorescence types, and pollinators. *Palicourea*
72 ranges from Mexico to northern Argentina, with some species in the Antilles (Taylor, 1997, 2018). Some
73 taxa in the genus have broad distributions across South America, while others are restricted to Central
74 America, the Atlantic Forest, the Andes, and the inter-Andean lowlands in northwestern South America.
75 Most species of *Palicourea* inhabit low-elevation, wet ecosystems with elevational ranges between 0–
76 1800 m.a.s.l (Taylor, 1997). However, some species extend up to the tree line in montane ecosystem
77 (>3000 m; <http://legacy.tropicos.org/Name/40000063?projectid=34>). This indicates that *Palicourea* exists
78 across various climatic niches. The genus was traditionally characterized for having thyriform
79 inflorescences with typically brightly colored, somewhat fleshy corolla tubes that are primarily visited by
80 hummingbirds but also by butterflies (Fig. 1A). Past molecular work has shown that several species
81 traditionally recognized as belonging to other genera, including *Cephaelis* and *Psychotria*, are nested in
82 *Palicourea* (Andersson & Rova, 1999; Nepokroeff *et al.*, 1999; Andersson, 2001; Fig. 1B,C). This has
83 resulted in an expanded circumscription of *Palicourea* including species with head-like inflorescences
84 subtended by red showy bracts (with characteristics associated primarily with butterflies but also
85 hummingbird pollination). The expanded circumscription also includes species with open to condensed
86 cymoid inflorescences with small to large white flowers subtended or not by bracts (characteristics
87 associated primarily with pollination by butterflies and other insects; Fig. 1). The diversity in characters
88 related to pollination, distribution patterns, and realized climatic niche in *Palicourea* offers a valuable
89 opportunity to explore the interplay of realized climatic niche evolution, biogeography, and inflorescence
90 type diversity in diversification of the Neotropical flora; the main goal of this study.

91 **Materials and Methods**

92 Sample collection, DNA extraction and Target Enrichment

93 We sampled a combination of silica dried and herbarium specimens of 116 specimens in the Rubiaceae
94 including 94 *Palicourea*, 10 *Psychotria*, and one sample each of *Rudgea cornifolia*, *Balmea stormiae*,
95 *Carapichea ipecacuanha*, *Carapichea guianensis*, *Cosmibuena grandiflora*, *Coutarea hexandra*,
96 *Eumachia boliviana*, *Hamelia patens*, *Hillia parasitica*, *Hoffmannia phoenicopoda*, *Notopleura*
97 *epiphytica*, and *Notopleura uliginosa* (Supplementary Table 1). All samples correspond to voucher
98 specimens obtained from the Missouri Botanic Garden (Supplementary Table 1). Total DNA was
99 extracted using a modified sorbitol protocol (Štorchová *et al.*, 2000). We generated target enrichment

100 sequence data for 2270 exons in 1059 loci using a Rubiaceae-specific probe set (Ball et al., *In press*).
101 Library preparation, target enrichment, and sequencing of targeted regions was conducted by
102 RapidGenomics (Gainesville, FL, USA). Samples that had high-molecular DNA (250-1000 ng) were
103 fragmented to a standard target size for Illumina sequencing. Enriched pools were combined, and probes
104 were hybridized to pools for target enrichment. 150 bp paired end reads were sequenced in an Illumina
105 HiSeq 3000.
106



107 Fig.1 Inflorescence types in *Palicourea*. A) Thyriform inflorescences, with characteristics associated with primarily
108 hummingbirds but also butterfly pollination. B) Head-like inflorescence subtended by showy bracts, traditionally associated with
109 butterfly visitors, but also believed to be visited by hummingbirds. C) Open to condensed cymoid inflorescences with small,
110 white flowers and reduced bracts. This inflorescence type has characteristics associated primarily with insect pollination (bees,
111 hawkmoths, flies), but hummingbird visitation has also been reported (Picture taken and modified from Barry E. Hammel).

112 Data processing and phylogenetic inference

113 FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) was used to quality-check the
114 demultiplexed reads prior to and after adapter removal with Trimmomatic v.0.39 (Bolger *et al.*, 2014).
115 Loci were assembled with HybPiper v.2.0.1 (Johnson *et al.*, 2016) using exons as reference sequence and
116 assembling single contigs instead of “supercontigs” to avoid assembling chimeric regions (i.e., contigs

