The interplay of biogeographic history, floral morphology, and climatic niche in *Palicourea* (Rubiaceae), a species-rich Neotropical plant radiation

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Supporting information: Tables S1–S12; Figures S1–S9

Summary

- Investigating how biotic and abiotic factors interact to shape species evolution is critical to understanding biodiversity patterns in the Neotropics, a region where an interplay of processes promoting diversification shaped the assembly of the world's richest flora.
- Using *Palicourea*, a species-rich and ecologically and morphologically diverse group of Neotropical plants in the coffee family, we aimed to identify patterns resulting from the interplay of biogeographic history, floral morphology, and climatic niche evolution. We inferred the most completely sampled multi-locus phylogeny of *Palicourea* to date, modeled species historical ranges, detected shifts in climatic and elevational preferences, and reconstructed the evolution of inflorescence morphology.
- Lowland Eastern-Northeastern South America and the montane Andean region are centers of species richness. Evolutionary dynamics in the two regions differ and result from a tradeoff between climatic niche disparity and lability of characters related to pollination. The latter is not related to climatic filtering of reliable pollinators across elevation.
- The interaction of biotic and abiotic factors has led to a continental radiation of plants that form an important ecological cornerstone of the Neotropics. Decades of botanical research in *Palicourea* and newly generated genomic data helped reveal patterns and processes shaping niche and range shifts in this diverse group.

Keywords: Biogeography, climatic niche, elevation gradients, floral morphology, Neotropics, phylogenetics

Introduction

Lineage diversification is shaped by the ability of species to move across the landscape and to adapt to changing conditions (Donoghue, 2008; Smith *et al.*, 2014). As evolution and speciation proceed, closely related species are generally expected to fill similar ecological niches and share phenotypic characteristics (Donoghue, 2008; Wiens *et al.*, 2010). However, the long-term co-occurrence of species (through either secondary contact or *in situ* speciation) requires that lineages diverge in one or more niche dimensions (Elton, 1946; Macarthur & Levins, 1967; Godoy *et al.*, 2014; Quintero & Landis, 2020). What determines macroevolutionary patterns across groups is thus a combination of range evolution and ecological processes, including niche conservatism (i.e., the retention of niche requirements of the common ancestor in closely related descendant taxa), niche evolution (e.g., the dispersal of species to new habitats with distinct climatic regimes), dispersal ability, and competition (Wiens & Donoghue, 2004). Investigating

the interplay of evolutionary and ecological processes is paramount to understanding the forces shaping current biodiversity patterns.

The Neotropics is a species-rich region encompassing several of the most biodiverse places on Earth including the Amazon, the Atlantic Forest, and the Andean mountains (Gentry, 1982a; Kreft & Jetz, 2007; Barthlott *et al.*, 2007; Ribeiro *et al.*, 2009). Notably, Neotropical plants constitute the world's richest flora (Gentry, 1982a; Raven *et al.*, 2020; Tietje *et al.*, 2022). Previous studies have indicated that Neotropical plant diversity has been generated by the interplay of abiotic and biotic factors including landscape heterogeneity, the formation of new habitats that triggered ecological opportunity, and pollinator shifts (Hughes & Eastwood, 2006; Madriñán *et al.*, 2013; Lagomarsino *et al.*, 2016; Antonelli *et al.*, 2018). The interaction of these factors resulted in adaptive radiations and replicated radiations in cloud forests and "sky island" páramos in the Andes, where lineages rapidly diversified in part by filling empty niches in recently formed habitats (Hughes & Eastwood, 2006; Simon *et al.*, 2009; Donoghue *et al.*, 2022; Vargas *et al.*, 2023a). Other processes shaping Neotropical plant diversification include the steady accumulation of niche-conserved species through time in climatically stable and isolated habitats like seasonally dry tropical forests (Särkinen *et al.*, 2012).

Past studies suggest that plant distributions are, in general, the result of retention of ancestral niche and dispersal into areas with climatic conditions for which species are pre-adapted (Sedio *et al.*, 2013; Frost *et al.*, 2017; Sanín *et al.*, 2022; Vargas *et al.*, 2023b). However, growing evidence also indicates that biome shifts played an important role in the evolution of several plant taxa (Frost *et al.*, 2022) and that those shifts, particularly across elevation gradients in tropical biomes, are accompanied by pollinator shifts (Dellinger *et al.*, 2023). Combining species-level phylogenies with spatial, morphological, and ecological data is fundamental to understanding of how biotic and abiotic factors play out at broad ecogeographic scales, shaping the assembly of the Neotropical flora (Vargas *et al.*, 2020).

Amongst Neotropical plants, *Palicourea* Aubl. (Rubiaceae) is a remarkably species-rich genus (~650 spp.), striking in its diversity of distribution patterns, climatic niche breadth, inflorescence and flower forms, and pollinators. *Palicourea* ranges from central Mexico to northern Argentina, with a number of species also in the Antilles (Taylor, 1997, 2018). Some species in the genus have broad distributions across South America, while others are restricted to Central America, the Atlantic Forest, the Andes, and the inter-Andean lowlands in northwestern South America. *Palicourea* inhabits various climatic niches: most species are found in low-elevation, wet forest ecosystems with elevational ranges between 0–1800 m.a.s.l (Taylor, 1997), but some species range up to the tree line in montane ecosystem (>3000 m; Rubiaceae project; http://legacy.tropicos.org/Name/40000063?projectid=34). Other species are found in seasonally dry forests and gallery and campina vegetation, and several appear to be specialists on acidic

substrates. The genus was traditionally characterized by thyrsiform (i.e., open and branched) inflorescences with brightly colored, tubular, often somewhat fleshy corollas that are primarily visited by hummingbirds but also by butterflies (Fig. 1A). The fruits are purple, blue, black or white, bird-dispersed drupes. However, morphological and molecular work has shown that several species traditionally recognized as belonging to other genera, notably *Cephaelis* Sw. and *Psychotria* L., are in fact nested in *Palicourea* (Fig. 1B,C) (Andersson & Rova, 1999; Nepokroeff *et al.*, 1999; Andersson, 2001). This recircumscription fundamentally changed the size and characterization of the genus, with an expanded definition that includes species with different flower and inflorescence forms such as head-like inflorescences enclosed by showy bracts (associated primarily with butterfly but also hummingbird pollination). Other species newly added to *Palicourea* have open to condensed cymose inflorescences with small to large, white flowers and small to large bracts (associated primarily with pollination by butterflies and other insects; Fig. 1; Taylor & Berger, 2026).

Here, we use the diverse plant genus *Palicourea* as a model to investigate how the interplay of biogeographic history, realized climatic niche, and inflorescence evolution have shaped macroevolutionary patterns in the Neotropics. Using target enrichment data and a strategy for paralog removal and orthology assessment, we inferred phylogenetic relationships of selected *Palicourea* species. The inferred phylogeny was used as a framework to assess the biogeographic history, evolution of traits related to pollination, and climatic preferences in the group. We then test if range evolution is linked to shifts in realized climatic niche or if dispersal tracks the climatic preferences of species —the latter is a pattern considered as prevalent in Neotropical plant evolution. To shed light on the sources of species richness in *Palicourea*, we further investigate if disparity in climatic niche or evolutionary lability of inflorescence type are prevalent within biogeographically structured clades. We test if the latter is driven by the expected preferred pollinators across elevation gradients. Finally, we established causal relationships between biogeography, climatic niche evolution, and shifts in inflorescence types favoring hummingbird pollination through phylogenetic path analysis.

