Phylogeny and biogeography of Neotropical flowering plant tribe Citharexyleae (Verbenaceae)

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Abstract

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The New World tropics, or Neotropics, located within tropical latitudes of North and South America, are one of the most diverse ecoregion in the world. However, this diversity is poorly understood in terms of described biodiversity—species numbers are uncertain and collection records are depauperate for many groups distributed in remote locations or dense forest—and patterns of evolution contributing to high diversity—factors underlying speciation in lineages are not well understood. A molecular phylogenetic and systematic study of the Neotropical flowering plant tribe Citharexyleae in the Verbena family (Verbenaceae), which also originated and diversified primarily in the Neotropics, was undertaken in order to describe diversity and understand patterns of evolution in an understudied Neotropical lineage. Chapter 1 comprises a systematic study of tribe Citharexyleae, which describes relationships between the three genera in the tribe (*Baillonia* (1 species), *Citharexylum* (*ca*. 70 species), and *Rehdera* (2 species)) as well as relationships within *Citharexylum*, the largest genus. *Baillonia* is included in

Citharexylum and *Rehdera* is retained distinct. A subgeneric classification including six subgenera system is proposed for *Citharexylum*, and morphological characters associated with each major clade described. In Chapter 2, the biogeographical patterns underpinning diversification in *Citharexylum* are explored. *Citharexylum* comprises ca. 70 species distributed widely throughout the Neotropics, from northern Mexico to southern Brazil and Argentina. Species occupy multiple biomes including continuously moist broadleaf forest, seasonally dry tropical forest, tropical coniferous forest, high elevation shrublands, and arid shrublands. The contributions of radiation within a biome and adaptation to/colonization of new biomes are compared. Both patterns have played important roles in the diversification of *Citharexylum,* and major lineages within *Citharexylum* display different patterns. The Mesoamerican clade exhibits a pattern of radiation within the ancestral biome with more recent, independent colonization events, whereas the South American clade exhibits a pattern of early colonization and radiation within biomes. Though diversification rates are higher in the Mesoamerican clade, both clades have produced similar taxonomic diversity in the same amount of time. Both patterns contribute equally to overall diversity in the genus. Chapter 3 incorporates molecular sequence data generated by the previous chapters with data generated in previous studies other lineages of Verbenaceae and newly generated data to produce an updated phylogeny of the Verbena family. Verbenaceae comprises *ca.* 770 species in 28 genera. A previous family-wide study sampled 7 chloroplast regions for 121 species. The expanded dataset encompasses twelve chloroplast regions, two nuclear ribosomal spacers, and eight low-copy nuclear loci for 366 species. Two new clades in Verbenaceae are described, including resurrection of the genus *Scleröon* Benth in Lindl.

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CONTENTS

CHAPTER 1:

Phylogeny, classification, and character evolution of tribe Citharexyleae (Verbenaceae)

ABSTRACT

- *Premise of the study*: Tribe Citharexyleae comprises three genera: *Baillonia*, *Citharexylum*, and *Rehdera*. While there is good support for these genera as a clade, relationships between genera remain unresolved due to low sampling of the largest genus, *Citharexylum*. A molecular phylogenetic approach was taken to resolve intergeneric relationships in Citharexyleae and infrageneric relationships in *Citharexylum*.
- *Methods*: Seven chloroplast regions, two nuclear ribosomal spacers, and six low-copy nuclear loci were analyzed for 64 species of Citharexyleae. Phylogenetic analyses were conducted using maximum likelihood, Bayesian inference, and Bayesian multi-species coalescent approaches. The following morphological traits were examined to identify diagnostic characters for clades within Citharexylum: habit, presence/absence of thorns, inflorescence architecture, flower color, fruit color, and geography.
- *Key results*: Intergeneric relationships are resolved as *Rehdera* sister to *Citharexylum* and *Baillonia* nested within *Citharexylum*. Two species, *C. oleinum* and *C. tetramerum*, fell outside of Citharexyleae. Six well-supported clades within *Citharexylum* are reconstructed; clades are best defined by geography, fruit color/maturation, and inflorescence architecture.
- *Conclusions: Baillonia* is included in *Citharexylum*; *Rehdera* is retained as a distinct genus. A subgeneric classification for *Citharexylum* is proposed.

Key words: *Baillonia*, *Citharexylum*, Citharexyleae, classification, microfluidic PCR, molecular phylogenetics, Neotropics, *Rehdera*, Verbenaceae

INTRODUCTION

Prior to molecular phylogenetics, there was disagreement on the division and definition of groups within Verbenaceae. Authorities employing different criteria (e.g., gynoecial structure, inflorescence architecture, fruit characteristics) arrived at different divisions of the family. Despite conflict in these morphology-based classifications of Verbenaceae, all recognized a group including *Citharexylum*, *Duranta*, and *Rhaphithamnus* (Bentham, 1839; Schauer, 1847; Briquet, 1895; Moldenke, 1971; Troncoso, 1974; Sanders, 2001; Atkins, 2004) on the basis of woody habit, fleshy fruit, and the presence of a staminode. Though these genera share a strong morphological affinity, molecular studies indicated that each genus is more closely related to other taxa that differ in one or more of these traits (Marx et al., 2010; Yuan et al., 2010; O'Leary et al. 2012).

Tribe Citharexyleae, as most recently circumscribed, comprises ca. 133 spp. of Neotropical trees and shrubs in three genera: *Baillonia* (1 spp.), *Citharexylum* (130 spp.), and *Rehdera* (2 spp.) (Marx et al., 2010). *Baillonia* is distributed in Brazil and Paraguay, *Citharexylum* is widespread throughout the Neotropics from Northern Mexico to Southern Brazil and Argentina, and *Rehdera* is distributed in Central America. All genera share a woody habit, extra-floral nectaries, terminal inflorescences, minute floral bracts, short pedicellate flowers, and the presence of a staminode. However, none of these traits are exclusive to these genera in Verbenaceae, and morphological synapomorphies that define the tribe have not been identified (Marx et al. 2010; O'Leary et al., 2012).

The three genera are distinguished from each other by carpel number and fruit type. *Citharexylum* and *Rehdera* have bi-carpellate ovaries that develop into fruits with two, two-

seeded mericarps. Species of *Rehdera* were previously classified as *Citharexylum*, but were segregated by Moldenke (1935) on the basis of having dry, dehiscent fruits. *Citharexylum* and *Baillonia* both produce fleshy, drupaceous fruits. *Baillonia* was traditionally classified in tribe Lantaneae on the basis of having one carpel that aborts during development, giving rise to a uni-carpellate, two-seeded fruit (Briquet, 1895). Its affinity for *Citharexylum* was suggested by Moore (1895) and Junell (1934). Additionally, species of *Citharexylum* exhibit cryptic dioecy (Tomlinson and Fawcett, 1972; Rocca and Sazima, 2006; Rueda and Hammil, in prep.). Flowers contain both stamens and pistils, but one whorl of reproductive parts is sterile so that individuals are functionally male or female. This characteristic has not been noted in other genera of Verbenaceae.

Molecular studies identified Citharexyleae as described above (Marx et al. 2010, O'Leary 2012), but, to date, no study has included sufficient sampling to confidently assess relationships among the tribe's genera. Marx et al. (2010), the only study to include all three genera and sample multiple species of *Citharexylum* and both species of *Rehdera*, inferred that *Baillonia* is included within *Citharexylum* and *Rehdera* is sister to *Citharexylum* with high support. However, because only 11 species in Citharexyleae were sampled and only chloroplast data were used, assessment of intergeneric relationships was left for further study. Greater taxonomic and genetic sampling is needed to fully resolve generic relationships.

Since the tribe is dominated by *Citharexylum*, resolution of generic relationships by necessity involves more thorough sampling of *Citharexylum*, which provides a further opportunity to understand relationships within the genus. Estimated to comprise 130 species (Atkins 2004), it is one of the largest genera of Verbenaceae, though there are suggestions that

this estimate is inflated (Sanders, 2001; O'Leary and Frost, in prep.). Despite the size of the genus, it has been poorly studied and described. Moldenke published a series of notes (1957-1959) preparatory to a monograph, but never completed a monograph of *Citharexylum*. Similarly, divisions of species into infrageneric groups have been suggested, but not validly published. Walpers (1845) divided taxa into two major sections: *Spinosa* and *Inermia*, based on the presence or absence of spines, respectively. Section *Inermia* was further divided by calyx morphology. Section *Spinosa* included five taxa that have been reduced to three taxa recognized today as *C. flexuosum*, *C. montevidense*, and *Rhaphithamnus spinosus* (now distinct from *Citharexylum*); section *Inermia* encompassed the remaining 29 taxa in the treatment. Moldenke (1957-1959) mirrored Walpers' sections in a dichotomous key, adding *C. andinum*, *C. herrerae*, and *C. weberbaueri* to section *Spinosa* and listing over 100 taxa in section *Inermia*. With the vast majority of taxa in section *Inermia*, this division provides little insight into relationships between species that further vary in traits such as habit, flower color, inflorescence architecture, and fruit color.

This study includes both greater taxonomic and genetic sampling than has been used previously. Microfluidic PCR was used to target seven chloroplast regions and two nuclear rDNA spacers. Broader sampling allows us to (1) resolve relationships between genera of Citharexyleae, (2) identify major clades of *Citharexylum* and the characters that unite them, (3) infer trait evolution within the tribe, and (4) establish a subgeneric classification for *Citharexylum*.

MATERIALS AND METHODS

***Taxon sampling*—**One hundred eighty-two accessions representing 67 species of Citharexyleae were included in this study: one accession of monotypic *Baillonia*, 176 accessions of 64 *Citharexylum* species, and five accessions of two *Rehdera* species. Nine species from across the Verbenaceae served as the outgroup. Vouchers and GenBank accession numbers may be found in Appendix S1 (see the Supplementary Data with this article). Herbaria acronyms follow Index Herbariorum (Thiers, constantly updated: http://sweetgum.nybg.org/science/ih/).

***Microfluidic PCR Primer Design and Validation*—**Targeted loci were selected for their phylogenetic utility and use in previous studies of Verbenaceae (O'Leary et al., 2009; Marx et al., 2010; Yuan et al., 2008, 2010; Lu-Irving and Olmstead, 2013; Lu-Irving et al. 2014; Frost et al., 2017). Seven chloroplast regions (cpDNA: *mat*K, *ndh*F, *rbc*L, *rpl*32, *rpo*C2, *trn*T-*trn*L, and *trn*L-*trn*F), two spacer regions (ETS and ITS) of nuclear 18S/26S rDNA (nrDNA), and five low-copy nuclear genes (waxy, PPR 24, PPR 62, PPR 70, and PPR 123) were included in this study.

Primer design and validation followed Uribe-Convers et al. (2016), with the exception that primer sequences were designed by eye from alignments of published data pulled from genbank (ncbi.nlm.nih.gov). Each primer pair was designed to amplify 500 +/- 100 bp DNA segments. Validation reactions were performed with four experimental samples: two *Citharexylum* species (*C. schottii* and *C. myrianthum*) to test performance in the in-group, one outgroup taxon (*Duranta coriacea*) to test the potential for family-wide utility, and a negative control. Sixteen primer pairs were selected for seven chloroplast regions. One primer pair was used for each of the nrDNA and low-copy nuclear loci except for waxy, which was amplified

with two. Primer pair sequences can be found in Appendix S2 (see the Supplementary Data with this article).