117 with stitched sequences derived from different paralogs or alleles; (Morales-Briones *et al.*, 2018, 2022).
118 Assembled regions with <50% sequence length recovered across <20% samples were removed. Paralog
119 identification was conducted by extracting loci with paralog warnings (putative paralogs) with the
120 `paralog_retriever` function of HybPiper. Loci without paralog warnings (no more than one sequence
121 recovered per sample) were aligned with MAFFT v7.505 (Katoh & Standley, 2013).
122 To maximize the amount of sequence data used in this study, orthology inference was conducted for
123 putative multi-copy loci following an automated tree-pruning approach (Morales-Briones *et al.*, 2022).
124 Briefly, all sequences flagged as paralogs (>1 contig assembled per target region) were aligned with
125 MACSE (Ranwez *et al.*, 2018), codon frameshifts were replaced with gaps, and columns with >90%
126 missing data were removed with Phyx (Brown *et al.*, 2017). Homolog trees were inferred with RAxML-
127 ng (Kozlov *et al.*, 2019) with a GTR+G model using an MRE-based bootstrapping method for estimation
128 of support (Felsenstein, 1985). Monophyletic and paraphyletic tips that belonged to the same taxon were
129 masked, keeping the tip with the most unambiguous characters in the alignments. Unusually long
130 branches, which are likely associated with spurious sequences, were removed with TreeShrink v1.3.9
131 (Mai & Mirarab, 2018). Final ortholog trees were obtained with an outgroup-aware (“monophyletic
132 outgroup”) strategy (Yang & Smith, 2014) keeping only ortholog groups with ≥ 35 ingroup taxa. The
133 “monophyletic outgroup” approach uses rooted gene trees (*Cosmibuena grandiflora*, *Eumachia boliviana*,
134 *Hamelia patens*, *Hillia parasitica*, *Hoffmannia phoenicopoda* used as outgroups) and for each gene tree, it
135 searches for the largest subtree with monophyletic and single copy outgroups. Gene trees with
136 paraphyletic or multicopy outgroups are discarded. Final fasta files were generated from ortholog trees
137 and used for alignment with MAFFT and gene tree inference was carried out with RAxML-ng, as
138 specified above. Loci with final alignments <200 bp were removed. Scripts used for data processing are
139 available at <https://bitbucket.org/ambedoyao/palicourea-analyses>.
140 We inferred phylogenetic relationships using a two-step coalescent aware species tree inference method
141 (ASTRAL-III v5.7.8; Zhang *et al.*, 2018) and used the previously inferred gene trees as input, collapsing
142 branches with <10% bootstrap support. When multiple accessions assigned to the same species were
143 included in the analyses, these were considered as separate operational taxonomic units because they
144 represent different populations of a given species for which monophyly has not been tested.

145

146 Inflorescence type, divergence dating, and modeling of ancestral biogeographic ranges

147 For each species of *Palicourea* included in this study, inflorescence type was coded into three different
148 categories: 1) thyriform with long, somewhat fleshy corollas in shades of blue, pink, red, orange, or
149 yellow; 2) head-like with small, white flowers subtended by showy bracts (“hot-lips” or “leafy”); 3) cyme
150 with pale flowers lacking showy bracts (*Psychotria*-like (Fig. 1). These three inflorescence types include

151 a broad spectrum of variation in floral characteristics since some *Palicourea* species have inflorescences
152 that have pale pink and purple coloration with colorful and well-developed floral axes (intermediate
153 between 1 and 3). Other species have inflorescences with more or less condensed inflorescences
154 subtended or not by pale bracts (like the former *Cephaelis*).

155 We estimated divergence times for our inferred species tree in RevBayes (Höhna *et al.*, 2014) using an
156 exponential relaxed clock model and a birth-death tree prior. Given computational limits to the number of
157 loci that can be analyzed jointly for divergence dating, we used a subset of 15 loci and specified a
158 partitioned GTR+G substitution model. We selected the loci with genesortR (Mongiardino Koch, 2021),
159 an R script for phylogenomic data subsampling using various standard criteria for evaluating sources of
160 systematic bias and phylogenetic signal across loci (e.g., average pairwise patristic distances, bootstrap
161 support, and Robinson-Foulds similarity to a target topology). We incorporated topological similarity to
162 the species tree as a subsampling criterion and evaluated loci with <10% missing sequences. When
163 multiple samples per species were present, we randomly selected one sample per clade. We used a
164 secondary calibration for the crown node of *Psychotria* and Palicoureeae derived from previous dating
165 analyses of Rubiaceae (Bremer & Eriksson, 2009; Manns *et al.*, 2012; Wikström *et al.*, 2015). A uniform
166 prior distribution with soft bounds (dnSoftBoundUniformNormal, min=20, max=46.9, sd=3.0, p=0.95)
167 was specified. A starting tree compatible with the calibration prior was inferred using penalized
168 likelihood (*chronos* function of R package *ape*; Paradis & Schliep, 2019) and the ASTRAL species tree.
169 We ran two independent chains for 300,000 generations, discarded a 0.25 fraction of posterior trees, and
170 generated a maximum clade credibility tree from the posterior distribution of inferred trees.

171 We modeled biogeographic range evolution on the maximum clade credibility time-calibrated tree using a
172 Dispersal-Extinction-Cladogenesis model (Ree & Smith, 2008). We defined five biogeographic regions
173 that reflect distribution patterns in the *Palicourea* species included in this study and that are compatible
174 with previous work on Psychotrieae and Palicoureeae (Sedio *et al.*, 2013). Biogeographic areas were
175 defined as follows: Central America and the Caribbean (C), trans-Andean lowland forests (L; i.e., Choco
176 region of western Colombia, Darién region of Eastern Panama, and inter-Andean valleys in northern
177 Colombia and Venezuela at elevation <1500 m), Andean region (A) (elevation >1500 m), Eastern South
178 America (E; Amazon and Orinoco basins, Guiana Shield region, and Cerrado), and the Atlantic Forest
179 (F). We allowed dispersal only between spatially adjacent areas.