Materials and Methods

Sample collection, DNA extraction and Target Enrichment

We sampled a combination of silica dried and herbarium specimens of 242 specimens in the Rubiaceae including 212 *Palicourea* (192/650 spp), 17 *Psychotria* including 7 suspected to be nested in *Palicourea* and awaiting nomenclatural transfer, and one sample each of the outgroups *Balmea stormiae, Carapichea affinis, Carapichea ipecacuanha*, *Carapichea guianensis, Cosmibuena grandiflora, Eumachia boliviana, Hamelia patens, Hillia parasitica, Hoffmannia phoenicopoda, Notopleura epiphytica*, *Notopleura uliginosa, Rudgea cornifolia,* and *Rudgea hostmanniana,* (Table S1). To capture general patterns across

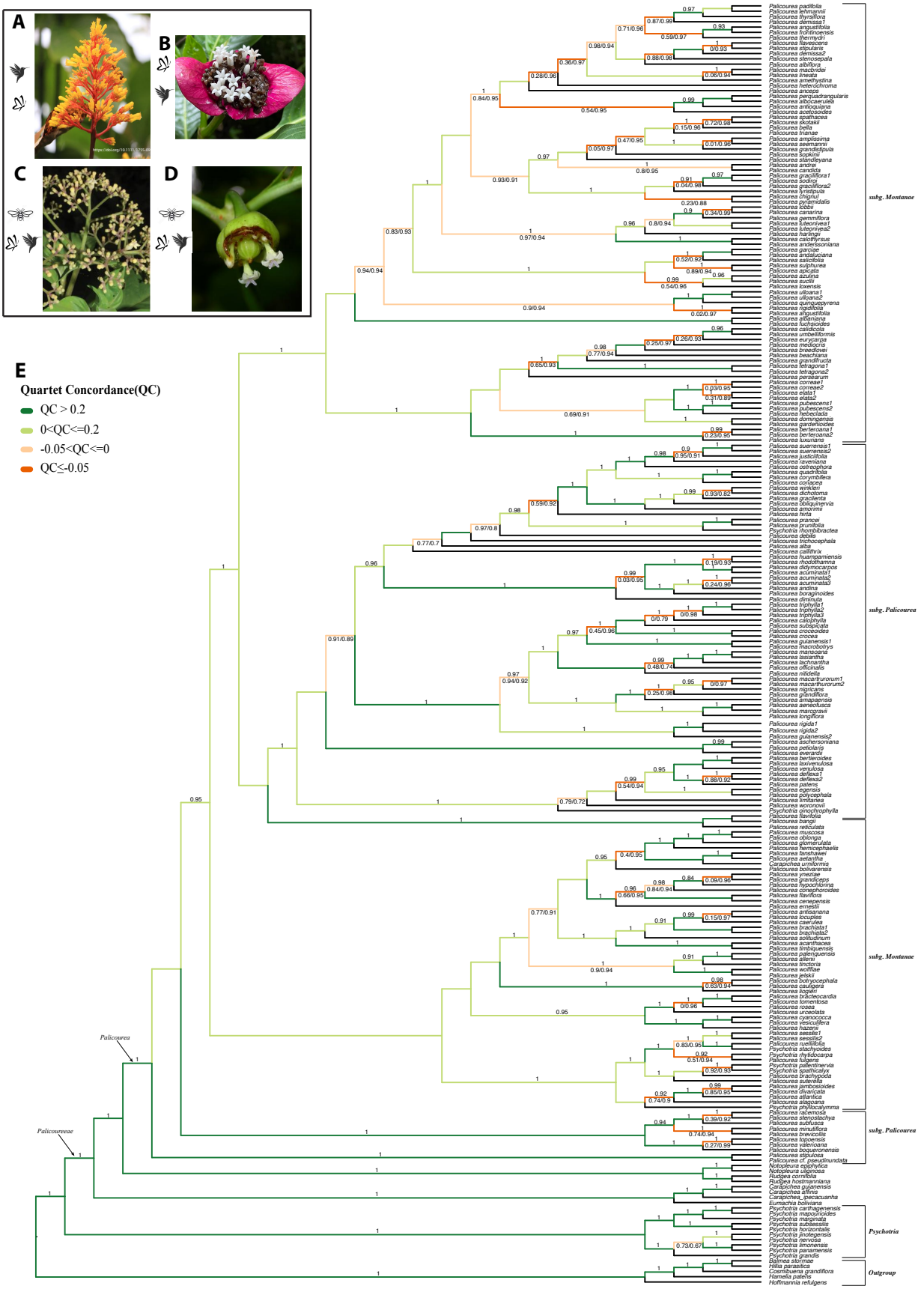
A
B
C
D
E
Quartet Concordance(QC)
QC > 0.2
0<QC<=0.2
-0.05<QC<=0
QC≤-0.05
Palicoureeae
Palicoureae
subg. Montanae
subg. Palicourea
subg. Montanae
subg. Palicourea
Psychotria
Outgroup

Figure 1 (previous page). Inferred species tree of *Palicourea* and inflorescence type as designated in this study and. A) Thyrsiform inflorescences, with characteristics associated with primarily hummingbirds but also butterfly pollination. B) Head-like inflorescence subtended by showy bracts, traditionally associated with butterfly visitors, but also believed to be visited by hummingbirds. C) Open cymoid inflorescences with small, white flowers and reduced bracts. This inflorescence type has characteristics associated primarily with insect pollination (bees, hawkmoths, flies), but hummingbird visitation has also been reported (Modified from Barry E. Hammel; Tropics.org. Missouri Botanical Garden. 17 April 2024 <http://www.tropicos.org/Image/100947249> CC-BY-NC-SA). D) Condensed cymoid inflorescences with small, white flowers and non-showy bracts (Modified from J. Homeier; Tropicos.org. Missouri Botanical Garden. 17 April 2024 http://www.tropicos.org/Image/86438 CC-BY-NC-SA). E). Inferred ASTRAL species tree. Branches are colored by Quartet Concordance score (QC). Local posterior probability > 0.9 is shown above branches. Quartet Differential and Quartet Informativeness scores are shown below branches (QD/QI) with low QC.

the species-rich *Palicourea*, our sampling was designed to broadly represent the current classification, morphological diversity, and geographic distribution of the group. This was led by CMT, the leading taxonomic expert of Neotropical Rubiaceae, including *Palicourea*. Most samples correspond to voucher specimens from the Missouri Botanical Garden. Total DNA was extracted using a modified sorbitol protocol (Štorchová *et al.*, 2000). We generated target enrichment sequence data for 2270 exons in 1059 loci using a Rubiaceae-specific probe set (Ball *et al.*, 2023), which has shown high target enrichment efficiency for Palicoureeae. Library preparation, target enrichment, and sequencing of targeted regions was conducted by RapidGenomics (Gainesville, FL, USA). Samples whose DNA was not degraded (e.g., retained 250-1000 bp fragments) were fragmented to a standard target size for Illumina sequencing. Enriched pools were combined, and probes were hybridized to pools for target enrichment. Paired end reads (150 bp) were sequenced in Illumina HiSeq 3000, Illumina NovaSeq S4, and Illumina NovaSeq platforms.

Data processing and phylogenetic inference

FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to quality-check the demultiplexed reads prior to and after adapter removal with Trimmomatic v.0.39 (Bolger *et al.*, 2014). Loci were assembled with HybPiper v.2.0.1 (Johnson *et al.*, 2016) using exons as reference sequence and assembling single contigs instead of "supercontigs" to avoid assembling chimeric regions (i.e., contigs with stitched sequences derived from different paralogs or alleles; Morales-Briones *et al.*, 2018, 2022). Assembled regions with <50% sequence length recovered across <20% samples were removed. Paralog identification was conducted by extracting loci with paralog warnings (putative paralogs) with the paralog_retriever function of HybPiper. Loci without paralog warnings (no more than one sequence recovered per sample) were aligned with MACSE (Ranwez *et al.*, 2018).

To maximize the amount of sequence data used in this study, orthology inference was conducted for putative multi-copy loci following an automated tree-pruning approach (Morales-Briones *et al.*, 2022). Briefly, all sequences flagged as paralogs (>1 contig assembled per target region) were aligned with MACSE (Ranwez *et al.*, 2018), codon frameshifts were replaced with gaps, and columns with >90% missing data were removed with Phyx (Brown *et al.*, 2017). Homolog trees were inferred with RAxML-ng (Kozlov *et al.*, 2019) with a GTR+G model using an MRE-based bootstrapping method for branch support estimation (Felsenstein, 1985). Monophyletic and paraphyletic tips that belonged to the same taxon were masked, keeping the tip with the most unambiguous characters in the alignments. Unusually long branches, which are likely associated with spurious sequences, were removed with TreeShrink v1.3.9 (Mai & Mirarab, 2018). Final ortholog trees were obtained with a "monophyletic outgroup" strategy (Yang & Smith, 2014) keeping only ortholog groups with ≥35 (>15%) ingroup taxa. The "monophyletic outgroup" approach uses rooted gene trees and for each gene tree, it searches for the

largest subtree with monophyletic and single copy outgroups. Gene trees with paraphyletic or multicopy outgroups were discarded. Final fasta files were generated from ortholog trees and used for alignment with MACSE. Gene tree inference was carried out with RAxML-ng, as specified above. Loci with final alignments <200 bp were removed. Scripts used for data processing are available at https://github.com/ambed0ya/Palicourea.