DNA extraction, Microfluidic PCR, and sequencing—Genomic DNA was extracted from field-collected, silica-gel-dried tissues using a modified CTAB method (Doyle and Doyle, 1987) or from herbarium specimens using a DNeasy plant mini kit (Qiagen, Valencia, California, USA). A total of 7603 bp of cpDNA were targeted for amplification and sequencing, including: 890 bp of matK in two segments, 1823 bp of ndhF in four segments, 1029 bp of rbcL in two segments, 525 bp of rpl32 in one segment, 1893 bp of rpoC2 in four segments, 512 bp of trnT in one segment, and 931 bp of trnLF in two segments. A total of 5,132 bp of nuclear DNA were targeted for amplification and sequencing, including: 859 bp of nrDNA (400 bp of ETS and 459 bp of ITS) and 4,274 bp for low-copy nuclear regions (332 bp of PPR24, 534 bp of PPR62, 341 bp of PPR70, 595 bp of PPR123, 967 bp of waxy haplotype 1, and 968 bp of waxy haplotype 2).

Microfluidic PCR was performed in two separate runs on an Access Array System (Fluidigm), one with a 48.48 Access Array IFC and another with a Juno 192.24 IFC. The same primer pairs were submitted for each IFC. Amplicons were harvested and pooled as described in Uribe-Convers et al. (2016). For each IFC, the resulting pools were multiplexed in an Illumina Miseq using the Reagent Kit v3 600 cycles. Microfluidic PCR in the Access Array System, downstream quality control, and Illumina sequencing were performed in the University of Idaho (Moscow, ID) Intstitute for Bioinformatics and Evolutionary Studies (ibest) Genomic Resources Core facility.

Data processing—Reads from the Illumina Miseq runs were processed using the Fluidigm2PURC pipeline for processing paired-end Illumina data generated using the Fluidigm platform (Blischak et al., in review). Reads were demultiplexed using the program dbcAmplicons (Uribe-Convers et al., 2016). Paired reads were separated by locus, with the fluidigm2purc script (github.com/pblischak/fluidigm2PURC). Each locus was processed using PURC's purc_recluster.py script (github.com/pblischak/fluidigm2PURC; Edgar, 2004, 2010; Camacho et al., 2009; Edgar et al., 2011; Martin 2011; Rothfels et al., 2016) to iteratively run chimera detection and sequence clustering to produce a set of putative haplotypes, or clusters. Reads were processed with clustering thresholds of 0.975, 0.99, 0.995, 0.997 and a size threshold of 5 (i.e. only clusters with ≥5 identical reads were retained). The clusters were run through the crunch-clusters script to infer haplotypes (github.com/pblischak/fluidigm2PURC).

Chloroplast regions were processed as haploid. Reads from nuclear loci were processed with "unknown" ploidy, meaning all possible haplotypes were kept and haplotype number could vary by sample. Gene trees were generated with RAxML ver. 8.2.11 (Stamatakis, 2014) to process the haplotypes for nuclear loci. For individuals with haplotypes that nested together—haplotypes that exhibited sequence similarity, but differed in amplicon length or a few heterogeneous sites—either the longest read or largest cluster was selected to represent the individual. For individuals with haplotypes that nested in different clades throughout the tree, all haplotypes were retained.

Raw reads for each locus were parsed into separate files by the purc_recluster.py script. In the instances that raw data were available, but with insufficient coverage to meet the filters set in purc_recluster.py, data were assessed and incorporated manually. For regions with a single

haplotype, a consensus sequence was generated for the available reads. For regions with multiple haplotypes, reads were analyzed individually in a phylogenetic context; consensus sequences were generated for reads that formed unique clades.

Four datasets were assembled for each region with different tiers of quality: 1) the original output from data processing, 2) the former plus individuals/haplotypes with ≥5 reads, 3) the former plus individuals/haplotypes with 2-4 reads, and 4) the former plus individuals/haplotypes with only a single read. Phylogenetic analyses were performed for each tier with RAxML ver. 8.2.11 to assess effects of the supplemented data on support values and topology. This supplementation technique was used only for individuals that had sufficient data to be included in the study, but were missing data for some loci due to low coverage. No additional individuals were added to the study as a result of these low-coverage reads.

Phylogenetic analyses

Gene tree analyses—Sequences were aligned using AliView ver. 1.18.1 (Larsson, 2014); alignments were inspected and manually adjusted where necessary. Individual alignments were analyzed with jModelTest 2.1.7 (Darriba et al., 2012; Guindon and Gascuel, 2003) to select the best-fit model of nucleotide substitution. The GTR + G model was selected and applied to all datasets. Alignments were concatenated using Geneious R7 7.0.6 (Biomatters, Auckland, New Zealand) and treated as a single dataset for regions with shared evolutionary histories: ndhF, rpoC2, trnT-trnL-trnF, rbcL, matK, and rpl32 are part of the haploid chloroplast genome (cp); ETS and ITS are tightly linked in the nuclear rDNA repeat (nr). In the instance of that multiple haplotypes were recovered for a region, haplotypes were separated and treated as independent

datasets. Eight independent datasets were analyzed: cp, nr, PPR24, PPR62, PPR70, PPR123, waxy haplotype1, and waxy haplotype 2.

Phylogenetic reconstructions were conducted using maximum likelihood and Bayesian approaches, as implemented in RAxML and MrBayes ver. 3.2.6 (Ronquist & Huelsenbeck, 2003), respectively, for individual datasets, low-copy nuclear genes combined, and all data combined. Maximum likelihood analyses consisted of bootstrap analyses with 500 replicates for each dataset. Datasets were partitioned into individual gene regions with individual parameters unlinked in Bayesian analyses. Analyses consisted of two replicate runs, each with four chains, which were run for at least one million generations, sampling every 200 generations. Runs were assessed for convergence and stationarity in Tracer v1.6.0 (Rambaut et al., 2014); runs were considered to have converged when effective sample sizes (ESS) for parameter values were above 200 for combined files. For each run, the first 20% of sampled trees were discarded as burn-in and the remaining trees were summarized into a majority rule consensus tree with all compatible groups.

Species tree analyses—Species tree estimation was performed on the ingroup using the multispecies coalescent (MSC) model implemented in *BEAST (BEAST v1.8.4; Drummond et al. 2012). Substituion models and clock models were unlinked for the fourteen individual alignments. The GTR+G substitution model, empirical base frequencies, and a random local clock (Drummond and Suchard, 2010) were applied to each. Trees were linked based on shared evolutionary history, in the case of cp and nr datasets, or based on gene tree topology for lowcopy nuclear genes. Two partitions were set for lowcopy nuclear genes: (1) those where

Central American species form a monophyletic group (PPR24, PPR123, waxy haplotype 2) and (2) those where Central American species form a paraphyletic group with respect to South American species (PPR62, PPR70, waxy haplotype 1). Chloroplast data were treated as an organellar (haploid) locus with half the effective population size of a bi-parentally inherited locus. The birth-death process was used as the species tree prior. Replicate runs were performed for 500 million generations, sampling every 25,000. Runs were assessed for convergence and stationarity in Tracer v1.6.0 (Rambaut et al., 2014) and considered to have converged when effective sample sizes (ESS) for parameter values were above 200 for combined files. Between 25 and 50 million sampled tree states were discarded from each run as burn-in. Accessions of *C. bourgeauianum*, *C. flabellifolium*, *C. guatemalense*, *C. oleinum*, *C. reticulatum*, and *C. tetramerum* were excluded from the species tree analyses due to large amounts of missing data or because they were inferred to belong with the outgroup.

Morphology—Characters examined were habit, presence/absence of thorns, inflorescence architecture, flower number, corolla color, fruit color, and geography. For habit, species were scored as shrubs or trees. Species were considered shrubs if less than six m tall and with multiple main stems. Species over six m tall and/or with a single trunk were considered trees. To better describe the variability in inflorescence architecture, terminal and axillary inflorescences were scored separately. Each inflorescence type was scored as absent, simple, or compound. Average flower number per inflorescence was categorized >20, 10-20, or <10. Corolla color was categorized as solid white, solid yellow, solid purple, or white/yellow with purple/orange on the abaxial surface of the petal lobes. Fruit color through maturation was

categorized as green to red-orange, green to red to black in a two-phase maturation process, or green directly to black. Geographic range was divided into three regions: Central America, Caribbean, and South America. Ancestral character reconstructions were performed for terminal inflorescence architecture, axillary inflorescence architecture, flower number, flower color, and fruit color using the R package phytools, which employs a continuous-time Markov chain model. Habit and geography were used as descriptive characters, but ancestral character reconstructions were not performed. The biogeography of *Citharexylum* is of interest and will be addressed in a separate, more detailed study. Character data were pooled from Moldenke (1957-1959); field observations; herbarium records via personal visits to HUH, F, MEXU, MO, and USM; and online databases GBIF, CV Starr Virtual Herbarium (NYBG), SEINET, and Tropicos.

RESULTS

***Dataset characteristics*—** The total concatenated dataset consisted of 12 927 aligned bp and 176 accessions, including 167 accessions in Citharexyleae. Among the characters, 4 140 were variable, of which 2 398 were potentially parsimony-informative. One hundred thirty-nine accessions had sequence data for at least five of the eight loci, with 82 of these having data for at least seven loci. All accessions included in the concatenated dataset had data for both cpDNA and nrDNA. Nine accessions of *Citharexylum* with insufficient data to be included in the concatenated analyses, but representing species not otherwise included, were retained in individual gene tree analyses. Characteristics of individual loci are shown in Table 1.

Phylogenetic analyses

Gene tree analyses—All gene trees support the monophyly of Citharexyleae, except for two species (*C. oleinum* and *C. tetramerum*), which nested in the outgroup. Within Citharexyleae, *Rehdera* was reconstructed as the sister to *Citharexylum,* and *Baillonia* nested within *Citharexylum* in all trees. *Citharexylum altamiranum* (clade *vi*) was reconstructed as sister to the rest of the genus (Figs. 2 and 3), which comprised two major clades (A and B; Fig. 3). Six smaller clades were consistently recovered in individual and combined gene trees, but relationships between these clades varied. Groups reconstructed within *Citharexylum* with high support for all datasets included: (*i*) a large clade including *C. ellipticum*, *C. hidalgense*, *C. moccinoi*, *C. roxanae*, and *C. schottii*; (*ii*) a clade composed of *C. brachyanthum*, *C. lycioides*, *C. racemosum* and *C. rosei*; (*iii*) a clade composed of *C. affine*, and *C. hintonii*; (*iv*) a clade including *Baillonia amabilis*, *C. microphyllum*, and *C. montanum*; (*v*) a clade/grade including *C. dentatum*, *C. flexuosum*, *C. herrerae*, and *C. montevidense*; and (*vi*) *C. altamiranum* (Figs. 2 and 3, but see Fig. 3 for tip labels). Clade A included clades *i*, *ii*, and *iii* in the chloroplast, lowcopy nuclear combined, and all data combined trees. In other trees, the placement of clades *ii* and *iii* varied. For the remainder of the paper Clade A will be referred to as the Central American clade (geography, Fig. 4) and will refer to the clade including clade *i*. In all trees, clade B included clade *iv* and clade/grade *v,* though relationships between taxa in clade/grade *v* differ among datasets (Supplemental trees with tip labels). Clade B is largely composed of South American species (geography, Fig. 4), and will from now on be referred to as the South American clade.