180 Niche range and pairwise climatic niche overlap estimation

181 We used the Climate and Niche Distribution Inference (CanDI; <https://github.com/abbyj-g/candi>) pipeline
182 to download climate data and gather and clean species occurrences from the Global Biodiversity
183 Information Facility (GBIF) and the Botanical Information and Ecology Network (BIEN). We removed
184 occurrence records that were duplicated, that are exactly at 0°, 90°, or ≥180°, or that fall in the ocean. We

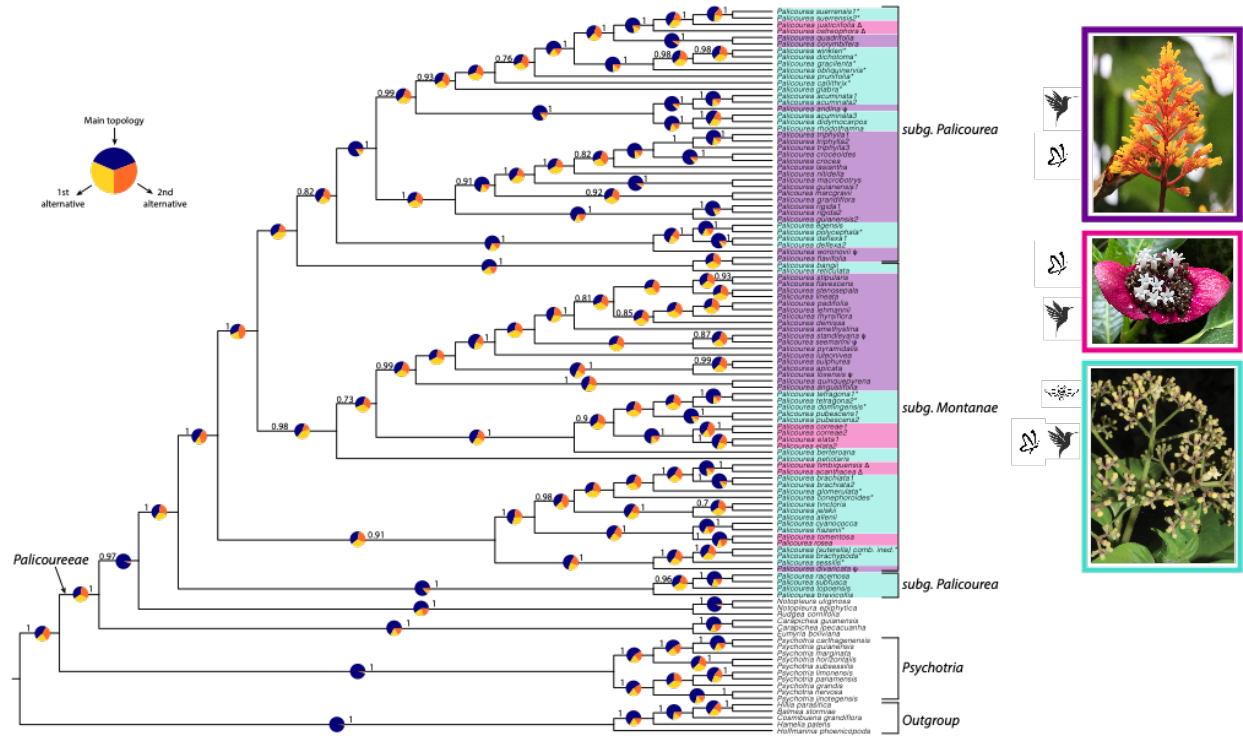
185 extracted associated bioclimatic variables for cleaned occurrence data from the WorldClim database (Fick
186 & Hijmans, 2017) and calculated a correlation matrix with the extracted values (Supplementary
187 Materials). To remove multicollinearity, only variables that had correlation coefficients <0.8 were kept
188 (annual mean temperature, maximum temperature of warmest month, minimum temperature of coldest
189 month, and annual precipitation). We used the R package NicheROVER
190 (<https://github.com/mlsy/nicheROVER>) to apply a Bayesian probabilistic method (Swanson *et al.*, 2015)
191 for estimating multidimensional niche regions (the 95% probability region in multivariate space for a
192 given species) and pairwise niche overlap (the probability that an individual from species A is found in
193 the niche region of species B) over 1,000 iterations. Pairwise niche overlap was estimated independently
194 for all possible pairs of *Palicourea* species in this study (n=5929).

195 **Results**

196 Data processing and phylogenetic inference

197 Locus recovery efficiency is shown in Supplementary Materials. A total of 810 exons passed all filters
198 (i.e., >20% samples at >50% sequence length and loci with >200 bp). These included 188 exons with no
199 paralog warnings (one contig assembled per locus) and 622 orthologous loci as inferred with the tree
200 pruning “monophyletic outgroup” approach. Three samples were removed based on the number of
201 paralogous loci and coverage (Supplementary Materials).

202 The species tree inferred with ASTRAL III (Fig. 2) recovered *Psychotria* as sister to Palicoureeae with
203 high support, in agreement with previous work (Manns *et al.*, 2012; Sedio *et al.*, 2013;
204 Razafimandimbison *et al.*, 2017). Subgenera *Montanae* and *Palicourea* are both paraphyletic, the former
205 due to the placement of *Pal. bangii* and *Pal. reticulata* and the latter due to the placement of a clade of
206 Brazilian species sister to the rest of the group. Gene tree discordance is identified across most nodes of
207 *Palicourea*.