We inferred phylogenetic relationships using a two-step coalescent aware species tree inference method (ASTRAL-III v5.7.8) (Zhang *et al.*, 2018) and used the previously inferred gene trees as input, collapsing branches with <10% bootstrap support following Zhang et al., 2018. When multiple accessions assigned to the same species were included in the analyses, these were considered separate operational taxonomic units as species monophyly has not been broadly tested. We used the Quartet Sampling method (Pease *et al.*, 2018) to characterize discordance in the inferred ASTRAL species tree topology and to distinguish lack of support from gene tree conflict. We estimated Quartet Concordance (QC; an entropy-like measure of how frequently the quartet that is concordant with the species tree occurs relative to the two discordant quartets for a given branch), Quartet Differential (QD; a measure of the disparity in the proportion of the two discordant quartet topologies for each node), Quartet Informativeness (QI; the proportion of inferred quartets that are informative for a given branch), and Quartet Fidelity scores (QF; an estimate of the proportion of times a given taxon produces a topology that is concordant with the species tree when sampled). We applied the method with 200 replicates (log-likelihood threshold cutoff=2). Final alignments were concatenated into a single super matrix and used as input for Quartet Sampling (https://github.com/FePhyFoFum/quartetsampling) alongside the previously inferred species tree.

Divergence dating, biogeographic modeling, and inflorescence type evolution

We estimated divergence times for our inferred species tree in Beast2 v2.7.6 (Bouckaert *et al.*, 2014). Due to problems with identifiability in the estimation of rates and divergence times when using a relaxed clock from relatively short loci and one secondary calibration point (see below), we used a strict clock model and a birth-death tree prior. Given computational limits to the number of loci that can be analyzed jointly for divergence dating, we used a subset of 15 loci and specified a partitioned HKY+G substitution model, which provided a consistent approximation across partitions as per model selection with ModelFinder (Kalyaanamoorthy *et al.*, 2017) as implemented in IQ-TREE v.3 (Wong *et al.*, 2025). The loci used for divergence dating were selected from the inferred orthologs dataset with genesortR (Mongiardino Koch, 2021), an R script for phylogenomic data subsampling using various standard criteria for evaluating sources of systematic bias and phylogenetic signal across loci (e.g., average pairwise patristic distances, bootstrap support, and Robinson-Foulds similarity to a target topology). Input files for genesortR were formatted with SEGUL v0.23.0 (Handika & Esselstyn, 2024). We incorporated topological similarity to

198 the species tree as a subsampling criterion and only evaluated loci with ≥200 bp and ≥100 sequences.
199 When multiple samples per species were present in the 15 loci filtered, we randomly selected one sample
200 per clade.
201 We used a secondary calibration for the crown node of *Psychotria* and Palicoureeae (i.e., normal prior
202 distribution with μ=32 and σ=4.2), derived from previous dating analyses of Rubiaceae (Bremer &
203 Eriksson, 2009; Manns *et al.*, 2012; Wikström *et al.*, 2015). A starting tree compatible with the
204 calibration prior was inferred using penalized likelihood (*chronos* function of R package *ape*; Paradis &
205 Schliep, 2019) on the ASTRAL species tree. We ran two independent chains for 200 million generations
206 and stored trees and states every 10,000. Chain convergence was assessed in Tracer v.1.7.1(Rambaut *et*
207 *al.*, 2018) and with the CODA package in R using convergence diagnostics (gelman R and ESS > 200
208 values) for all default parameters. We discarded a 0.25 fraction of posterior trees for each run before
209 combining inferred trees from both chains and generated a maximum clade credibility tree from the
210 resulting posterior distribution. To assess the influence of tree priors and sequence data on divergence
211 time estimates, we conducted a prior-only analysis and an analysis with a Yule tree prior (as opposed to a
212 birth-death tree prior). We ran two independent chains (200 million generations each) for each additional
213 analysis and assessed convergence as specified above.
214 We inferred biogeographic range evolution on the maximum clade credibility time-calibrated tree under
215 Dispersal-Extinction-Cladogenesis in RevBayes (Höhna *et al.*, 2016a), modeling anagenetic and
216 cladogenetic changes, and assuming colonization and vicariance as equally important to range evolution
217 and cladogenesis (Ree & Smith, 2008). We defined five biogeographic regions that reflect distribution
218 patterns in the *Palicourea* species included in this study and previous work on Psychotrieae and
219 Palicoureeae (Sedio *et al.*, 2013). Biogeographic areas were defined as follows: Central America (C),
220 lowland forests west of the Andes (L; i.e., Chocó region of western Colombia, Darién region of Eastern
221 Panama, and coastal areas and inter-Andean valleys in northern Colombia and Venezuela predominantly
222 at <1500 m.a.s.l.), high-elevation Andean region (A; predominantly at ≥1500 m.a.s.l.), primarily lowland
223 Eastern-Northeastern South America (E; Amazon and Orinoco basins, Guiana Shield region, and
224 Cerrado), and the Atlantic Forest (F). The elevation used to define the high-elevation Andean region—
225 which encompasses mid- and upper montane forests and low páramo habitats— followed Sedio et al.,
226 2013, whose cutoff is based on floristic studies of Rubiaceae; 1500 m is also a recognized elevation where
227 the Andean flora transitions towards higher proportion of highland-associated elements (Kessler *et al.*,
228 2011; Galbraith *et al.*, 2014). However, as the lower limits of montane forests vary locally, our area
229 coding was revised and confirmed by CMT, the leading taxonomic authority in *Palicourea*. Code used for
230 the above analyses is available at https://github.com/ambed0ya/Palicourea .

For each species of *Palicourea* included in this study, inflorescence type was coded into four different categories: 1) thyrsiform cyme with relatively long, somewhat fleshy corollas in shades of blue, pink, red, orange, or yellow ("*Palicourea*-like"; Fig. 1A); 2) head-like form with small, white flowers subtended by enlarged showy bracts ("*Cephaelis*-like"; Fig. 1B); 3) open cyme with pale flowers and reduced bracts ("open *Psychotria*-like"; Fig. 1C); and 4) condensed cyme with pale flowers and non-showy bracts ("condensed *Psychotria*-like"; Fig. 1D). These simplified inflorescence types each include a range of variation, and intermediate forms are found in some *Palicourea* species. We inferred ancestral states with RevBayes (Höhna *et al.*, 2016b) under an independent rates and an equal rates model, assuming an exponential prior distribution for transition rates (see https://github.com/ambed0ya/Palicourea for parameter settings and scripts). The two MCMC analyses were run for 25,000 generations and models were compared via Bayes Factors.

Climatic niche and elevation data preparation

We used the Climate and Niche Distribution Inference (CanDI; https://github.com/abbyj-g/candi) pipeline to download climate data and gather and clean species occurrences from the Global Biodiversity Information Facility (GBIF) and the Botanical Information and Ecology Network (BIEN). We removed occurrence records that were duplicated, that are exactly at 0°, 90°, or ≥180°, or that fall in the ocean. Species with >10 filtered occurrence records were kept. Although not all publicly available data are authoritatively checked (Maldonado *et al.*, 2015; Goodwin *et al.*, 2015), intense data curation and validation by taxonomic experts (including CMT) minimized potential geographic inaccuracies and taxonomic uncertainties in our dataset. We extracted associated bioclimatic variables for cleaned occurrence data from the WorldClim database (Fick & Hijmans, 2017), log transformed all values. We selected a subset of bioclimatic variables based on their biological interpretability and ecological relevance to *Palicourea*, while removing multicollinearity. A correlation matrix was calculated from the extracted values (Fig. S1) and log-transformed variables that had correlation coefficients <0.8 were kept. We extracted elevation data from our curated occurrences using a raster file manually downloaded from WorldClim (worldclim.org); the species median value for each abiotic variable was used in all subsequent analyses.