Citharexylum spinosum, the type species for the genus, nested within different clades for different datasets. All three accessions nested within the Central American clade in cpDNA,

PPR70, and PPR123 trees. Accessions were split, with some nesting in the Central American clade and some in the South American clade, in nrDNA, PPR24, and PPR62 trees. Two accessions of *C. spinosum* had an additional copy for each of the waxy of haplotypes (i.e. four total copies of the waxy gene, two copies of haplotype 1 and two copies of haplotype 2). For each haplotype, one copy nested within the Central American and another nested within the South American clade. One accession also showed this pattern in PPR62.

Species tree analyses— The species tree topology was consistent with the topology reconstructed by the all combined dataset (Fig. 4). The same six major clades were inferred with high support in the species tree as in the individual and combined gene trees. The split between the Central American clade and the South American clade was also well supported in the species tree. *Citharexylum spinosum*, which nested in both the Central American and South American clade in gene tree analyses, is placed in the Central American clade by the MSC.

Morphology—Observations were available for all characters for all species except fruit color in *C. racemosum* (Fig. 4). Thorns were present only in species in the Andean shrub clade. Ancestral character estimations supported two origins of thorns in clade *v* (Appendix S9), the first either in a clade or grade—depending on the analysis—including *C. andinum*, *C. flexuosum*, *C. joergensenii*, *C. kobuskianum*, *C. montevidense,* and *C. weberbaueri* and the second in *C. herrerae*. Terminal inflorescences were lost in *C. subflavescens* and *C. montanum* (Appendix S10). Terminal inflorescences varied within species as whether they were simple or compound. Only *Rehdera penninervia* was found to consistently have compound terminal inflorescences.

Similarly, there was variation within species for presence/absence of axillary inflorescences. Almost half (27) of the species included individuals with axillary inflorescences. Of those, 12 species were observed to consistently have axillary inflorescences, and 6 of those species included individuals with compound axillary inflorescences. Again, *Rehdera penninervia* was the only species for which members consistently had compound axillary inflorescences. Axillary inflorescences were lost in the ancestor of the two major clades of *Citharexylum* and regained nine times (Appendix S11). Reduction of flower number per inflorescence from >20 to <20 is estimated to have occurred in *C. altamiranum*, *C. alanii* and *C. mircophyllum*, clade *ii*, and clade *v* (Appendix S12). Flowers with at least partial purple coloration arose six times throughout the genus (Appendix S13). Red fruits were estimated to be the ancestral fruit color in *Citharexylum*; black fruits arose in *C. altamiranum* and two or three times in clade *v* (Appendix S14). Ancestral reconstructions supported three separate origins of black fruits with a red-orange intermediate in the Central American clade. Red fruits were estimated to be the most likely ancestral state of this clade. Black fruits with a red-orange intermediate was the second-most probable ancestral state in the reconstructions.

DISCUSSION

Intergeneric relationships—Phylogenetic reconstructions infer *Rehdera* to be sister to *Citharexylum* and support the inclusion of *Baillonia* in *Citharexylum*. This is consistent with previous studies of Verbenaceae and is bolstered by increased sampling of species in Citharexyleae. Two species of *Citharexylum*, *C. oleinum* and *C. tetramerum*, nested with the

outgroup; preliminary results suggest these species belong to Duranteae, but further investigation is needed.

Infrageneric relationships— The Mexican species *Citharexylum altamiranum* is sister to the rest of the genus. The remaining species of *Citharexylum* form two major clades defined by geography: (A) the Central American clade and (B) the South American clade. Each of these major clades contains about half of the taxonomic diversity of *Citharexylum*. The Central American clade comprises three smaller, well-supported clades (clades *i*, *ii*, and *iii* in previous sections). The largest Central American subclade, clade *i*, contains 25-30 species of moist to seasonally dry forest trees and shrubs. *Citharexylum bourgeauianum* and *C. guatemalense*, which were not included in MSC analyses due to large amounts of missing data, were also placed in this clade in gene tree analyses. These relationships are supported by geography and morphology. Clade *ii* includes four species of arid-adapted shrubs distributed in Central Mexico, and clade *iii* is made up of two to three species of purple-flowered trees. Based on gene tree results, *Citharexylum flabellifolium* belongs to the Central American clade, but its placement in a subclade is unresolved. In the nrDNA tree, *C. flabellifolium* nests within clade *i*. The same accession nests in clade *iii* in the PPR70 tree. *Citharexylum flabellifolium* is an arid-adapted species distributed in Baja California Sur and Sonora; it is morphologically distinct from other species in the genus. Like *C. affine* and *C. hintonii,* the other species in clade *iii*, *C. flabellifolium* has purple flowers. However, there are also purple-flowered species in clade *i*, making it difficult to place without better phylogenetic resolution.

Two well-supported clades are inferred within the South American clade: (*iv*) a clade of tall, mesic-forest trees with many flowers, and (*v*) a clade of Andean shrubs with reduced inflorescences. With the exception of two Caribbean shrub species, clade *iv* includes large-leaved species distributed in Amazonia, the Brazilian Atlantic coast forest, and mid elevations of the northern Andes. Not sampled in the MSC, *C. reticulatum* is placed in this moist forest clade by nrDNA. This species would be difficult to place by morphology alone, since it shares characters of both the moist forest trees and the Andean shrubs. Distributed from Ecuador to Peru, it grows at higher elevations and is smaller in stature than is typical of the South American moist forest clade. The species does have long, many-flowered inflorescences with white flowers, which are typical of the moist forest trees. However, it is reported to have black fruits (Moldenke, 1957-1959), which are common in the Andean shrub clade, instead of red fruits typical of the moist forest clade.

Morphology— *Rehdera* is the earliest diverging lineage of Citharexyleae and is distinguished by having dry schizocarpous fruits, whereas *Citharexylum* and *Baillonia* have fleshy drupes. As suggested by Marx et al. (2010), *Baillonia amabilis* appears to be a species of *Citharexylum* in which only two of four ovules mature. It is ambiguous as to how the two mature ovules in *Baillonia* are derived (O'Leary et al., 2012). Traditionally, *Baillonia* is described as having one carpel abort early in development, the remaining carpel with two ovules matures. However, dissection of fruits from Rojas 7368 (SI) reveal two carpels, each with one mature ovule. Fruit dissections of closely related species *C. poepiggii* reveal most individuals have four mature ovules, as expected, but one individual, Phillipson 1380 (US), appears to have one

abortive carpel (Nataly O'Leary, personal communication). Further investigation of mature carpel/ovule number is needed in *Baillonia* and *Citharexylum* to determine how prevalent abortion of carpels/ovules is throughout the genus.

Citharexylum represents an independent origin of fleshy fruits in Verbenaceae (O'Leary et al., 2012). Fruits either mature from green to red-orange, green to red to black in a two-phase maturation process, or green directly to black. The two major clades of *Citharexylum* are distinguished by fruit maturation. The South American clade consists of species with a single mature fruit color; fruits either mature to red or to black without an intermediate color. The moist forest trees typically have red fruits; black fruits are common in the Andean shrubs. The Central American clade includes species with a two-step fruit maturation process. Red-orange is an intermediate color as the fruits mature to black, and both colors of fruits are observed simultaneously in the infructescences. In some Central American species, only red-orange has been recorded as the mature fruit color; it is unclear if fruits of those species mature at the red-orange stage, or if observations for fully mature black fruits are lacking. Likewise, *C. altamiranum* is only reported as having black fruits; it is uncertain if the fruits mature from green to black or if the intermediate color has not been observed. It seems probable that single-phase maturation would be the ancestral state of the genus, and that *C. altamiranum*, as the earliest diverging lineage, would mature to black without an intermediate color. Without more observations, it cannot be concluded if two-phase maturation was lost in the South American clade or arose in the Central American clade

Dioecy needs further investigation throughout *Cithareyxlum*. Species have been confirmed as dioecious in the literature, including *C. spinosum* (Tomlinson and Fawcett, 1972),

C. myrianthum (Rocca and Sazima, 2006), Costa Rican species (Rueda and Hammil, in prep.), and dioecy has been observed in the field for many others (L. Frost, personal observation). Dioecy can be inferred in *Citharexylum* because in male plants the entire flower disarticulates from the pedicel at senescence, leaving naked racemes. In female plants, the calyx becomes lignified as the fruits mature and is retained on the pedicel, even as fruits are eaten or fall off. This pattern is observed in specimens of *Baillonia* (see Palmer 1853-6 (Orrell and Howell, 2018) and Hassler 2683a (MNHN, 2018a) for putative male and Prance et al. 15 (Tulig, 2017) and Weddell 3193 (MHNH, 2018b) for putative female specimens), but more detailed study is needed to determine if the species is dioecious.

A broader study is also needed to determine if all members of *Citharexylum* are dioecious or only some species/clades. Variation in inflorescence architecture provides further evidence of dioecy for some species. Functionally male plants often have greater investment in production of attractive, reproductive structures, which can translate as a greater number of flowers produced overall than female individuals (Mayer and Charlesworth, 1991). Variation within species of *Citharexylum* for presence/absence of compound or axillary inflorescences may be related to sex. In these variable species, male individuals could increase flower production either through the development of axillary inflorescences, or compound inflorescences, or both. Variation between sexes is easily observed for species with large, many-flowered inflorescences, but is difficult to detect in shrub species with reduced inflorescences. These species have simple, few-flowered inflorescences that terminate short, axillary branches. One individual does not obviously produce more flowers or more inflorescences than another. It is also more difficult in general to infer the

sex of individuals in these few-flowered species using the observations described above. Further investigation of dioecy in the Andean and arid-adapted Mexican shrubs is warranted.

In addition, cryptic dieocy is not always readily observable and may be more widespread in the family. A hand-written note on the label of G. Diggs & M. Nee 2473 (F) collection of *C. oleinum* reads, "4 epipetalous, ± sessile stamens. Style short, functionally a male flower?" *Citharexylum oleinum* and *C. tetramerum* consistently fall outside of Citharexyleae. Further work is needed to determine the exact placement of these species in Verbenaceae, but our evidence suggests that these species do not belong in *Citharexylum*. *Citharexylum* is the only genus in Verbenaceae in which dioecy has been documented, but *C. oleinum* and *C. tetramerum*, if found to be dioecious, would represent a separate origin in Verbenaceae.

Spines or thorns, arising as modified axillary shoots, were the first character used to suggest a subgeneric classification scheme for *Citharexylum*. Thorns are only present in the Andean shrub clade and may have arisen twice in that group. They may have evolved as a defense mechanism against large herbivores in the more open vegetation of inter-Andean dry valleys. Thorns are retained in the more mesic, low-land tree species *C. montevidense*, which is distributed in eastern Argentina, Paraguay, and Uruguay. The second origin of thorns is in *C. herrerae*, which is nested within a group of unarmed Andean cloud forest shrubs. Beyond the production of thorns, *C. herrerae* is morphologically distinct in this clade by having red fruits, whereas the unarmed shrubs have black fruits, and stems that remain green for several years (the latter trait is also distinct within the genus, shared only by *C. roxanae*, a distantly-related, arid-adapted shrub distributed in Baja California Sur and Sonora). Though not technically thorn-bearing, some species outside of the Andean shrub clade also have sharp defenses. *Baillonia*

amabilis, which inhabits the Brazilian cerrado*,* has spiny sterigmata at leaf bases (Moldenke, 1941), and the arid-adapted Mexican shrubs (*C. brachyanthum*, *C. flabeillifolium*, *C. lycioides*, *C. racemosum*, and *C. rosei*) have relatively short, pointed, branches.