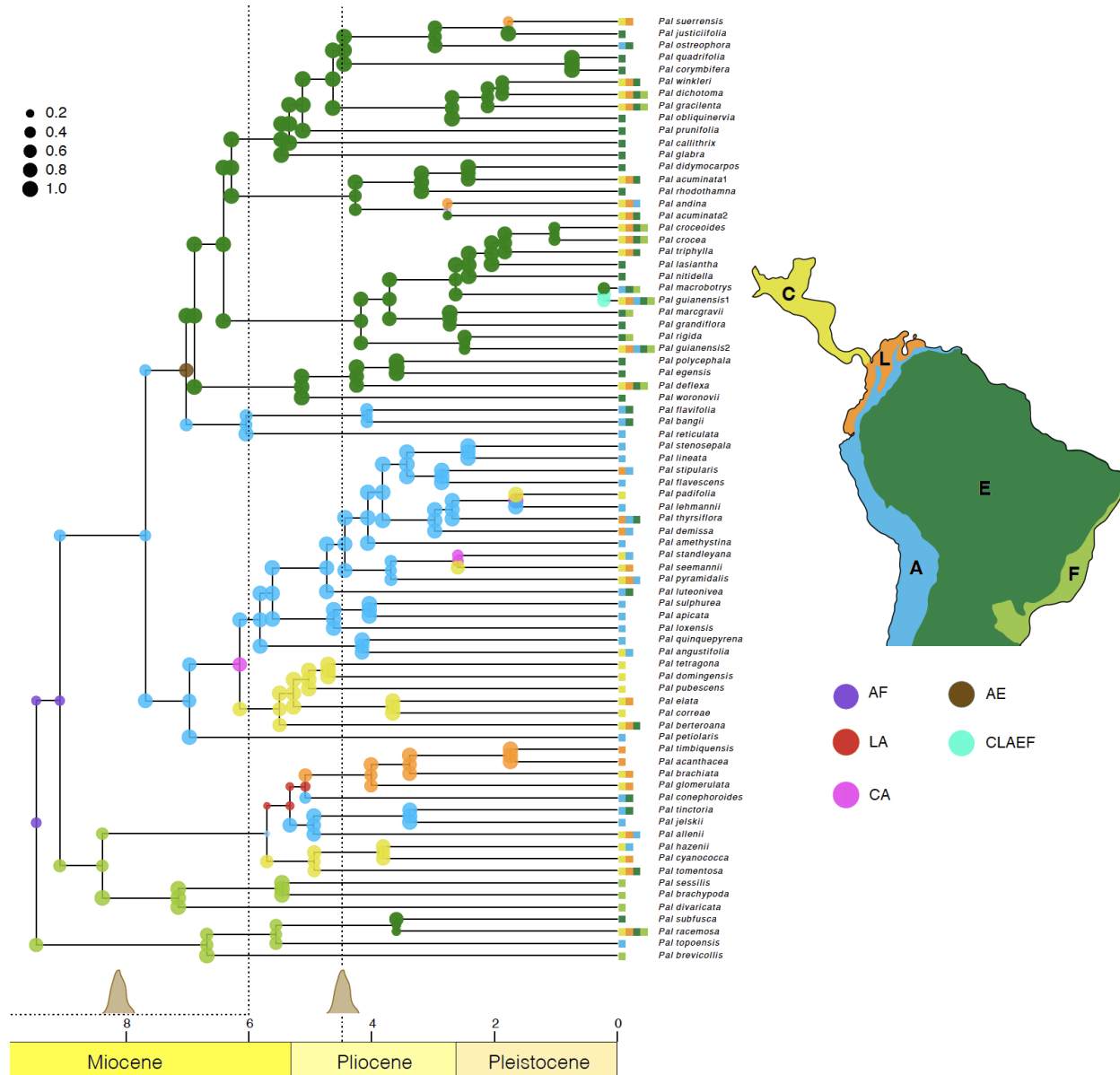
208
 209 Figure 2. ASTRAL species tree of *Palicourea* species in this study, including outgroups. The normalized quartet score
 210 (proportion input gene tree quartets compatible with the species tree are shown (dark blue), as well as the quartet scores for the
 211 two alternative topologies at each node (orange and yellow). Local posterior probabilities >0.7 are shown above branches.
 212 Inflorescence types and the flower visitors that are typically associated with them are color-coded for each species. Species with
 213 intermediate inflorescence morphologies are indicated (*). Species coded in red and Δ have head-like inflorescences subtended
 214 by leafy colorful bracts but are distinct from the characteristic “hot-lips” morphology. Species coded in blue and * have pale
 215 cymes with small to large bracteate flowers that are somewhat condensed. Species coded in purple and Ψ have inflorescences
 216 with somewhat small and pale pink or purple corollas with well-developed, colorful floral axes.
 217

218 Inflorescence type, divergence dating, and modeling of ancestral biogeographic ranges

219 The three broad inflorescence types have evolved multiple times in *Palicourea* (Fig. 2), implying parallel
 220 evolution of traits related to pollination in the group. Large showy bracts (as characterized many former
 221 members of *Cephaelis*) have evolved multiple times, with the true “hot lips” morphology originating at
 222 least twice (once in *Pal. elata* + *Pal. correae*, and again in *Pal. tomentosa* and *Pal. rosea*) and other
 223 showy configurations arising independently. Similarly, the “typical” *Palicourea* and “typical” *Psychotria*
 224 morphologies, with elongated floral axes and showy flowers and open or condensed cymes with small
 225 pale flowers respectively, have evolved multiple times.