Evolution of climatic niche and elevation and its link to biogeographic patterns

To investigate the lability in the evolution of climatic and elevation optima and its relationship to the biogeographic history of *Palicourea,* we addressed three complimentary questions. 1) Is diversification in climatic niche space or niche conservatism characteristic of major biogeographically centered clades of *Palicourea*? 2) Are shifts in the climatic niche preference of species associated with biogeographic range

evolution? 3) Are transitions across elevational gradients accompanied by shifts in climatic preferences of species in *Palicourea*? First, we estimated disparity in climatic variables in *Palicourea* across five biogeographically structured monophyletic groups and one biogeographically unstructured clade identified in our ancestral range reconstruction (Fig. 2). Taxa from the lowland forests west of the Andes were not included in this study as the largest clade included only three species. Several of the biogeographically structured clades used in the disparity analysis include lineages that have moved out and no longer occur in the biogeographic area characteristic of each clade. To ensure that inference of disparity reflected within-region patterns, the analysis was restricted to species that both originated and remained within each clade's defining biogeographic area. We used scores from the first two axes of a principal components analysis (*prcomp*) of abiotic variables (PC1=temperature proxy; PC2=precipitation and temperature seasonality proxy; Table S2) to estimate disparity using the average squared pairwise distance metric with the R package *dispRity* (Guillerme, 2018). To account for the differences in clade size, we conducted bootstrapping (n=1000 bootstraps) with rarefaction (n=4) and tested for significance of clade differences using the Bhattacharyya coefficient (*test.dispRity;* Guillerme, 2018) with Bonferroni correction following Dellinger et al., 2023. To visually inspect the variation in realized abiotic (climatic) niche, we performed a phylogenetic principal component analysis (*phyl.pca* function of Phytools v2.1; Revell, 2024). Here too, we restricted the analysis to species inferred to have both originated and remained in one of our defined biogeographic areas.

Second, we tested if shifts in climatic niche preferences of species are associated with range evolution using a multi-optima Ornstein-Uhlenbeck model in *l1ou* (Khabbazian *et al.*, 2016) in which all the abiotic variables were jointly analyzed; model selection was conducted using the phylogenetic Bayesian information criterion (pBIC) (Khabbazian *et al.*, 2016). All other settings were kept at default values. Convergent regimes were estimated with the *estimate_convergent_regimes function*.

Third, we tested if species realized climatic niches are correlated with elevation using phylogenetic generalized lineal models implemented in the R package *phylolm* (Tung Ho & Ané, 2014). We ran the analysis using scores from the first two axes of the principal components analysis as for estimates of disparity above (PC1=temperature proxy; PC2=precipitation and temperature seasonality proxy; Table S2). Elevation was calculated as the mean elevation across occurrence records for each species.

Interplay of range evolution, environmental preferences, and inflorescence type

To investigate the interplay of abiotic (climatic niche and biogeography) and biotic (inflorescence type associated with probable pollinators) factors on the evolution of *Palicourea*, we used phylogenetic path analysis (PPA; Hardenberg & Gonzalez-Voyer, 2013; Gonzalez-Voyer & Von Hardenberg, 2014). We evaluated alternative causal hypotheses linking biogeography (BG), climatic niche (Clim), and

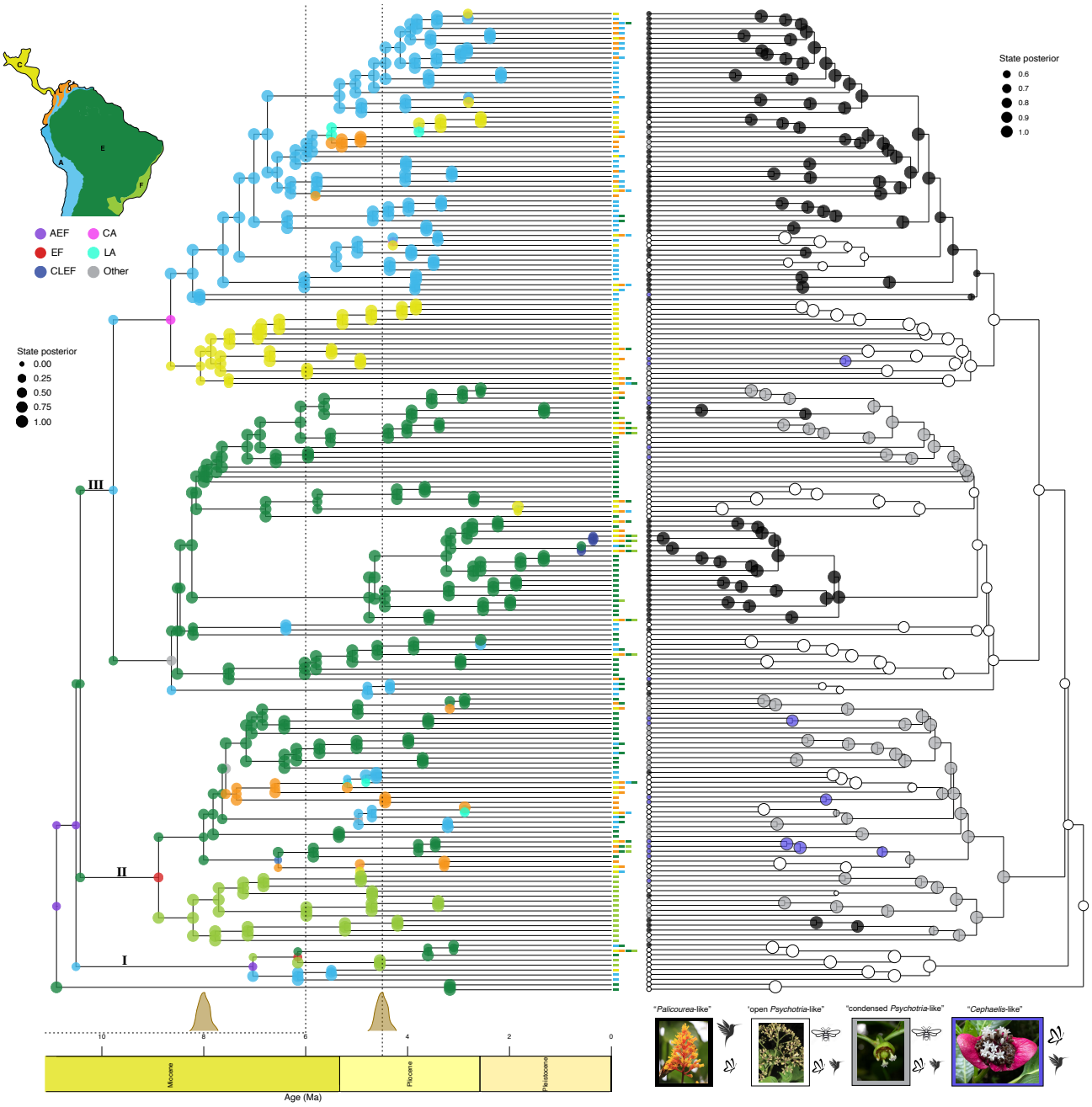

Figure 2. Results for biogeographic modeling (left) and ancestral state reconstruction of inflorescence type (right) in *Palicourea*. Major pulses of Andean uplift (~12–6 and ~4.5 Ma) are shown on the map. The probability of inferred ancestral states at the nodes are proportional to the symbol size. C) Central America. L) lowland forests west of the Andes). A) High elevation Andes. E) primarily lowland Eastern-Northeastern South America. F) Atlantic Forest. Presence of species across defined biogeographic areas is shown at the tips.

inflorescence type (Infl). As elevation is strongly correlated with our climatic niche proxies (see results section below), we did not include it in PPA analyses. We binarized biogeographic area as Andean or extra-Andean (see "Divergence dating, biogeographic modeling, and inflorescence type evolution" section above for definition of high-elevation Andes biogeographic area). Inflorescence type was also binarized with "*Palicourea*-like" inflorescences as primarily hummingbird and "*Cephaelis*-like" and both open and condensed "*Psychotria*-like" inflorescences as primarily insect pollinated. This categorization is based on previous pollination studies in the group (e.g., Feinsinger *et al.*, 1988; Ree, 1997; Taylor *et al.*, 2010; Valois-Cuesta *et al.*, 2011; Betancourt *et al.*, 2023). Climatic niche was represented by the first two axes of the principal components analysis (See section above. PC1=temperature proxy; PC2=precipitation and temperature seasonality proxy; Table S2). We built and evaluated two sets of our models, one including PC1 and another with PC2. Competing causal models were compared using phylogenetic path analysis implemented in the R package *phylopath* (Van Der Bijl, 2018) following (Dellinger *et al.*, 2024). We used Pagel's lambda for the regressions on our continuous variable, and maximum penalized likelihood for the binary variables. Model fit was assessed using Fisher's C statistic ($p>0.05$=model fit to data). We conducted model selection using the corrected C-statistic Information Criterion (CICc; CICc>2=support for the better model). Standardized path coefficients of regressions including a continuous variable were estimated for the best-supported model (see https://github.com/ambed0ya/Palicourea for code).