Classification—We present a subgeneric classification of the revised *Citharexylum* including *Baillonia*, which was transferred recently to *Citharexylum* (Christenhusz et al., 2018). Six well-supported, monophyletic subgenera are identified. The type species *C. spinosum* is suspected to be of hybrid origin with parental lineages from separate subgenera. This poses a taxonomic problem, since two clades contain representatives of the type. However, chloroplast DNA, MSC analyses, fruit morphology, and a predominant distribution in the Caribbean (including southern Florida) support the placement of *C. spinsoum* in clade *i* of the Central American clade.

Citharexyleae Briquet

I. *Citharexylum* L., Sp. Pl. 1: 625. 1753. Type: *Citharexylum spinosum* L.

1. C. subgenus ***Citharexylum*** L.A. Frost, subg. nov. Type: *Citharexylum spinosum* L.

   Trees and shrubs, thorns absent; inflorescences terminal or terminal and axillary, simple or compound; flowers many, corolla white, pale yellow-green, or purple; fruits maturing from orange to black.

   Distribution: Central America, Caribbean, Northern South America

   Estimated species: 25-30

2. C. subgenus ***Mexicanum*** L.A. Frost, subg. nov. Type: *Citharexylum brachyanthum* (A.Gray ex Hemsl.) A.Gray

Shrubs, thorns absent; inflorescences terminal and simple, terminating very short branchlets; flowers few, corolla white; fruits red-orange at maturity or maturing from orange to black.

Distribution: Central Mexico

Estimated species: 4

3. C. subgenus ***Affine*** L.A. Frost, subg. nov. Type: *Citharexylum affine* D.Don.

Trees, thorns absent; inflorescences terminal and axillary, simple or compound; flowers many, corolla purple; fruits maturing from orange to black.

Distribution: Central America (primarily Mexico)

Estimated species: 2-3

4. C. subgenus ***Sylvaticum*** L.A. Frost, subg. nov. Type: *Citharexylum myrianthum* Cham.

Trees or shrubs, thorns absent; inflorescences terminal, axillary, or terminal and axillary, simple or compound; flowers few or many, corolla white; fruits red.

Distribution: South America, the Carribbean

Estimated species: 15 - 18

5. C. subgenus ***Andinum*** L.A. Frost, subg. nov. Type: *Citharexylum flexuosum* Moldenke

Shrubs, thorns present or absent; inflorescences terminal and simple; flowers few to submany, corollas white to pale yellow-green, some with lobes tipped purple or orange in bud; fruits red or black.

Distribution: Andean South America

Estimated species: 14-16

6. C. subgenus ***Altamiranum*** L.A. Frost, subg. nov. Type: *Citharexylum altamiranum* Greenm.

Shrubs, thorns absent; inflorescences terminal and axillary, simple; flowers few, corollas purple; fruits black.

Distribution: Mexico

Estimated species: 1

II. *Rehdera* Moldenke, Repert. Spec. Nov. Regni Veg. 39: 48–50. 1935. Type: *Rehdera trinervis* S.F. Blake

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LITERATURE CITED

Atkins, S. 2004. Verbenaceae. Pp. 449-468 In: The Families and Genera of Flowering Plants, Vol. 7 (J. W. Kadereit, ed.). Springer-Verlag, Berlin.

Bentham, G. 1839. Enumeration of plants collected by Mr. Schomburgk in British Guiana. *The Annals and Magazine of Natural History* 2:441-451.

Blischak, P. D., M. Latvis, D. F. Morales-Briones, J. C. Johnson, V. S. Di Stilio, A. D. Wolfe, and D. C. Tank. In Review. Fluidigm2PURC: automated processing and haplotype inference for double-barcoded PCR amplicons. *bioRxiv*. doi: 10.1101/242677.

Briquet, J. 1895. Verbenaceae. In Die naturlichen Pflanzenfamilien (A. Engler and K. Prantl, eds), Tiel 4/3a, pp. 132-182. Engelmann, Leipzig, Germany.

Camacho, C., G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, et al. 2009. BLAST+: Architecture and applications. BMC Bioinformatics 10: 421.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and high-performance computing. *Nature Methods* 9(8): 772.

Doyle, J. J., and J. L. Doyle. 1987. A rapid isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Drummond, A. J., and M. A. Suchard. 2010. Bayesian random local clocks, or one rate to rule them all. *BMC Biology* 8:114. doi: 10.1186/1741-7007-8-114

Drummond, A. J., M. A. Suchard, D. Xie, and A. Rambaut. 2012. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29(8): 1969–1973. doi: 10.1093/molbev/mss075

Edgar, R.C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792-1797.

Edgar, R.C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26(19), 2460-2461

Edgar, R.C., B.J. Haas, J.C. Clemente, C. Quince, R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection, Bioinformatics 27(16), 2194-2200.

Frost, L. A., Tyson, S. M., Lu−Irving, P., O'Leary, N. and Olmstead, R. G. (2017), Origins of North American arid−land Verbenaceae: More than one way to skin a cat. American Journal of Botany, 104: 1708-1716. doi:10.3732/ajb.1700292

Guindon, S., and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52(5): 696-704.

Höhna, S., T.A. Heath, B. Boussau, M.J. Landis, F. Ronquist, J.P. Huelsenbeck. 2014. Probabilistic graphical model representation in phylogenetics. *Systematic Biology* 63:753–771.

Höhna, S., M.J. Landis, T.A. Heath, B. Boussau, N. Lartillot, B.R. Moore, J.P. Huelsenbeck, and F. Ronquist. 2016. RevBayes: Bayesian Phylogenetic Inference Using Graphical Models and an Interactive Model-Specification Language. *Systematic Biology* 65(4): 726-736.

Junell, S. 1934. Zur Gynaceummorphologie und Systematik der Verbenaceen und Labiaten. Symb. Bot. Upsal. 4: 1-219.

Larsson, A. (2014). AliView: a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics* 30(22): 3276-3278. doi:10.1093/bioinfor.

Lu-Irving, P., and R. G. Olmstead. 2013. Investigating the evolution of Lantaneae (Verbenaceae) using multiple loci. *Botanical Journal of the Linnean Society* 171: 103-119. doi:10.1111/j. 1095-8339.2012.01305.x

Lu-Irving, P., O'Leary, N., O'Brien, A. and R. G. Olmstead. 2014. Resolving the genera *Aloysia* and *Acantholippia* within the tribe Lantaneae (Verbenaceae), using chloroplast and nuclear sequence data. *Systematic Botany* 39: 644-655. doi: 10.1600/036364414X680816

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10-12.

Marx, H., N. O'Leary, Y.-W. Yuan, P. Lu-Irving, D. Tank, M.E. Múlgura, and R. G. Olmstead. 2010. A molecular phylogeny and classification of Verbenaceae. *American Journal of Botany* 97(10): 1647–1663.

MNHN - Museum national d'Histoire naturelle. 2018a. The vascular plants collection (P) at the Herbarium of the Muséum national d'Histoire Naturelle (MNHN - Paris). Version 69.53. Occurrence Dataset https://doi.org/10.15468/nc6rxy accessed via GBIF.org on 2018-03-05. https://www.gbif.org/occurrence/1019625478.

MNHN - Museum national d'Histoire naturelle. 2018b. The vascular plants collection (P) at the Herbarium of the Muséum national d'Histoire Naturelle (MNHN - Paris). Version 69.53. Occurrence Dataset https://doi.org/10.15468/nc6rxy accessed via GBIF.org on 2018-03-05. https://www.gbif.org/occurrence/439496996.

Moldenke, H. N. 1941. A monograph of the genus Baillonia. Darwiniana, 5: 166-177.

Moldenke, H. N. 1958a. Materials towards a monograph of thegenus Citharexylum I. – Phytologia 6: 242–256.

Moldenke, H. N. 1958b. Materials towards a monograph of the genus Citharexylum II. – Phytologia 6: 262–320.

Moldenke, H. N. 1958c. Materials towards a monograph of the genus Citharexylum III. – Phytologia 6: 332–368.

Moldenke, H. N. 1958d. Materials towards a monograph of the genus Citharexylum IV. – Phytologia 6: 383–432.

Moldenke, H. N. 1959a. Materials towards a monograph of the genus Citharexylum V. – Phytologia 6: 448–505.

Moldenke, H. N. 1959b. Materials towards a monograph of thegenus Citharexylum VI. – Phytologia 7: 7–48.

Moldenke, H. N. 1959c. Materials towards a monograph of the genus Citharexylum VII. – Phytologia 7: 49–73.

Moldenke, H. N. 1971. A fifth summary of the Verbenaceae, Avicenniaceae, Stilbaceae, Dicrastylidaceae, Symphoremataceae, Nyctanthaceae, and Eriocaulaceae of the world as to valid taxa geographic distribution, and synonymy. 2 vols. Publ. by the author.

Moore, Sp. Le M. 1895. The Phanerogamic Botany of the Matto Grosso Expedition, 1891-92. Transactions of the Linnean Society of London (Botany) Ser. 2 (4).

O'Leary, N., Y.-W. Yuan, A. Chemisquy, and R. G. Olmstead. 2009. Reassignment of Species of Paraphyletic Junellia s. l. to the New Genus Mulguraea (Verbenaceae) and New Circumscription of Genus Junellia: Molecular and Morphological Congruence. *Systematic Botany*, 34(4): 777-786.

O'Leary, N., C. I. Calviño, S. Martínez, P. Lu-Irving, R. G. Olmstead, and M. E. Múlgura. 2012. Evolution of morphological traits in Verbenaceae. *American Journal of Botany*, 99: 1778–1792. doi:10.3732/ajb.1200123.

Orrell T, Hollowell T (2018). NMNH Extant Specimen Records. Version 1.14. National Museum of Natural History, Smithsonian Institution. Occurrence Dataset https://doi.org/10.15468/hnhrg3 accessed via GBIF.org on 2018-03-05. https://www.gbif.org/occurrence/1322362840.

Rambaut, A., M. A. Suchard, D. Xie, and A. J. Drummond. 2014. Tracer v1.6. Available from http://beast.bio.ed.ac.uk/Tracer.

Rocca, M., and M. Sazima. 2006. The dioecious, sphingophilous species *Citharexylum myrianthum* (Verbenaceae): Pollination and visitor diversity. *Flora* 201: 440-450.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19(12): 1572–1574. doi:10.1093/bioinformatics/btg180

Rothfels, C.J., K.M. Pryer, and F-W. Li. 2016. Next-generation polyploidy phylogenetics: rapid resolution of hybrid polyploid complexes using PacBio single-molecule sequencing. New Phytologist

Sanders, R. W. 2001. The genera of Verbenaceae in the southeastern United States. *Harvard Papers in Botany* 5: 303-358.

Schauer, J. C. 1847. Verbenaceae. In: A. P. De Candolle, Prodr. 11: 522-700.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9): 1312–1313. doi:10.1093/bioinformatics/btu033.

Tomlinson, P.B., & Fawcett, P. 1972. Dioecism in *Citharexylum* (Verbenaceae). Journal of the Arnold Arboretum. 53(3): 386-389.

Troncoso, N. S. 1974. Los generos de Verbenacea de Sudamerica extratropical. *Darwiniana* 18: 295-412.

Tulig M, Ramirez J, Watson K (2017). The New York Botanical Garden Herbarium (NY). The New York Botanical Garden. Occurrence Dataset https://doi.org/10.15468/6e8nje accessed via GBIF.org on 2018-03-05. https://www.gbif.org/occurrence/1097308086.