226 Inference of divergence times indicates that *Palicourea* originated in the late Miocene (Fig. 3), in line
 227 with previous estimates for the group (Bremer & Eriksson, 2009; Manns *et al.*, 2012; Wikström *et al.*,
 228 2015). Divergence dating further shows that *Palicourea* is biogeographically structured, with one clade

229 originating in Eastern South America (E), a grade in the Atlantic Forest (F), at least two dispersals into
 230 followed by diversification within montane influenced Andean ecosystems (A), a clade that likely
 231 originated in trans-Andean lowland forests (L), and several dispersals from South America into Central
 232 America (C). Notably, dispersal into new regions has frequently been followed by *in situ* speciation and
 233 accompanied by range expansion mostly into adjacent areas. At least four long-distance dispersal events
 234 from the Andes into Central America are inferred to have taken place in the Late Miocene and in the
 235 Pliocene. The ancestral area of *Palicourea* is not inferred with certainty.

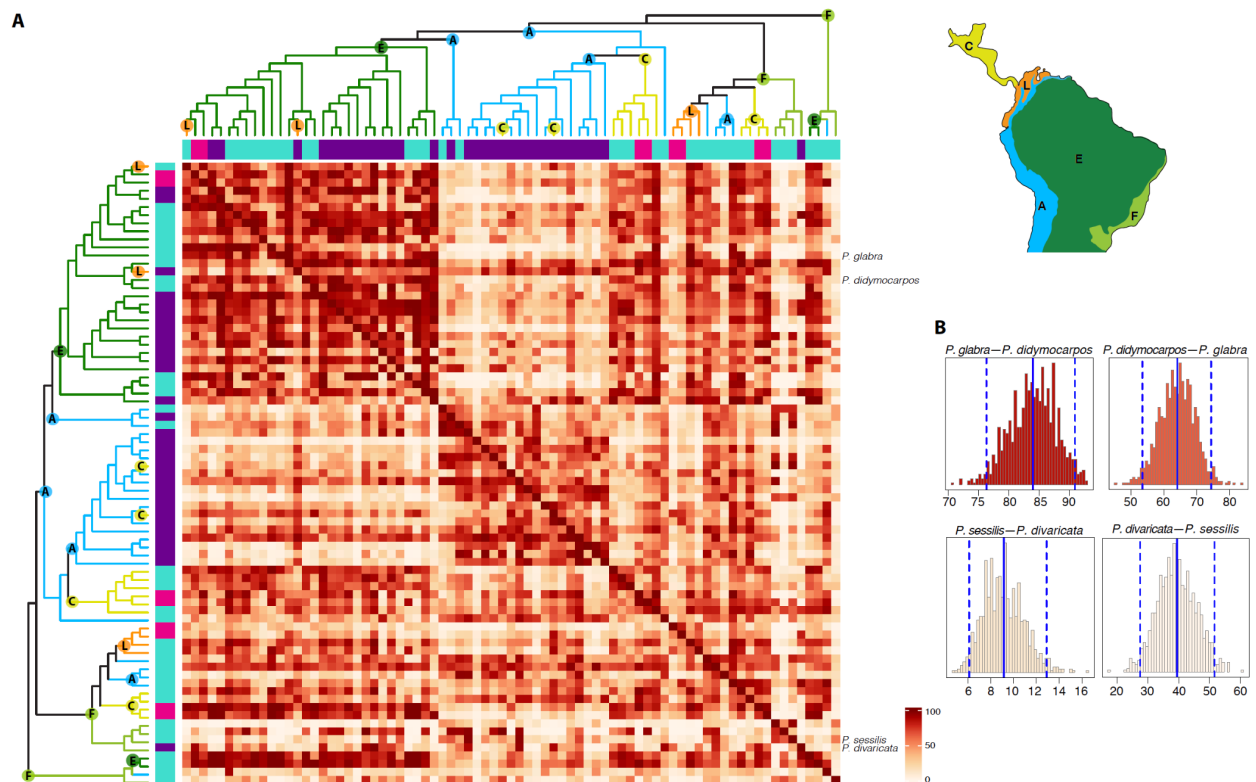


236
 237 Figure 3. Biogeographic reconstruction of *Palicourea* showing time in million years. Major pulses of Andean uplift (~12–6 and
 238 ~4.5 Ma are shown). Biogeographic areas are shown on the map. The probability of the ancestral ranges inferred at the nodes are
 239 proportional to the symbol size. A) Andes. C) Central America. E) Eastern South America. F) Atlantic Forest. L). Trans-Andean
 240 lowland forests. Current distribution of species across defined biogeographic areas is shown at the tips.