We constructed and compared fourteen models to test for the PPA (Fig. S9; Table S8). Four models test for a direct impact of occupation of the Andean region on the evolution of an inflorescence primarily pollinated by hummingbirds, either alone (H1, H3, H4) or mediated by initial climate niche shifts (H2). Four models broadly test if the evolution of inflorescences primarily pollinated by hummingbirds was driven by shifts in climatic niche, either directly (H5, H7, H8) or mediated by initial biogeographic shifts into the Andes (H6); this latter model is consistent with Cruden's 1972 hypothesis that dropping temperatures resulting from increased elevation in the Andean region favors vertebrate pollination. Additional models test if inflorescence evolution is driven by the combined effects of occupation of montane Andes and shifts in climatic niche (H9) or that there is no link abiotic factors and inflorescences associated with hummingbird pollination (H10, H11). Three final models test if inflorescence type drives evolution of abiotic factors (H12-14). Some of our specified models imply the same conditional independencies (i.e., H2/H4, H6/H8, H10/H11, and H3/H7/14) and hence they cannot be statistically distinguishable through PPA. However, we included all models for further interpretation of results.

We further explored if inflorescence type evolution is linked to variation in elevation occupation. For this we applied phylogenetic ANOVA (*phylANOVA;* Garland *et al.*, 1993; Revell, 2024) followed by Levene's test (*leveneTest;* Fox & Weisberg, 2019). We characterized inflorescence type as "primarily hummingbird" and "primarily insect pollinated" as specified above. Finally, we estimated how the proportion of primarily hummingbird pollinated species varied with elevation using linear regression (*lm*). For this, we counted the number of taxa in each of the two primary pollinator categories in 50 m bins (e.g., 0–50, 51-100) and estimated the proportion of taxa with primarily hummingbird pollinators across bins. Eleven records over 4000 m were binned together (4001–4700 m).

Results

Data processing and phylogenetic inference

Locus recovery efficiency is shown in Table S3 and Fig. S2. A total of 1,059 exons passed all filters (i.e., >20% samples at >50% sequence length and loci with >200 bp), including 184 exons with no paralog warnings and 875 inferred orthologs. Exon filtering results and the proportion of exons retained with our filtering criteria are in Table S4, Fig. S3.

The inferred species tree (Fig. 1E) recovered *Psychotria* as sister to Palicoureeae with high support, in agreement with previous work (Manns *et al.*, 2012; Sedio *et al.*, 2013; Razafimandimbison *et al.*, 2017). Seven species of *Psychotria* that are currently pending name transfer to *Palicourea* based on morphology are confirmed to fall within *Palicourea* in our inferred species tree. Subgenera *Montanae* and *Palicourea* as currently circumscribed were both found as paraphyletic. The Quartet Concordance (QC) score is shown as a measure of branch support. The metric considers the number of non-zero counts among the three candidate quartet topologies for a given branch in the species tree, and the total number of replicates where the optimal resolution exceeded the minimum likelihood threshold (DL=2) (Pease *et al.*, 2018). QC takes on the range [-1, 1] and approaches 1 when the number of counts of the quartet topology concordant with the species tree >0 and the two discordant quartet topologies occur at about similar proportions. Negative values indicate support for one of the two alternative topologies. A QC> 0.2 can be interpreted as good support for the species tree (Pease *et al.*, 2018).

The inferred quartet topologies were often concordant with the ASTRAL species tree, as indicated by the Quartet Concordance (QC) and Quartet Differential (QD) scores (Fig. 1 and Table S5). Areas of low support are predominantly in shallow nodes and in the subgenus *Montanae* (Fig. 1 and Table S5), where there is weak (QC<0.2) and counter-support for branches in the species tree (negative QC values and QD<0.3). The proportion of informative replicates across branches is summarized by the quartet

informativeness (QI) score (0.67–1; μ = 0.94; Table S5). The high QI estimated values indicate that conflict across branches is not the result of lack of information in our data.

Divergence dating, biogeographic modeling, and inflorescence type evolution

Sensitivity analyses (i.e., "prior only" and "Yule tree prior") resulted in poor mixing of the "prior only" analysis, indicating limited identifiability of rate and diversification parameters in the absence of sequence information (Fig. S4). In addition, the posterior distribution of the crown age of *Palicourea* differed from the prior-only distribution, indicating that sequence data contributed information to the posterior distribution of estimated node ages beyond the calibration priors. Divergence time inference was also robust to tree-prior specifications, as the use of a Yule tree prior yielded estimates broadly consistent with those obtained under the Birth–Death prior. Despite identifiability issues of relaxed-clock analyses with our dataset, the evidence that posterior node-age estimates were informed by sequence data and remained stable across tree priors, supports the robustness of our strict clock analysis.

Palicourea originated in the late Miocene (ca. 11 Ma; 95% HPC=7.7, 14.07 Ma; Fig. 2, left and Fig. S5), in line with previous estimates for the group (Bremer & Eriksson, 2009; Manns *et al.*, 2012; Wikström *et al.*, 2015). The smaller clade I occupies the full biogeographic range of *Palicourea* and is relatively unstructured. The core radiation of *Palicourea* (clades II and III) have stronger biogeographic structure, with one large clade that diversified in montane Andean ecosystems (defined here as >1500 m), two larger clades that diversified in Eastern-Northeastern South America, and two smaller clades that originated and radiated in Central America and in the Atlantic Forest. Species presence in inter-Andean lowlands of northwestern South America results from at least five independent dispersal events from the Andes and from Eastern-Northeastern South America. Dispersal is predominantly between adjacent biogeographic regions, with at least five long-distance dispersal events from the Andes into Central America, of which one was inferred to have taken place in the Late Miocene and four in the Pliocene after the second pulse of Andean uplift. Notably, early dispersal into new regions was frequently followed by *in situ* diversification and accompanied by range expansion mostly into adjacent areas.

The independent rates model was a better supported model than the equal rates model (ln*BF*=3.8184) for ancestral state reconstruction of inflorescence type. Our results show that *Palicourea* has undergone multiple shifts from its ancestral "*Psychotria*-like" open inflorescence with small, white flowers towards more specialized morphologies (Fig. 2, right; Rubiaceae project; http://legacy.tropicos.org/Name/40000063?projectid=34) (Ree, 1997; Valois-Cuesta *et al.*, 2011; Furtado *et al.*, 2023). While we inferred six transitions to "condensed *Psychotria*-like" inflorescences that likely share generalist insect pollination with the ancestor of *Palicourea*, the showier "*Cephaelis*-like" and "*Palicourea*-like" inflorescence types have evolved independently at least 11 and 12 times respectively.

The former is found primarily at low elevation (<1500 m; Fig. 2), while the latter is associated with subsequent diversification in both lowland and montane habitats. Six reversals from "*Palicourea*-like" to *"Psychotria*-like*"* are also reported, all within clade III. Among the species that occur in the montane Andes in our sampling (n=71), ~66% (n=47) have inflorescence types associated primarily with hummingbird pollination.

Evolution of climatic niche and elevation and its link to biogeographic patterns

Phylogenetic PCA resulted in the two first two components together explaining 93.4% of variation in the data. PC1 primarily encompasses a gradient from climates with high to low temperature seasonality. PC2 mainly reflects a gradient from cold and dry to warm and wet climates. We aimed to test whether diversification in climatic niche space or niche conservatism is characteristic of major biogeographic clades of *Palicourea.* Our phylogenetic PCA results show that taxa in different biogeographic areas occupy a gradient from more to less seasonal temperature, starting with the Atlantic Forest, followed by Central American, Eastern-northeastern South America, lowland forests west of the Andes, and Andean taxa, the latter four of which broadly overlap (Figs. 3D and S6; Table S6). Most Andean species occupy drier and colder climates relative to species found in other biogeographic areas, as well as a broader precipitation and average temperature spectrum. The spread of species within biogeographic areas along climatic gradients shown in our phylogenetic PCA is further captured by disparity analyses. Disparity in species preferences for temperature is higher in the Andes, with low overlap in disparity between the Andean clade with all other biogeographic clades, except the unstructured clade (Fig. 3B-C; Table S6). No significant overlap in disparity in temperature seasonality and precipitation was inferred between the Atlantic Forest clade and clades in other biogeographic areas (Table S6).