Uribe-Convers, S., M. L. Settles, and D. C. Tank. 2016. A Phylogenomic Approach Based on PCR Target Enrichment and High Throughput Sequencing: Resolving the Diversity within the South American Species of Bartsia L. (Orobanchaceae). *PLOS ONE* 11(2): e0148203. doi: 10.1371/journal.pone.0148203

Yuan, Y.-W., and R. G. Olmstead. 2008a. A species-level phylogenetic study of the *Verbena* complex (Verbenaceae) indicates two independent intergeneric chloroplast transfers. *Molecular Phylogenetics and Evolution* 48: 23-33.

Yuan, Y.-W., C. Liu, H. E. Marx, and R. G. Olmstead. 2009. The PPR (pentatricopeptide repeat) gene family, a tremendous resource for plant phylogenetic studies. *New Phytologist* 182: 272-283.

Yuan, Y.-W., C. Liu, H. E. Marx, and R. G. Olmstead. 2010. An empirical demonstration of using PPR (pentatricopeptide repeat) genes as phylogenetic tools: phylogeny of Verbenaceae and the Verbena complex. *Molecular Phylogenetics and Evolution* 54: 23-35.

TABLES

Table 1. Characteristics of individual datasets

Alignment	No. of taxa	Proportion of taxa	Alignment length (bp)	% missing data	No. of variable sites	Proportion of variable sites	No. of parsimony informative sites	Proportion of parsimony informative sites	GC content
all comb.	645	1.000	27794	74.00	11139	0.401	7270	0.262	0.392
cpDNA	627	0.972	16961	69.45	5176	0.305	3175	0.187	0.358
nrDNA	580	0.899	1401	43.94	906	0.647	764	0.545	0.592
PPR11	204	0.316	1461	29.76	866	0.593	621	0.425	0.401
PPR62	151	0.234	1452	44.65	717	0.494	457	0.315	0.411
PPR70	202	0.313	1128	52.35	697	0.618	464	0.411	0.382
PPR81	123	0.191	1180	7.96	652	0.553	433	0.367	0.395
PPR90	72	0.112	986	3.29	314	0.318	182	0.185	0.405
PPR91	45	0.070	1306	8.74	524	0.401	335	0.257	0.394
PPR97	62	0.096	747	5.47	266	0.356	158	0.212	0.41
PPR123	241	0.374	1394	31.18	739	0.53	533	0.382	0.405

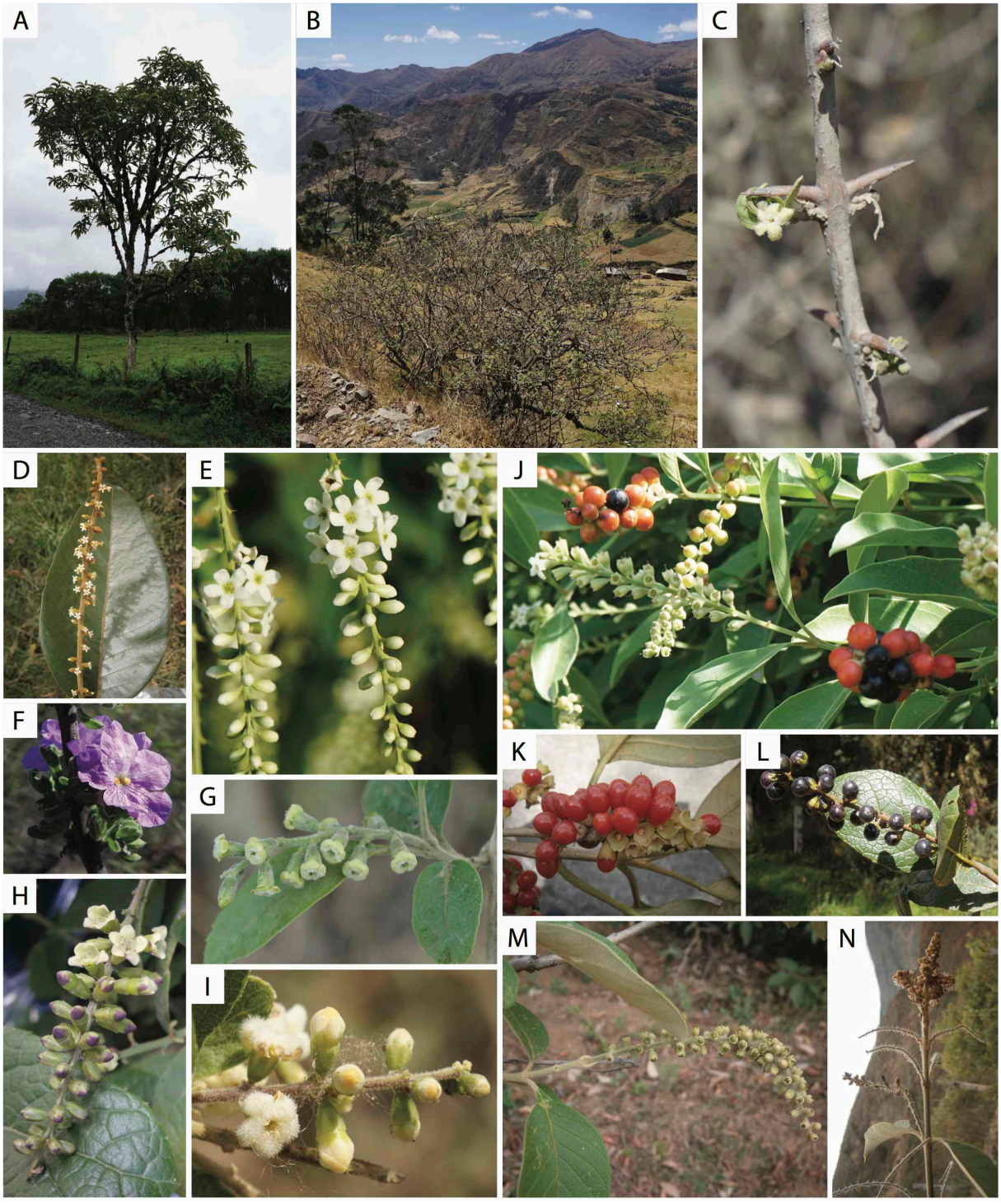
FIGURE CAPTIONS

Fig. 1. Morphological diversity of *Citharexylum*. Habit (A-B): (A) tree (*C. montanum*), (B) shrub (*C. weberbaueri*); Thorns (C; *C. flexuosum*); Flower color (D-I): (D, E) white (*C. subflavescens, C. hexangulare*), (F) purple (*C. flabellifolium*), (G) pale yellow-green (*C. kobuskianum),* (H) pale with purple-tipped buds (*C. sulcatum*), (I) white with orange-tipped buds (*C. peruvianum*); Fruit color (J-L): (J) two-phase maturation from green to orange to black (*C. ellipticum*), (K) green to red (*C. montanum*), (L) green directly to black (*C. sulcatum*); Inflorescence (M-N; *C. kunthianum*): (M) female inflorescence with simple terminal raceme (N) male inflorescence with compound terminal racemes and simple axillary racemes.

Fig. 2. Gene tree cartoons for cp, nr, lowcopy nuclear combined, and individual lowcopy nuclear datasets showing topological differences among gene trees. Roman numberals on branches correspond to clades of the tree recognized in the text; R marks the genus *Rehdera*. For gene trees in which one clade nested within another, the numeral for that clade is placed within the inclusive clade (e.g. clade iii in PPR24 and PPR123). Data for species in clade iii were missing for PPR62, and for species in clade ii for PPR123. Solid lines on branches indicate clades; dashed lines indicate grades. Tip labels may be found in Fig. 3.

Fig. 3. Bayesian consensus tree for all data combined. Thickened branches indicate high support (≥ 70 bootstrap support from maximum likelihood (ML) analyses or ≥ 90 % posterior probability from Bayesian inference (BI)). Support for nodes are plotted as bootstrap/posterior probability (percent). Roman numerals, curved lines at the tips, and branch colors correspond to clades recognized in the text. R indicates the genus *Rehdera*.

Fig. 4. *BEAST multi-species coalescent tree. Thickened branches indicated nodes with high support (posterior probability ≥ 0.95). Colored boxes represent character states of species. First column from the left—habit: tree (green) or shrub (yellow); thorns: present (black) or absent (white); terminal inflorescence: compound (black), simple (gray), or absent (white); axillary inflorescence: compound (black), simple (gray), or absent (white); flower number: >20 (black), 10-20 (gray), or <10 (white); flower color: literal (white, pale yellow, purple, pale yellow with purple lobes (split yellow and purple boxes), white with orange lobes (split white and orange box)); mature fruit color: literal (red with no intermediate, black with no intermediate, black with red/orange intermediate (split orange and black boxes)), or dry fruits (brown); Geography: Central America (pink), Caribbean (orange), or South America (blue), boxes with multiple color inhabit multiple regions. Roman numerals correspond to clades identified in previous figures and the proposed subgenera of *Citharexylum*: (i) *C.* subg. *Citharexylum*, (ii) *C.* subg. *Mexicanum*, (iii) *C.* subg. *Affine*, (iv) *C.* subg. *Sylvaticum* (v) *C.* subg. *Andinum* (vi) *C.* subg. *Altamiranum*. R indicates the genus *Rehdera*.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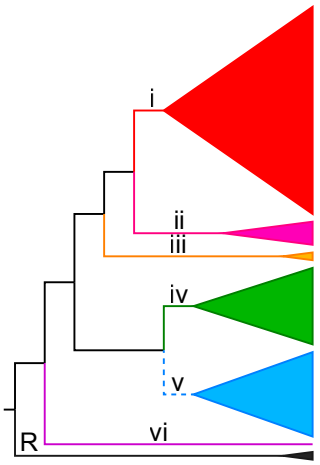

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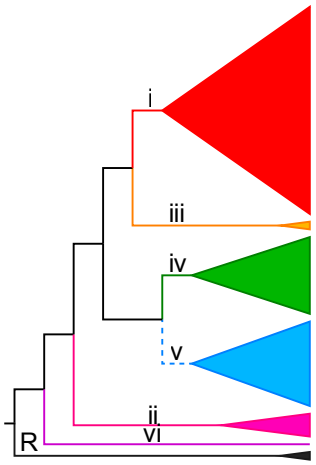

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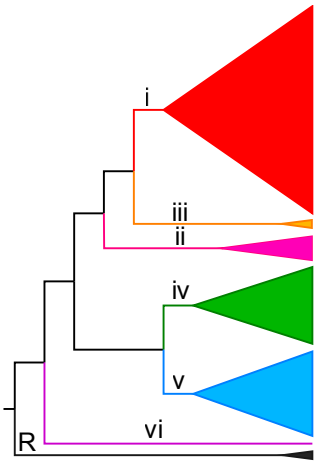

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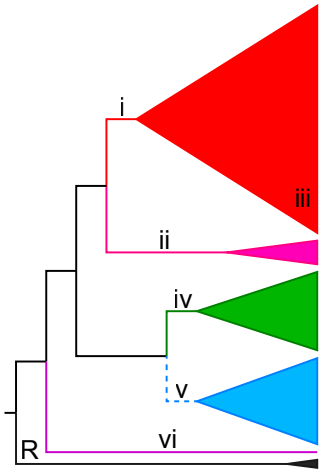
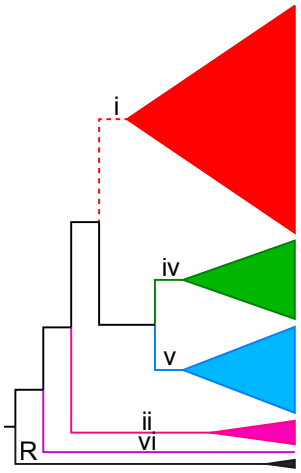