241 Many species ranges are characterized by disjunction between two regions. We observe that this
242 sometimes follows a pattern where the disjunct distribution occurs across an area that is interrupted by a
243 climatically distinct region. For example, the large clade inferred to have originated in primarily humid
244 lowland Eastern South America (E) includes eight species with disjunct distributions in lowlands of South
245 and Central America, interrupted by the Andean mountains (A) (Fig. 3). Similarly, a large clade inferred
246 to have evolved in the Andes (A) includes two species (*Pal. angustifolia* and *Pal. standleyana*) with
247 disjunct distributions that also occur in Central American premontane forests. The grade inferred to be
248 from the Atlantic Forest includes a clade restricted to that biogeographic region, as well as species
249 currently restricted to E, A, and a species broadly distributed in lowlands interrupted by A.
250

251 Pairwise climatic niche overlap estimation

252 Results for the estimation of the pairwise probability of realized climatic niche overlap across all species
253 pairs is shown in Fig. 4. Mean values calculated for each pairwise comparison are shown (Fig. 4A),
254 together with the posterior distribution of niche overlap for two selected species pairs (Fig. 4B). Given
255 that each pairwise estimate was calculated independently, the estimated values are not relative to
256 comparisons across other species pairs. Species inferred to have the same ancestral range, particularly in
257 Eastern South America and the Andes, have a high probability of niche overlap (Figs. 4a and 4B, top),
258 despite climatic heterogeneity in our demarcated regions. Species with ancestral ranges in Eastern South
259 America have a high niche overlap with species with inferred ancestral ranges in Central America and
260 some species with ancestral ranges in inter-Andean lowlands (Figs. 3 and 4). Overall, probability of niche
261 overlap is low across pairwise comparisons including species with ancestral ranges in the Andes and in
262 Eastern South America, respectively. There are varying degrees of niche overlap among Atlantic Forest
263 species. In these species, the probability of niche overlap is lowest for species with differing inflorescence
264 types than for species with the same inflorescence structure.
265



266
 267 Figure 4. A) Heatmap showing the estimated mean values of climatic niche overlap across all pairwise comparisons, where the
 268 probability of overlap of the species in the rows to species in columns are shown. Each square constitutes an independent
 269 measure of niche overlap between a species pair. Values are clustered as per our inferred species tree. Ancestral ranges inferred
 270 and inflorescence type are shown as coded in Figs. 2 and 3. B) Posterior distribution of the probability of niche overlap (shown as
 271 percentage) estimated for 2 species pairs. 95% confidence intervals (dashed lines) and mean values (solid line) are shown.
 272

273 Discussion

274 The diversity in geographic ranges, characters related to pollination biology, and climatic niches of
 275 *Palicourea* species make the group a compelling clade for understanding the drivers of evolution in
 276 Neotropical plants. Using *Palicourea* as a model system, we identified a pattern of species coexistence in
 277 biogeographic regions that is mediated by climatic niche conservatism and the evolution of different
 278 inflorescence types that are likely linked to pollinator shifts, suggesting that an interplay of abiotic and
 279 biotic factors explains the clade's high species richness.

280 This study constitutes the most comprehensive multi-locus phylogenetic tree for *Palicourea*, an
 281 ecologically important group of Neotropical plants. Our inclusion of hundreds of nuclear loci vastly
 282 expands upon a previous phylogeny of the group, which used one nuclear marker (ITS; Sedio *et al.*,
 283 2013). This allowed us to model common biological processes (ILS) and identify areas of conflict across
 284 loci when inferring species relationships. The sources of gene tree discordance (gene flow of incomplete

285 lineage sorting) across many nodes of our inferred species tree should be further investigated. Our
286 phylogeny supports a recent broader circumscription of the genus (Taylor *et al.*, 2010; Delprete &
287 Lachenaud, 2018; Taylor, 2019) and points towards minor taxonomic adjustments that will render both
288 subgenera of *Palicourea* monophyletic. The resulting phylogeny is a useful framework for researchers
289 interested in the evolution of Neotropical plant diversity and of Rubiaceae, the fourth largest family of
290 flowering plants.

291

292 *Biogeographic history of Palicourea*

293 Divergence times estimated for *Palicourea* indicate that the genus originated in the Late Miocene (Fig. 3),
294 after globally warm temperatures of the Middle Miocene Climatic Optimum had dropped (Zachos, 2001).
295 At that time, montane ecosystems and their elevational gradients were also likely in place in northern
296 South America as a result of Andean uplift (Jaramillo, 2019; 2023). Mountain building would continue to
297 change the landscape through the Pliocene (Gregory-Woodzicki, 2000; Garziona *et al.*, 2008; Mora *et al.*,
298 2010; Hoorn *et al.*, 2010; Anderson *et al.*, 2016). This uplift process set the stage for diversification of
299 *Palicourea*. The ancestral area of the genus is inferred (although with low probability) to be a broad
300 distribution in the Andes and Atlantic Forest (both mountain-influenced regions) and a major lineage
301 originated in Andean mountains within the first million years of *Palicourea*'s diversification.

302 The evolutionary history of *Palicourea* is marked by episodic events of dispersal into biogeographic
303 regions with distinct climates. Despite occurring across nearly all Neotropical ecosystems, there have
304 been relatively few biogeographic movements, with many resulting *in situ* speciation and range expansion
305 between adjacent areas (Figs. 3 and 4). Among these key dispersal events, *Palicourea* has colonized
306 Central America from South America on multiple occasions, each within the last ~6 Ma, well after the
307 inferred closure of the Central American Seaway with the emergence of the Isthmus of Panama (Montes
308 *et al.*, 2015; Fig. 3). Movement into Central America may have occurred gradually over land, as seed
309 dispersal by small birds, who are attracted to the fleshy and colorful fruits, is characterized by short
310 dispersal distances and limited gene flow (Taylor, 1989; Theim *et al.*, 2014).