Testing if shifts in the climatic niche preference of species are associated with range evolution resulted in 38 inferred evolutionary shifts in climatic and elevation optima, with 20 involving lineages from the montane Andes. We found 12 of these evolutionary shifts to be associated with four convergent regimes (Fig. 3A). Temperature and precipitation optima tend to be lower in Andean lineages than those from other regions, with some overlap with the Atlantic Forest clade. However, Andean and Atlantic Forest groups differ in temperature seasonality regimes. *Palicourea* occupies primarily lowland ecosystems across areas outside the Andean region. Phylogenetic PCA further supports these patterns (Figs. 3D and S6; Table S7).

Finally, testing if the climatic preferences of species are correlated with elevation revealed a strong negative effect of elevation on the temperature preference of *Palicourea,* with elevation largely explaining the variation in PC1, and strong phylogenetic signal of temperature preferences in the group ($p=2.2e-16$; $R^2=0.9205$; $\lambda=0.825$; Fig. S7). On the other hand, while the correlation of elevation with PC2

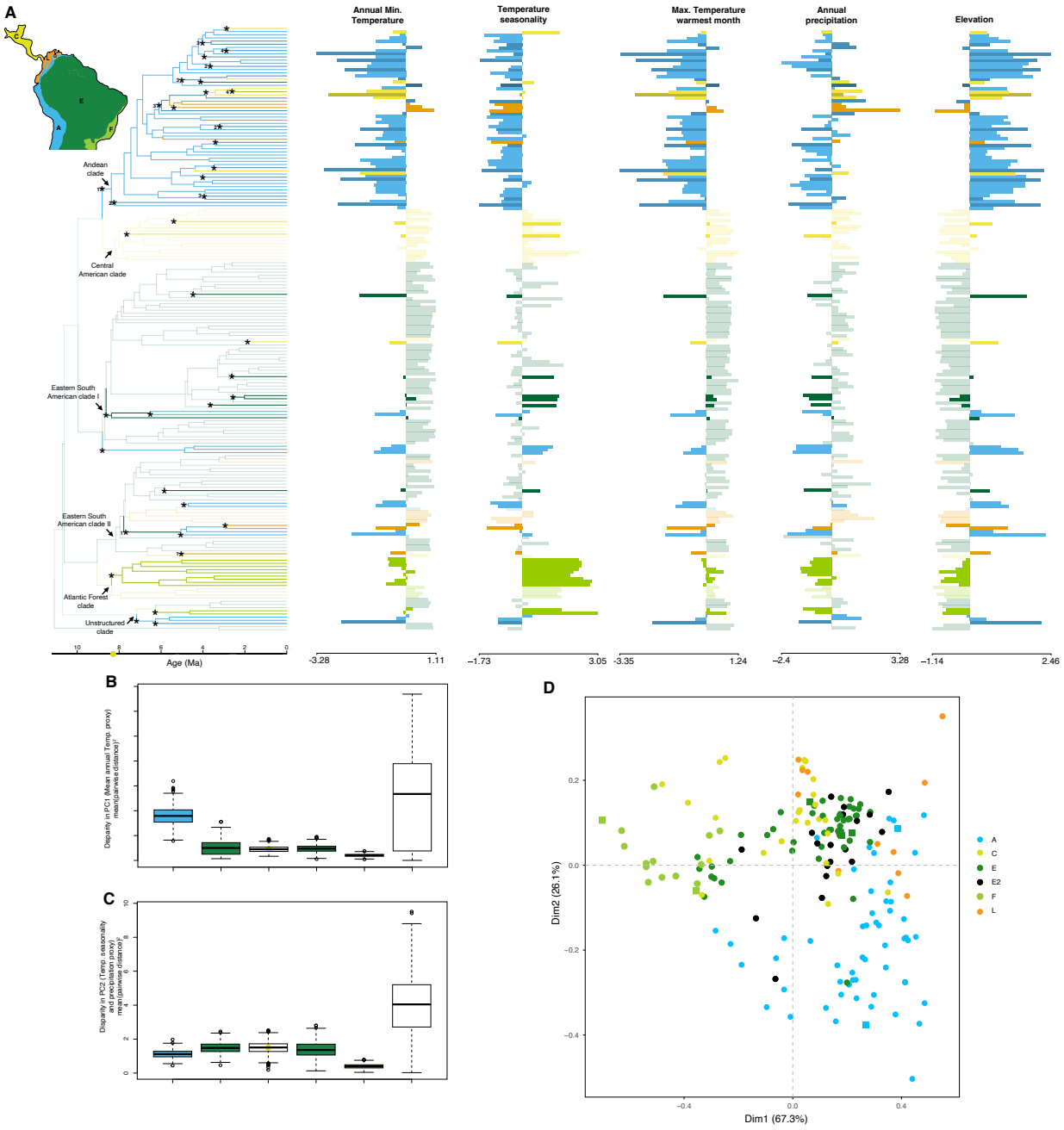

Figure 3. A) Detected shifts in abiotic (climatic and elevation) preferences of *Palicourea*. The location of shifts (* and opaque colors) are indicated along branches; transparent branches indicate no shifts from the ancestral optima. Convergence to the same climatic niche optima across branches is shown with numbers on branches. Biogeographically structured groups are highlighted according to our biogeographic reconstruction results. Clades used in disparity analyses (B, C) are highlighted. Disparity results across biogeographically structured clades for PC1 (B) and PC2 (C) are shown. D) Phylogenetic Principal Component Analysis results are shown with colors coded as in the l1ou analysis.

(precipitation and temperature seasonality proxy) was significant, this relationship is weak and also supports climatic niche conservatism in *Palicourea* (p=0.0014; R^2=0.055; λ=0.95; Fig. S8).

Interplay of range evolution, environmental preferences, and inflorescence type

We found a significant difference in elevation between species inferred to be primarily pollinated by hummingbirds vs. insects (p-value=0.05; Fig. 4A) and the variances across groups (Levene's test) are statistically different (p-value=0.006). Species that may be pollinated by hummingbirds occupy higher elevations and a broader range of elevations than species likely pollinated by insects. The estimated proportion of taxa pollinated primarily by hummingbirds increases with elevation (p-value 1.364e-10; Fig. 4B).

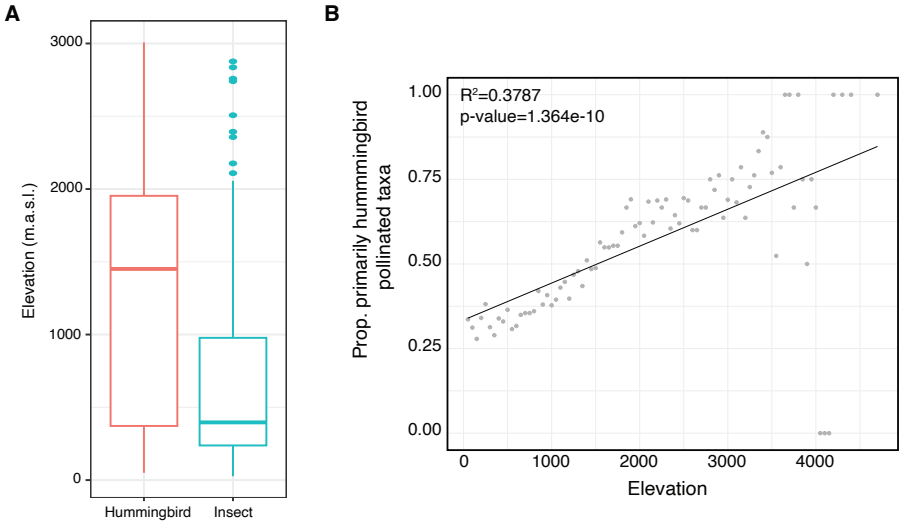

Figure 4. A) Phylogenetic ANOVA for the difference in the average elevation between species with primarily hummingbird pollinated ("*Palicourea*-like") and primarily insect pollinated ("*Psychotria*-like" and "*Cephaelis*-like") inflorescence types. B) Correlation between elevation and the proportion of taxa with primarily hummingbird pollinated inflorescence types.