PPR24



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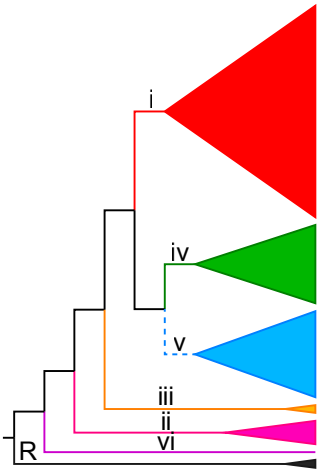

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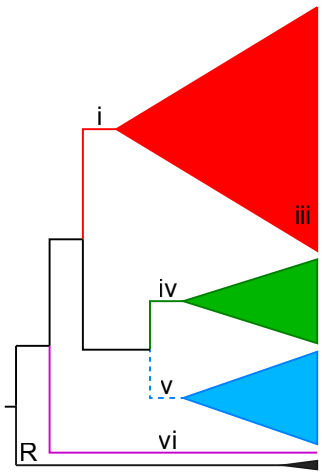

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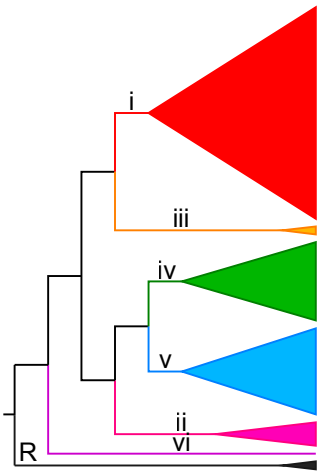
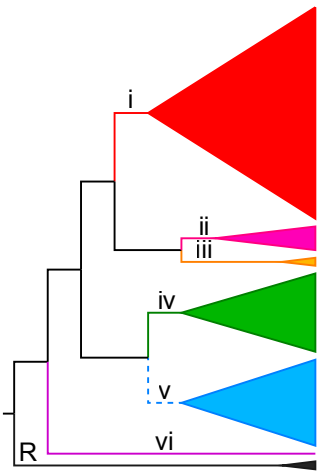
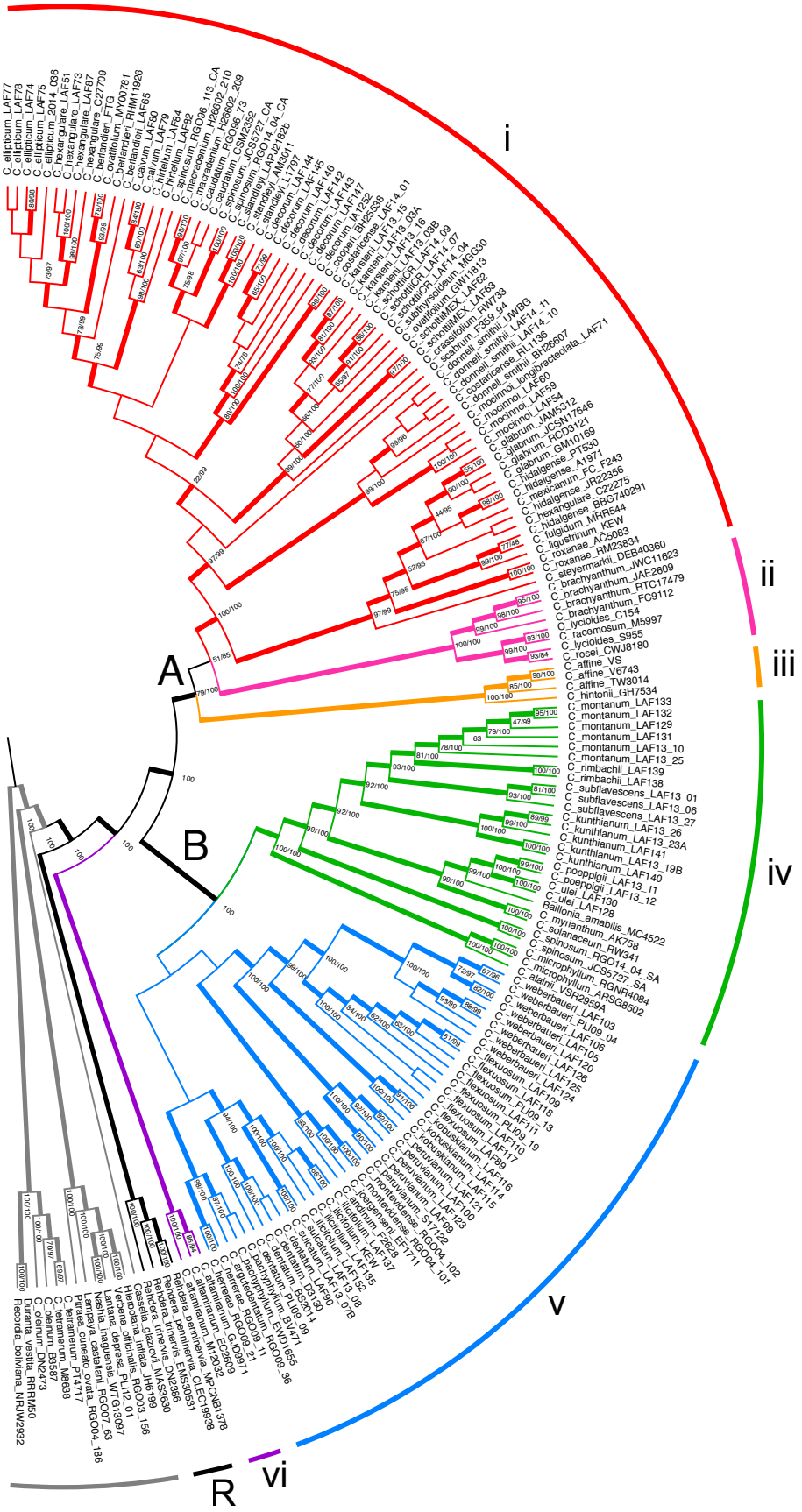


waxy haplotype 1



waxy haplotype 2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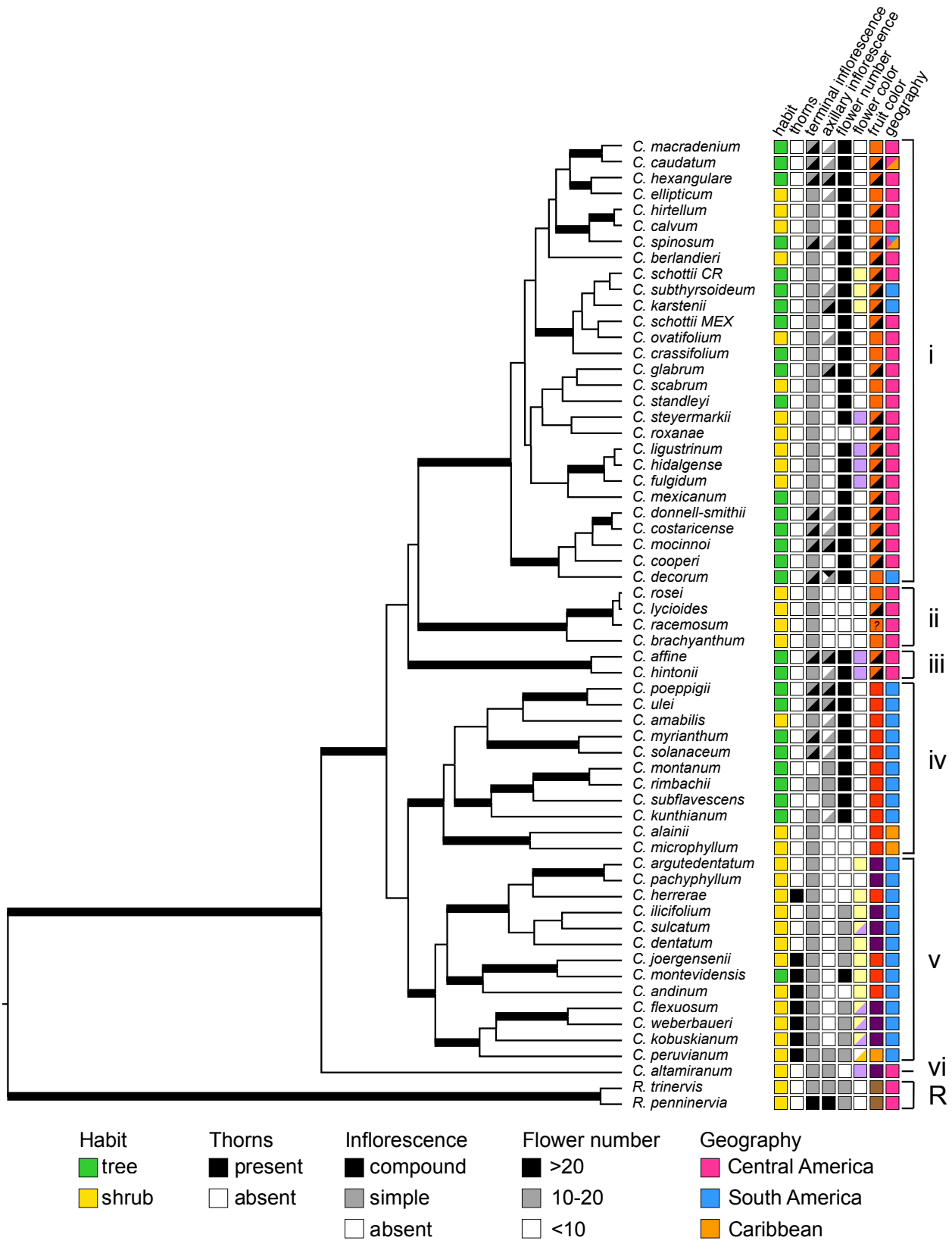
ii
iii
iv
v
vi
A
B
R


habit
thorns
terminal inflorescence
axillary inflorescence
flower number
flower color
fruit color
geography
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C. hexangulare
C. ellipticum
C. hirtellum
C. calvum
C. spinosum
C. berlandieri
C. schottii CR
C. subthyrsoideum
C. karstenii
C. schottii MEX
C. ovatifolium
C. crassifolium
C. glabrum
C. scabrum
C. standleyi
C. steyermarkii
C. roxanae
C. ligustrinum
C. hidalgense
C. fulgidum
C. mexicanum
C. donnell-smithii
C. costaricense
C. mocinnoi
C. cooperi
C. decorum
C. rosei
C. lycioides
C. racemosum
C. brachyanthum
C. affine
C. hintonii
C. poeppigii
C. ulei
C. amabilis
C. myrianthum
C. solanaceum
C. montanum
C. rimbachii
C. subflavescens
C. kunthianum
C. alainii
C. microphyllum
C. argutedentatum
C. pachyphyllum
C. herrerae
C. ilicifolium
C. sulcatum
C. dentatum
C. joergensenii
C. montevidensis
C. andinum
C. flexuosum
C. weberbaueri
C. kobuskianum
C. peruvianum
C. altamiranum
R. trinervis
R. penninervia
i
ii
iii
iv
v
vi
R
Habit
tree
shrub
Thorns
present
absent
Inflorescence
compound
simple
absent
Flower number
>20
10-20
<10
Geography
Central America
South America
Caribbean

SUPPLEMENTAL FIGURES

Fig. S1. Gene trees for cpDNA. Right: maximum likelihood (ML) tree from RAxML; left: Bayesian consensus tree (BI) from MrBayes.