311 Many species occur in humid lowland forests on either side of the Andes, resulting in disjunct
312 distributions. The distribution of these species (e.g., *Pal. winkleri*, *Pal. dichotoma*, *Pal. gracilenta*, *Pal.*
313 *acuminata*, *Pal. croceoides*, *Pal. crocea*, *Pal. triphylla*, *Pal. deflexa*, *Pal. standleyana*, *Pal. berteriana*,
314 *Pal. hazenii*, *Pal. tomentosa*, and *Pal. racemosa*) is the result of either long-distance dispersal or local
315 extinction across the Andean mountains. While these events took place ~6–2 Ma (Fig. 3) when most of
316 the Andes were already in place, trans-Andean lowlands, like the present-day Táchira Depression, could

317 have served as corridors for movement of lowland species across the Andes. Geological and biological
318 evidence support that such lowland breaks across the Andes existed through the Pliocene (Hungerbühler
319 *et al.*, 2002; Antonelli *et al.*, 2009; Hazzi *et al.*, 2018; Montes *et al.*, 2021; Sanín *et al.*, 2022b). Passage
320 of lowland species could have also been facilitated through northeastern South America, since the Mérida
321 cordillera did not complete its uplift until 3–5 Ma (Audemard M, 2003). Phylogenetic evidence from
322 other plant groups, as well as frogs, mammals, and non-avian reptiles, is consistent with low-land
323 passages facilitating dispersal across the Andes through the Pliocene, when continuous ranges were
324 formed (Antonelli *et al.*, 2009; Torres Jimenez *et al.*, 2021; Rodriguez-Muñoz *et al.*, 2022). The role of
325 climatic niche conservatism and niche evolution in facilitating dispersal in *Palicourea* is discussed below
326 in the section on the interplay of biogeography, inflorescence structure, and climatic niche overlap.

327

328 *Evolution of inflorescence types*

329 *Palicourea* has experienced repeated evolution of inflorescence types, suggesting multiple shifts in
330 pollinators. The likely ancestor of Palicoureeae had small, white flowers in bractless inflorescences (Fig.
331 1C) that may have relied on various generalist small insects for pollination, as in the former *Psychotria*
332 subgenus *Heteropsychotria* (now embedded within *Palicourea*). Inflorescence morphology within
333 *Palicourea* shows a trend toward increased showiness, both via modification of bracts and evolution of
334 larger, more colorful flowers. This increased showiness has likely been associated with increased
335 importance of hummingbird pollination, which are known to be important pollinators of both the open,
336 thyriform, colorful inflorescences of “typical” *Palicourea* and the “hot lips” morphology of many species
337 including *Pal. elata* (Fig. 1A, B; Ree, 1997; Valois-Cuesta *et al.*, 2011; Furtado *et al.*, 2023).

338 All biogeographic regions are home to at least two inflorescence classes, often resulting from a shift
339 within the region, though there are differences in the patterns of inflorescences across the Neotropics (Fig.
340 4). There are proportionally more species with inflorescence types typically associated with insect
341 pollination (bees, hawkmoths, and flies) in the clade inferred to have originated in Eastern South
342 America. This biogeographic area is primarily dominated by lowlands of the Amazon forest, but also
343 constitutes a heterogeneous landscape with savannas, wetlands, and the Guiana highlands. Our results
344 indicate that this heterogeneity in the landscape of Eastern South America has resulted in the evolution of
345 all inflorescence types across species of *Palicourea* nested in the clade with inferred ancestral range in the
346 region (Fig. 3). In contrast, most Andean species, all of which are adapted to montane ecosystems, have
347 brightly colored flowers in open inflorescences with characteristics linked to visitation by hummingbirds.
348 These results follow Cruden’s 1972 hypothesis that montane environments drive a turnover from insect to
349 vertebrate pollination at high elevations. This hypothesis has recently been empirically tested, supporting

350 the idea that in the tropics, the probability of hummingbird pollination increases with elevation (Dellinger
351 *et al.*, 2023). Future work should focus on documenting effective pollinators in *Palicourea*, and testing
352 the causality of pollinator shifts and dispersal along elevational gradients in the group.

353 *Palicourea* is marked by extreme variation in inflorescence traits, including overall size, elongation of
354 axes (from capitate to long thyrses), presence and size of bracts, and colors of axes, bracts, and flowers
355 themselves. While we have grouped this variation into three main categories to explore broad trends and
356 patterns in the evolution of *Palicourea*, we also warn that this incredible variation is not entirely captured
357 by our codings, including many intermediate forms between our established categories. For example, *Pal.*
358 *justiciifolia*, *Pal. ostreophora*, *Pal. timbiquensis*, and *Pal. acanthacea* have flowers subtended by showy,
359 colorful “leafy” bracts, though these are different from the characteristic “hot-lips” morphology of *Pal.*
360 *elata*. Similarly, some species (e.g., *Pal. gracilentia*) have somewhat congested cymes subtended by
361 small-to-large bracts, distinct from the typical *Psychotria* inflorescence type characterized by open cymes
362 with small bracts (Fig. 2). Species like *Palicourea divaricata* have inflorescences with somewhat small
363 and purple corollas, an intermediate morphology between the traditional *Palicourea* and *Psychotria*
364 inflorescence types. While our future research will examine this variation in more detail and attempt to tie
365 it to reproductive and evolutionary ecology, the broad trends that we identify unquestionably point
366 towards dynamic evolution of inflorescences in *Palicourea*’s history.