Comparison of our fourteen models and model selection through PPA confirms this pattern. Phylogenetic Path Analysis using PC1 (temperature proxy) resulted in two best-fit models (H5 and H9; Fig.5, S9, Table S9). While these two models have different causal structures, they are statistically equivalent because they imply the same conditional independencies. Model H4 indicates that presence in the montane Andes has a direct negative effect on the temperature preference of species (β =-1.41; Table S10) and a direct positive effect on the presence of inflorescence type primarily pollinated by hummingbirds (β =1.55; Table S10)

independently. Model H2 represents an alternative casual structure in which climatic niche shifts mediated movement into the montane Andes and this in turn drove the evolution of inflorescences primarily pollinated by hummingbirds (β =1.55; Table S10). These effects are statistically significant ($p<0.05$; Table S10). Under these preferred models, the temperature preferences of species had no direct effect on inflorescence type (primarily hummingbird or insect pollinated; Fig. 5, S9; Table S8). Although H9 includes a pathway that links climatic niche shifts mediating movement into the montane Andes and subsequently inflorescence type evolution, the estimated effect was weak (β =-0.08; Table S10), providing little evidence that climatic niche shifts substantially influence the evolution of primarily hummingbird pollinated inflorescence types through shifts into the montane Andes. Phylogenetic Path Analysis using PC2 (precipitation and temperature seasonality proxy) resulted in one best-fit model (H6; Table S11). However, the direct effect of PC2 on the presence of inflorescence type primarily pollinated by hummingbirds is weak and not statistically significant (β =-0.14; p=0.2595; Table S12). In sum, these results show that the presence in the montane Andes has a direct and significant effect on the evolution of inflorescence type primarily pollinated by hummingbirds. This effect is not mediated by climate. In addition, there is a strong, direct, and negative relationship between presence in the montane Andes and the temperature experienced by *Palicourea*, with Andean species inhabiting colder climates.

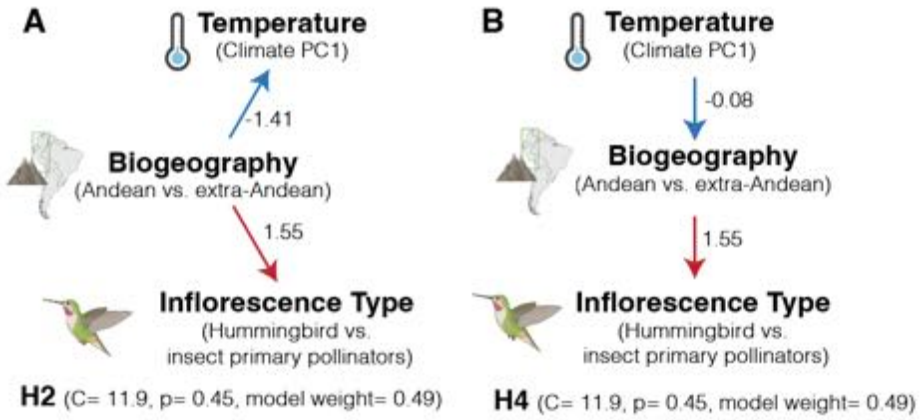

Figure 5. Best fit models from phylogenetic path analysis modeling interaction between climate, biogeography and inflorescence type. Numbers next to arrows represent the path coefficients (β). The model weight, C-statistic, and p-value of the C-statistic are also reported for each model. A) represents Hypothesis 2 (H2) and B) represents Hypothesis 4 (H4). See Table S9 for a description of each tested and Figure S9 for their visualization. Clip art from Biorender.

Discussion

Palicourea's diversity in geographic ranges, morphological characters related to pollination biology, and climatic niche makes it a compelling clade for understanding the drivers of evolution in Neotropical plants. This study constitutes the most comprehensive multi-locus phylogenetic tree for the genus, which is a particularly important contribution for understanding global diversity patterns as *Palicourea* is one of the only 86 "big genera" of plants (i.e., >500 species; Moonlight *et al.*, 2024). Our inclusion of hundreds of nuclear loci vastly expands upon a previous phylogeny of the group, which used only one nuclear marker (Sedio *et al.*, 2013). This allowed us to model a common biological processes (ILS) and identify areas of conflict across loci when inferring species relationships. Our phylogeny supports the recent broadened circumscription of the genus (Taylor *et al.*, 2010; Razafimandimbison *et al.*, 2017) and provides a new insight into the systematics of the group.

We also gain insights into the drivers of diversity in this ecologically important Neotropical plant group. We identify repeated species radiations following dispersals into new biogeographic areas (Fig. 2), which are accompanied by shifts in climatic niche mainly when they involve transitions from lowlands to montane regions (Fig. 3A). Species-rich lowland and montane clades display contrasting evolutionary dynamics. Species radiations in Lowland Eastern-Northeastern South America are associated with low disparity in temperature and precipitation (Fig. 3B-D) and lability in inflorescence type evolution (Fig. 2). On the other hand, the species-rich high-elevation Andean clade (A) is characterized by higher disparity in temperature and precipitation (Fig. 3B-D), the highest number of shifts in climatic regimes than any other biogeographic clade, and few shifts in inflorescence type. The factors likely responsible for this pattern are discussed below.

Biogeographic history of Palicourea

Divergence times estimated for *Palicourea* indicate that the crown group of the genus originated in the Late Miocene (Fig. 2), after globally warm temperatures of the Middle Miocene Climatic Optimum had dropped (Zachos, 2001). At that time, montane ecosystems with steep elevational gradients were likely in place in northern South America as a result of Andean uplift (Jaramillo, 2023). Mountain building would continue to change the landscape through the Pliocene (Gregory-Woodzicki, 2000; Garzione *et al.*, 2008; Mora *et al.*, 2010; Hoorn *et al.*, 2010), and the resulting landscape heterogeneity set the stage for the evolution and diversification of *Palicourea*. While there is uncertainty in our estimate of the ancestral area for *Palicourea*, our results suggest that it colonized the Andes within a million years of its origin. As documented in many Andean plant clades, the Andean radiation *Palicourea* is characterized by high gene

tree discordance (Figs. 1 and 2), either due to high rates of ILS during the rapid accumulation of species or frequent gene flow across closely related and co-occurring taxa. Future research should focus on the relative contribution of ILS and introgression on gene tree conflict in regions of the phylogeny that we identified as having highest gene tree discordance (Fig. 1).

The evolutionary history of *Palicourea* is marked by episodic events of dispersal into new biogeographic regions, with several of these movements resulting in *in situ* speciation and range expansion to adjacent areas (Fig. 2, left). Among key dispersal events, *Palicourea* has colonized Central America from South America on multiple occasions within the last ~8 Ma, well after the inferred closure of the Central American Seaway (Fig. 2, left; Montes *et al.*, 2015). Recent dispersals between lowlands west of the Andes and Central America (Fig. 2) are consistent with past evidence supporting stronger floristic affinities of the Chocó with Central America than with Amazonia (Gentry, 1982b; Zamora *et al.* 2004). This is also in line with previous findings of ancestral climatic niche conservatism in the assembly of local communities in Central American *Psychotria* and *Palicourea* (Sedio *et al.*, 2013), as well as in other groups of plants, birds and other terrestrial vertebrates (Leigh *et al.*, 2014). Movement into Central America may have primarily occurred gradually over land, as seed dispersal by small birds, who are attracted to the fleshy and colorful fruits, is characterized by short dispersal distances and limited gene flow (Taylor, 1989; Theim *et al.*, 2014). Notably, the structured biogeographic patterns that we inferred in *Palicourea* contrast with other studies that document more frequent historical long-distance dispersal in woody plants (Willis *et al.*, 2014; Dexter *et al.*, 2017).

Evolution of inflorescence type

Palicourea has experienced repeated evolution of inflorescence types, suggesting multiple shifts in pollinator strategy (Fig. 2, right). The ancestral state for *Palicourea* is small, white flowers in open inflorescences with small green bracts (Fig. 1C), which attract various generalist insect pollinators (Mesquita-Neto *et al.*, 2018). Inflorescence evolution shows a trend toward increased showiness in *Palicourea*, both via modification of bracts and evolution of larger, more colorful flowers. This increased showiness is associated with hummingbird pollination, which is prevalent in species with open, thyrsiform, colorful inflorescences ("*Palicourea*-like" (Betancourt *et al.*, 2023)) and with head-like inflorescences subtended by showy bracts ("*Cephaelis*-like"; Fig. 1A,B (Feinsinger *et al.*, 1988; Ree, 1997; Valois-Cuesta *et al.*, 2011; Furtado *et al.*, 2023).