Fig. S2. Gene trees for nrDNA. Right: maximum likelihood (ML) tree from RAxML; left: Bayesian consensus tree (BI) from MrBayes.

Fig. S3. Gene trees for PPR24. Right: maximum likelihood (ML) tree from RAxML; left: Bayesian consensus tree (BI) from MrBayes.

Fig. S4. Gene trees for PPR62. Right: maximum likelihood (ML) tree from RAxML; left: Bayesian consensus tree (BI) from MrBayes.

Fig. S5. Gene trees for PPR70. Right: maximum likelihood (ML) tree from RAxML; left: Bayesian consensus tree (BI) from MrBayes.

Fig. S6. Gene trees for PPR123. Right: maximum likelihood (ML) tree from RAxML; left: Bayesian consensus tree (BI) from MrBayes.

Fig. S7. Gene trees for waxy haplotype 1. Right: maximum likelihood (ML) tree from RAxML; left: Bayesian consensus tree (BI) from MrBayes.

Fig. S8. Gene trees for waxy haplotype 2. Right: maximum likelihood (ML) tree from RAxML; left: Bayesian consensus tree (BI) from MrBayes.

Fig. S9. Ancestral character reconstructions for presence/absence of thorns. Pie charts at nodes indicate the probability of a given state at that node—present (black) or absent (white).

Fig. S10. Ancestral character reconstructions for terminal inflorescences. Pie charts at nodes indicate the probability of a given state at that node—compound (black), simple (gray) or absent (white).

Fig. S11. Ancestral character reconstructions for axillary inflorescences. Pie charts at nodes indicate the probability of a given state at that node—compound (black), simple (gray) or absent (white).

Fig. S12. Ancestral character reconstructions for flower number per raceme. Pie charts at nodes indicate the probability of a given state at that node—>20 (black), 10-20 (gray) or <10 (white).

Fig. S13. Ancestral character recontructions for flower color. Pie charts at nodes indicate the probability of a given state at that node—literal (white, pale yellow, orange, or purple).

Fig. S14. Ancestral character recontructions for fruit color. Pie charts at nodes indicate the probability of a given state at that node—red with no intermediate (red), black with no intermediate (black), red with black intermediate (blue), or dry fruits (brown).

Fig. S1. cpDNA


ML
C_spinosum_RGO96_113
C_hirtellum_LAF82
C_hirtellum_LAF84
C_spinosum_JCS5727
C_spinosum_RGO14_04
C_hexangulare_LAF73
C_hexangulare_LAF87
C_hexangulare_LAF51
C_calvum_LAF79
C_calvum_LAF80
C_berlandieri_LAF65
C_caudatum_RGO96_73
C_macradenium_BH26603
C_hexangulare_C27709
C_macradenium_BH26602
C_hidalgense_BBG740291
C_berlandieri_RHM11926
C_standleyi_LAPJ2182b
C_standleyi_L1797
C_standleyi_AM3011
C_scabrum_F359_94
C_glabrum_JCSN17646
C_glabrum_JAM5312
C_guatemalense_IC1827
C_caudatum_CSM2352
C_ellipticum_LAF78
C_ellipticum_LAF75
C_ellipticum_LAF77
C_ellipticum_LAF74
C_ellipticum_EC14571
C_fulgidum_MRR544
C_ligustrinum_KEW
C_ovatifolium_MY00781
C_berlandieri_FTG
C_mocinnoi_LAF54
C_mocinnoi_LAF60
C_mocinnoi_LAF59
C_mocinnoi_longibracteolatum_LAF71
C_decorum_LAF144
C_decorum_LAF146
C_decorum_LAF145
C_decorum_LAF142
C_decorum_LAF147
C_decorum_LAF143
C_decorum_IA1252
C_donnell_smithii_UWBG
C_costaricense_RL1136
C_donnell_smithii_LAF14_10
C_donnell_smithii_LAF14_11
C_donnell_smithii_BH26607
C_cooperi_BH25538
C_costaricense_LAF14_01
C_karstenii_LAF13_03A
C_karstenii_LAF13_16
C_karstenii_LAF13_03B
C_karstenii_LAF13_15
C_schottiiMEX_LAF63
C_costaricense_E626
C_subthyrsoideum_MGG30
C_ovatifolium_GW11813
C_schottiiMEX_LAF62
C_mexicanum_S625
C_crassifolium_RW733
C_schottiiCR_LAF14_07
C_schottiiCR_LAF14_04
C_schottiiCR_LAF14_09
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C_hexangulare_C22275
C_glabrum_RCD3121
C_glabrum_GM10169
C_steyermarkii_DEB40360
C_roxanae_AC5083
C_roxanae_RM23834
C_hidalgense_JR22356
C_hidalgense_A1971
C_hidalgense_PT530
C_brachyanthum_JAE2609
C_brachyanthum_JWC11623
C_lycioides_C154
C_lycioides_S955
C_racemosum_M5997
C_rosei_CWJ8180
C_brachyanthum_RTC17479
C_brachyanthum_FC9112
C_affine_VS
C_affine_TW3014
C_affine_V6743
C_hintonii_GH7534
C_dentatum_LAF90
C_dentatum_D3130
C_dentatum_BS2014
C_dentatum_PLI09_09
C_sulcatum_LAF13_07B
C_sulcatum_LAF13_08
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C_ilicifolium_LAF135
C_ilicifolium_KEW
C_ilicifolium_LAF137
C_flexuosum_LAF118
C_flexuosum_LAF109
C_flexuosum_LAF89
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C_flexuosum_LAF117
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C_montevidense_RGO04_102
C_joergensenii_EF1711
C_andinum_F2628
C_weberbaueri_LAF103
C_weberbaueri_PLI09_04
C_weberbaueri_LAF124
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C_weberbaueri_LAF105
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C_weberbaueri_LAF120
C_peruvianum_LAF123
C_peruvianum_LAF121
C_peruvianum_S17122
C_peruvianum_LAF100
C_peruvianum_LAF99
C_kobuskianum_LAF116
C_kobuskianum_LAF115
C_kobuskianum_LAF114
C_montanum_LAF132
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C_montanum_LAF129
C_montanum_LAF13_25
C_montanum_LAF13_10
C_montanum_LAF131
C_subflavescens_LAF13_06
C_subflavescens_LAF13_01
C_subflavescens_LAF13_27
C_rimbachii_LAF139
C_rimbachii_LAF138
C_poeppigii_LAF13_11
C_poeppigii_LAF13_12
C_ulei_LAF128
C_ulei_LAF130
Bailonia_amabilis_MC4522
C_solanaceum_RW341
C_myrianthum_AK758
C_kunthianum_LAF13_23A
C_kunthianum_LAF13_26
C_kunthianum_LAF13_19B
C_kunthianum_LAF141
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Hierbotana_inflata_JH6199
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Lampayo_castellani_RGO07_63
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Cassella_glaziovii_MAS3630
Hierbotana_inflata_JH6199
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Lantana_depresa_PLI12_01
Nashia_inaguensis_WTG13097
Lampayo_castellani_RGO07_63
Pitraea_cuneato_ovata_RGO04_186
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Duranta_vestita_RRRM50
Recordia_boliviana_NRJW2932

Fig. S2. nrDNA

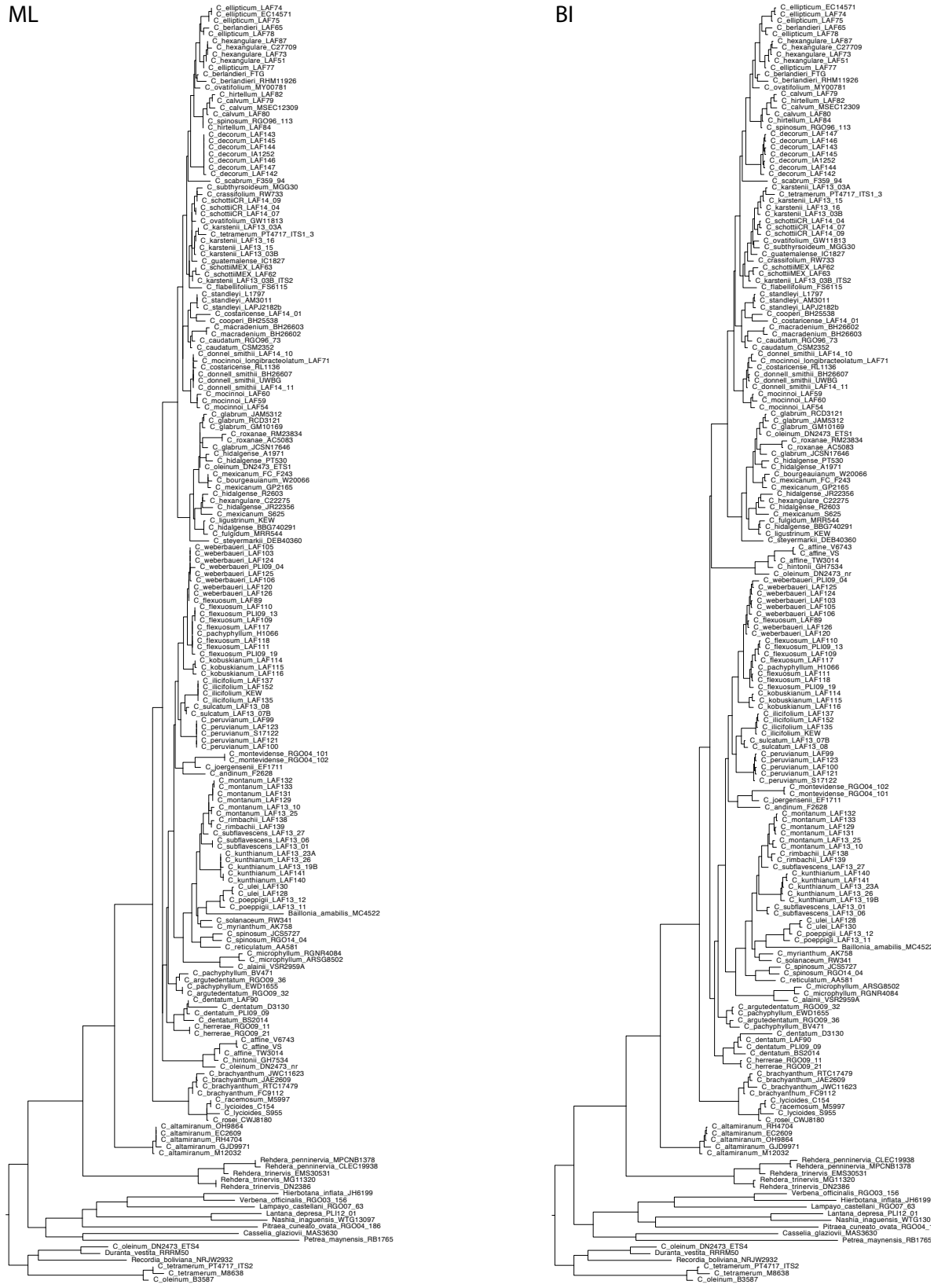
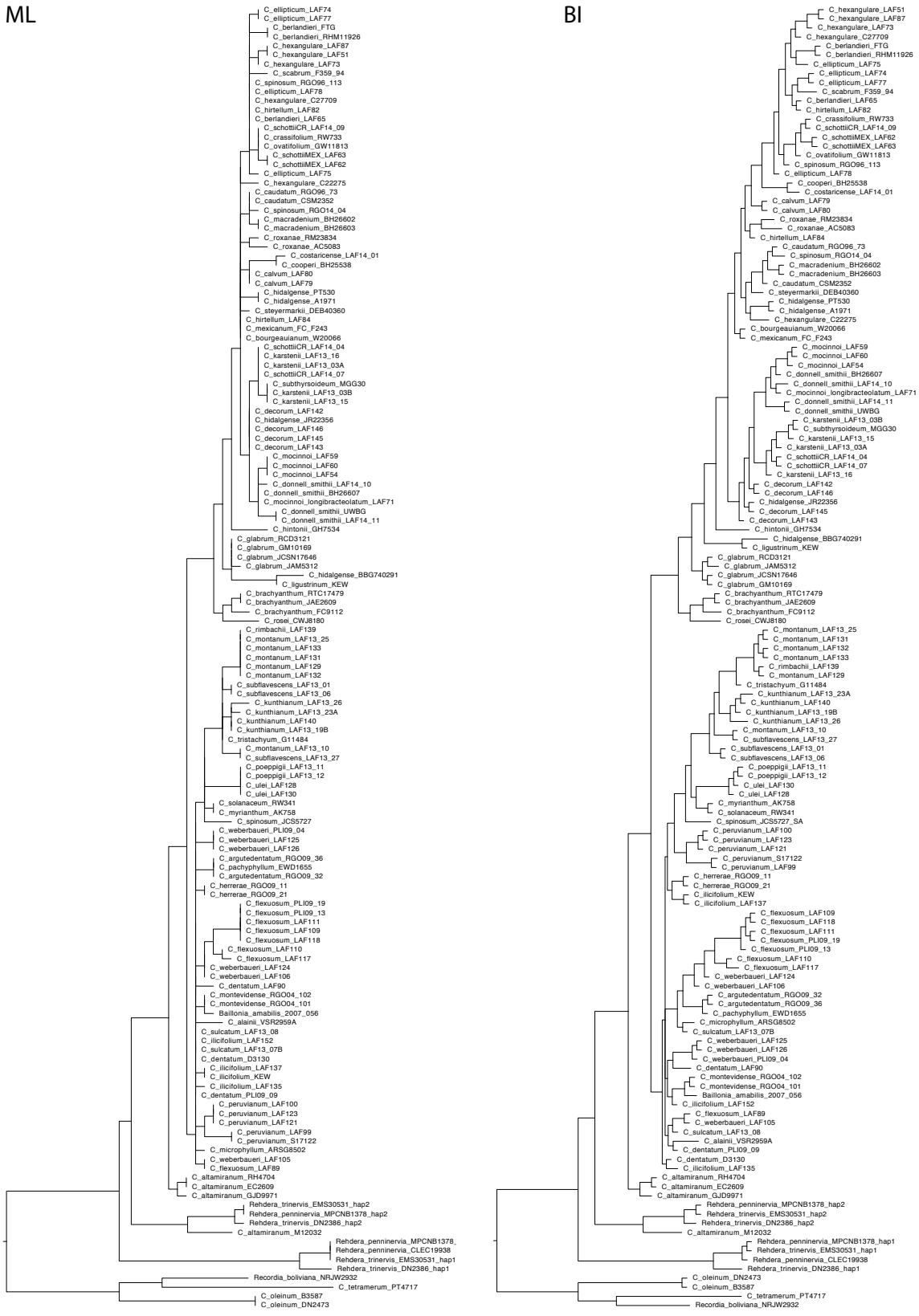