367

368 *Climatic niche overlap in Palicourea and its interplay with biogeography and inflorescence type*

369 While *Palicourea* occurs across a wide range of climates and habitat types, we find strong evidence for
370 niche conservatism within the group. Our results indicate that dispersal events associated with major
371 shifts of the climatic niche followed by *in situ* radiation of taxa with broadly similar tolerances, have
372 resulted in most extant species of *Palicourea* (i.e., the eastern South American and Andean clades; Fig.
373 4). We also find evidence in support of climatic niche conservatism mediating transcontinental dispersals
374 in *Palicourea*. Dispersals of the genus to Central America frequently track ancestral adaptation to ranges
375 with hot, humid climates in low-elevation ecosystems, as the two core radiations of Central American
376 *Palicourea* have a high probability of climatic niche overlap with species in lowland eastern South
377 America (Figs. 3 and 4). This is further supported by the fact that various species (e.g., *Pal. triphylla*, *Pal.*
378 *winkleri*, *Pal. berteriana*, and *Pal. racemosa*) have disjunct distribution across biogeographic areas
379 primarily represented by low-elevation ecosystems. Finally, we identified climatic niche conservatism
380 mediating dispersal from South America into Central America across high elevation habitats. Namely,
381 three species (i.e., *Pal. standleyana*, *Pal. angustifolia*, and *Pal. hazenii*) have disjunct distributions in
382 Central America and the high-elevation Andes, and another (*Pal. padifolia*) is embedded within the

383 Andean radiation and is found in montane regions of Central America. These species are primarily
384 distributed in mountainous regions and have an overall high probability of niche overlap with species
385 restricted to the high-elevation Andean region. While lability in niche evolution has allowed *Palicourea*
386 to colonize most habitats of the Neotropics, it appears that niche conservatism is the norm in the genus.

387 The repeated evolution of inflorescence forms within each biogeographic region (Fig. 4) suggests that the
388 biotic niche may be more labile than climatic niche in the evolutionary history of *Palicourea*. It is
389 possible that the co-existence of many closely related species with similar climatic niches, often in
390 communities that include multiple sympatric congeners, is facilitated by shifts in dominant pollinator
391 species. However, phylogenetic scale is a critical aspect in assessing niche evolution (Quintero *et al.*,
392 2022), as is spatial scale, and the regions we have defined (such as eastern South America) are large
393 heterogeneous regions usually with many distinct ecosystems. Expanding our study to investigate
394 community-level differences in climatic niche evolution would provide insights into the mechanisms that
395 have generated the patterns we identify. Species restricted to the Atlantic Forest (i.e., *Pal. sessilis*, *Pal.*
396 *brachypoda*, *Pal. suterella*, *Pal. brevicollis*, *Pal. divaricata*) are a good case study; in this grade, a higher
397 probability of niche overlap was estimated across species pairs inhabiting lowlands and with
398 inflorescence types associated primarily with insect pollination (*Pal. sessilis*, *Pal. brachypoda*, *Pal.*
399 *suterella*, *Pal. brevicollis*), as compared to those pairwise comparisons involving *Pal. divaricata*, a
400 species with light-purple flowers that could be visited by both insects and hummingbirds and distributed
401 in high elevations; Fig. 4B, bottom; Supplementary Materials). These findings indicate that while at a
402 broader phylogenetic and geographic scale niche conservatism is prevalent in species occurring in the
403 same biogeographic region, at a smaller scale both climatic niche divergence and shifts in inflorescence
404 morphology may play a role in the coexistence of species.

405 *Palicourea* is a remarkably species-rich genus of Neotropical plants. Using data derived from herbarium,
406 including genome-wide DNA sequences, occurrence data and morphology, we infer a densely sampled
407 phylogeny of *Palicourea* and demonstrate that species richness in the group resulted from repeated
408 species radiations following dispersal into new geographic areas. This has resulted in a phylogeny that is
409 very biogeographically structured (Fig. 3) and characterized by a high degree of niche conservatism (Fig.
410 4). Species that occur in the same biogeographic region have high niche overlap and exhibit frequent
411 divergence of characters related to pollinator shifts, though traits associated with hummingbird pollination
412 are proportionally more common in montane regions. Processes driving the remarkable diversity of
413 *Palicourea* may include niche specialization, genetic divergence due to isolation in fragmented habitats,
414 local adaptation to extreme climates, and pollination shifts across a dynamic landscape matrix. Future
415 work identifying the contribution of these processes to diversification in the group should include

416 expansion of taxon representation to test hypotheses with the use of comparative phylogenetic methods,
417 as well as ecological field studies documenting the role of inflorescence, fruit, and metabolomic traits in
418 determining biotic interactions.

419

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427

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