All biogeographic regions recognized here are home to at least two inflorescence forms, often resulting from shifts within the region. However, there are differences in the distribution of inflorescence form across the Neotropics (Fig. 2). There are proportionally more species with showy "*Palicourea*-like"

inflorescences in Andean lineages, with the large Andean clade experiencing few shifts to other inflorescence types. This contrasts with a higher diversity of inflorescence types and larger number of shifts in most lowland regions, most of which are from the ancestral "*Psychotria*-like" morphology to another form. Shifts in lowland regions may reflect adaptation to local pollinator pools across environmentally heterogeneous regions, or pollinator partitioning to avoid competition among co-occurring species (Barreto *et al.*, 2024).

Palicourea is marked by a wide variation in inflorescence traits, including overall size, elongation of axes (from capitate to long thyrses), development and form of bracts, and colors of axes, bracts, and flowers. While we have grouped this variation into four main categories to explore broad trends, the full diversity of *Palicourea* is incompletely captured by our categories. For example, species such as *P. divaricata* have inflorescences with somewhat small and purple corollas, an intermediate morphology between the "*Palicourea*-like" and "*Psychotria*-like" inflorescence forms. Also, some species here coded with "open *Psychotria*-like" inflorescences can be somewhat condensed (i.e., *P. andina, P. conephoroides, P. egensis, P. polycephala, P. tetragona,* and *P. winkleri*). While the inflorescence morphology of *Palicourea* species corresponds roughly to established pollination syndromes, there are relatively few empirical pollination biology studies for this group, and the majority focus on conspicuous and/or widespread species; documenting effective pollinators for a diversity of species would be invaluable to understanding the functional significance of inflorescence morphology in *Palicourea*. Despite this limitation, the broad trends that we identify unquestionably point towards lability of morphological evolution of inflorescence traits in *Palicourea*.

Biogeographic, inflorescence type, and climatic lability, and their interplay

While *Palicourea* ranges widely across climates and habitat types, biogeographic structure is broadly characteristic of the group. The degree of relative biogeographic stability in *Palicourea* is complemented by climatic niche overlap and degree of disparity of species in all biogeographic regions except the Andes (Fig. 3A, B, D). Further highlighting the important role that environmental gradients in montane regions play in the disparification of lineages is the fact that the Andean clade is characterized by higher disparity in temperature preferences than all other biogeographic clades (Fig. 3B, D). While we identify many shifts in abiotic niche, the majority of these occur in Andean lineages and represent differences in magnitude of the shifts in the selection optimum, not direction of the evolutionary regimes (Fig. 3A). With the exception of montane Andean *Palicourea*, these results are broadly consistent with previous findings in *Psychotria* (sister to Palicoureeae) in which climatic niche conservatism is the norm (Sedio *et al.*, 2013). This is further supported by the *phylolm* analyses testing for the correlation of elevation and

595 climatic niche, which showed that elevation explains most of the variation in temperature preference
596 across species ($p = 2.2 \times 10^{-16}$; $R^2 = 0.9205$) and that temperature preference exhibited strong
597 phylogenetic signal ($\lambda = 0.825$; Fig. S7). Further documenting low niche disparity in lowland *Palicourea*,
598 we find support for dispersal and range expansion that tracks ancestral climatic preferences and elevation
599 across lowland regions; for example, there are many range expansions from the lowland Eastern-
600 Northeastern South America clade into climatically similar areas of Central America (based on similar
601 disparity estimates and higher probability of overlap in climatic preference of groups; Fig. 3B,D; Table
602 S6). Together, these results suggest that climatic niches are largely conserved in *Palicourea* and that
603 much of the observed climatic differentiation in *Palicourea* reflects the colonization of elevational
604 gradients in the Andes rather than repeated independent shifts in climatic preference.

605 We identify the signature of mountains on the evolution of inflorescence type. Although *Palicourea*
606 species that are primarily pollinated by hummingbirds occur across elevations, primarily hummingbird
607 and primarily insect-pollinated species significantly differ in the average elevation that they occupy (Fig.
608 4A). Furthermore, the proportion of hummingbird pollinated taxa increases with elevation and most
609 species in the high-elevation, species-rich Andean clade (A) have inflorescence types linked to primarily
610 hummingbird pollination (Fig. 2).These results are in line with Cruden's 1972 hypothesis and previous
611 empirical pollination studies demonstrating that harsh conditions in high-elevation environments drive
612 turnover from ecothermic insect to endotermic vertebrate pollination (Cruden, 1972; Dellinger *et al.*,
613 2023), including in Caribbean Rubiaceae (Lehmann *et al.*, 2019). However, phylogenetic path analyses
614 identified that transitions to hummingbird pollination are not tied to climate and instead are linked to the
615 occupation of montane Andean environments alone (Fig. 5, Tables S8-S12). Thus, we do not find support
616 for Cruden's hypothesis, and our results point to a different mechanism underlying shifts to primary
617 hummingbird pollination in *Palicourea*.

618 Non-climatic features of Andean environments that may underlie repeated transitions toward
619 hummingbird-associated inflorescences include habitat heterogeneity, geographic isolation, and
620 ecological opportunity linked to young geological age. Hummingbird pollinators may help maintain the
621 population connectivity of Andean *Palicourea* species by ensuring gene flow across fragmented
622 landscapes and long distances, relative to bee pollination (Gamba & Muchhala, 2023), protecting species
623 from extinction or extirpation. For other lineages, reliance on widespread hummingbird pollinators may
624 have facilitated establishment in new Andean regions by ensuring effective reproduction after dispersal,
625 even when isolated from ancestral populations, resulting in speciation in allopatry. Because
626 hummingbirds (McGuire *et al.*, 2014) and *Palicourea* diversified concurrently during the geologically
627 recent Andean uplift in the last 10 million years, both groups likely responded to similar shared

paleoenvironmental conditions and diversified into newly opened niches, potentially influencing one another through diffuse coevolution. In addition, Andean hummingbird communities are species-richness (Stiles, 1981; McGuire *et al.*, 2014; Rueda-Uribe *et al.*, 2025) and functionally diverse (Sonne *et al.*, 2016) compared to lowland environments, which may further contribute to the relationship between Andean occupation and hummingbird associated inflorescences in *Palicourea*. Hummingbirds are very effective pollinators that carry high pollen loads (Furtado *et al.*, 2023), transport pollen over long distances (Gamba & Muchhala, 2023), and groom infrequently (Castellanos *et al.*, 2003). A recent study on Neotropical *Costus* demonstrates that a prevalence of hummingbird pollination at higher elevations may be driven by increased hummingbird visitation rates and greater per-visit pollination efficiency, rather than by a decline in bee visitation resulting from colder temperatures (Juárez *et al.*, 2026)— a pattern that may hold true in Andean *Palicourea*. As many *Palicourea* species are known to be visited by both insects and hummingbirds, the genus is well-suited to study how pollinator efficacy changes along elevational gradients and what selection pressures lead to pollination specialization or shifts in pollination syndrome.

Conclusions

We document an important role of both abiotic and biotic factors in the evolution of *Palicourea* and the sum of our evidence suggests that the forces that underlie diversification may differ in lowland and montane clades. In the Andes, uniformity in pollination strategy and shifts in climatic regimes that accompanied the biogeographic transition from lowlands underly species radiation (Figs. 2 and 3B), while in the lowlands, climatic niche is conserved and inflorescence type evolution is dynamic (Figs. 2 and 3B). We find that shifts to inflorescences associated with hummingbird pollination are linked to presence in the Andes, but not climatic niche, suggesting that the ecological opportunities and pollinator communities of young montane environments, rather than a direct climatic filter on pollinator physiology, position the Andes as a driver of floral evolution through mechanisms beyond temperature and cloud cover alone. Expanding our study to include deeper taxon sampling, community and population-level phylogenetic and ecological studies, and exploration of other dimensions of niche space (e.g., phytochemical) would provide insights into the evolutionary processes that generated the patterns we unveiled. The breadth of natural history observations in *Palicourea* accumulated over decades, coupled with recently generated genomic data, present a great opportunity to continue to shed light on the patterns and processes shaping niches and range shifts in Neotropical plants.

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Author Contribution

AMB, CMT, and LPL designed the study. CMT designed taxon sampling. AM and SO generated sequence data. AMB processed and analyzed data and wrote the first draft of this manuscript. AM and LDB contributed to data analysis. AMB, CMT, LDB, and LPL collaborated on writing and editing of the document ahead of submission, and all authors approved the final submission.

Data Availability

All sequence data in this study are deposited at the National Center for Biotechnology Information (NCBI) Sequence Read Archive under Bioproject PRJNA970862 (available upon initial acceptance.) All alignments, trees, and code generated in this study are available at https://github.com/ambed0ya/Palicourea

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