Fig. S3. PPR24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C_hirtellum_LAF82
C_berlandieri_LAF65
C_schottiiCR_LAF14_09
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C_schottiiMEX_LAF62
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C_mexicanum_FC_F243
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Fig. S4. PPR62

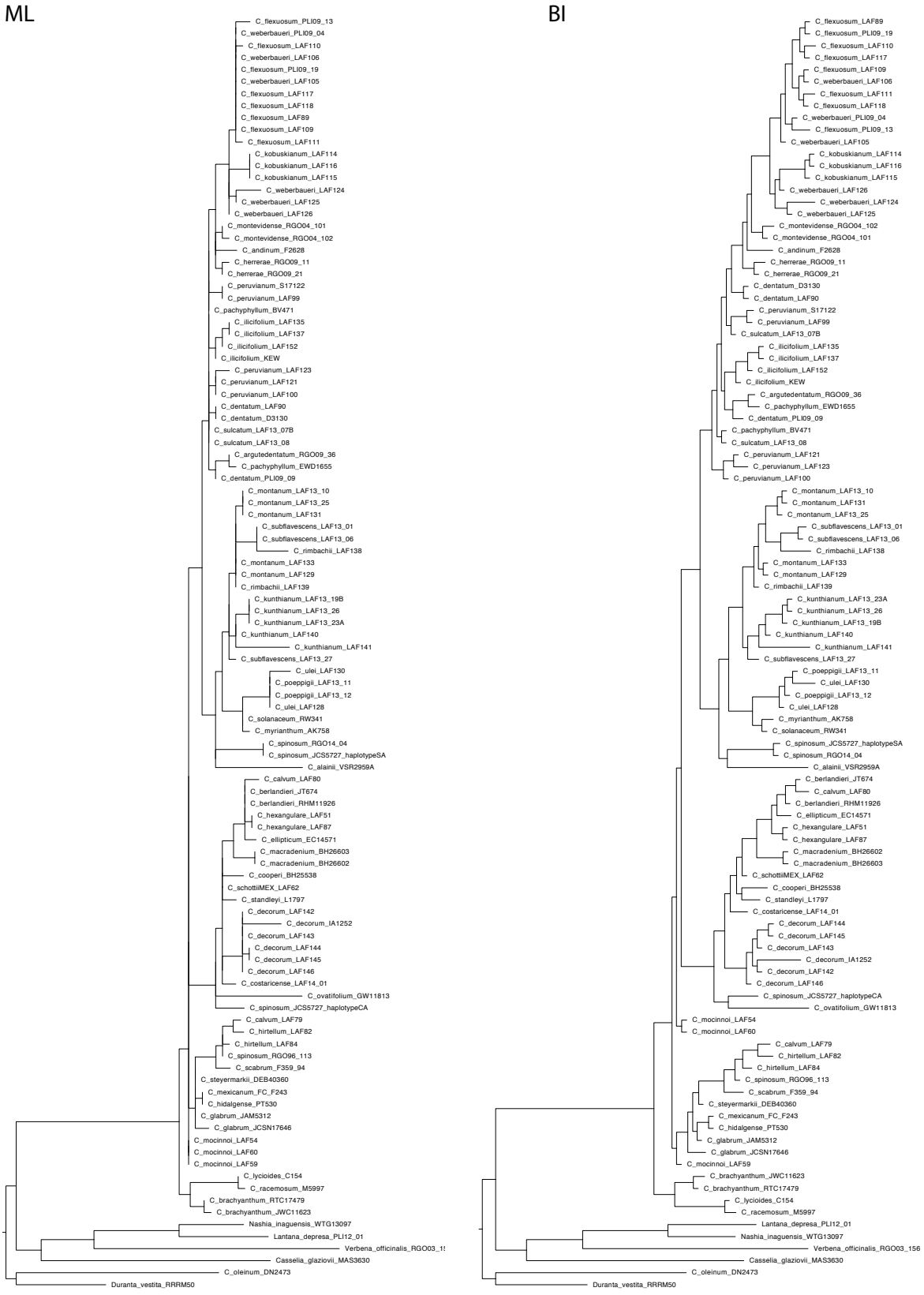
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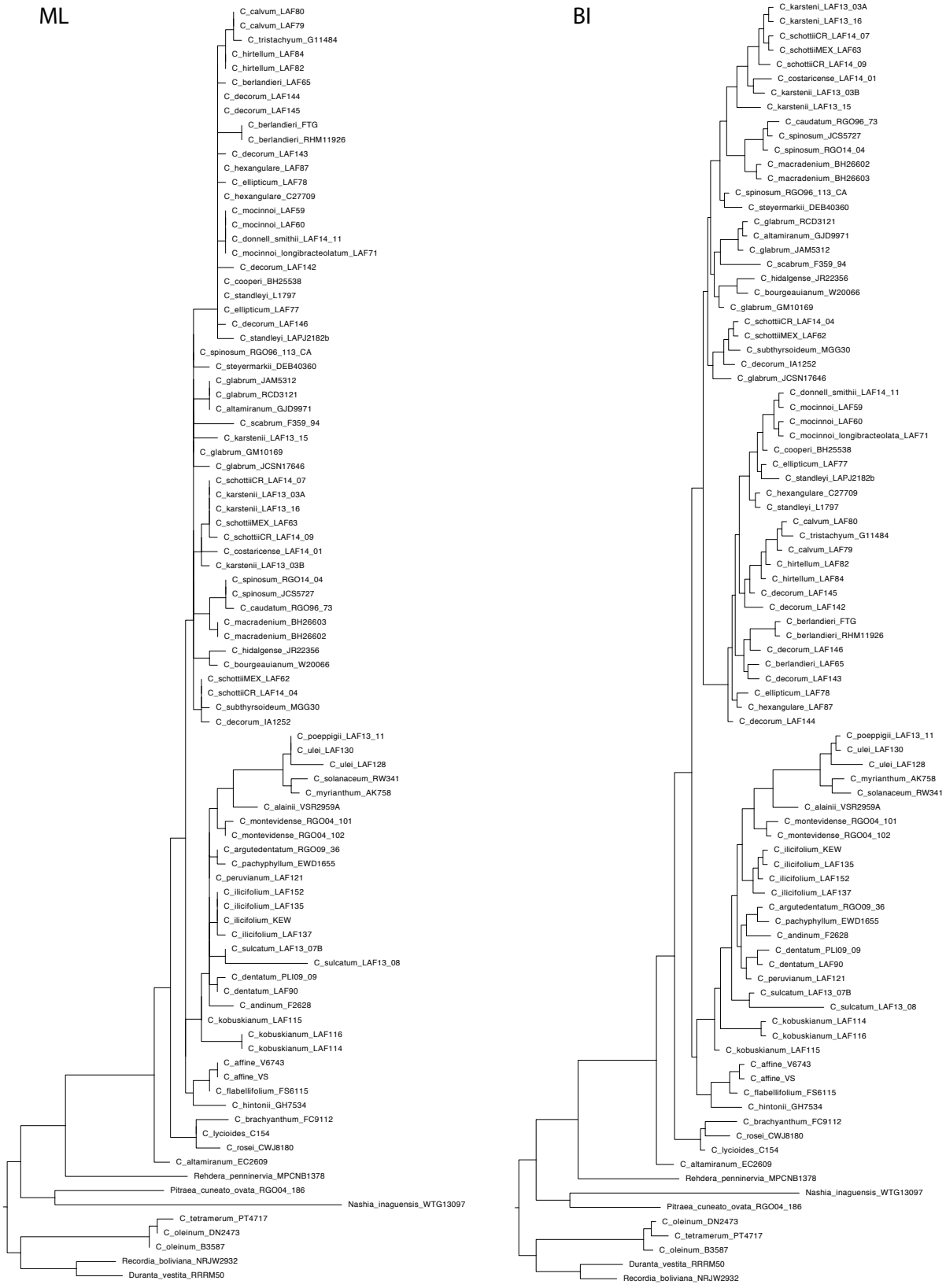
Fig. S5. PPR70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Pitraea_cuneato_ovata_RGO04_186
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C_tetramerum_PT4717
C_oleinum_B3587
Duranta_vestita_RRRM50
Recordia_boliviana_NRJW2932

Fig. S6. PPR123

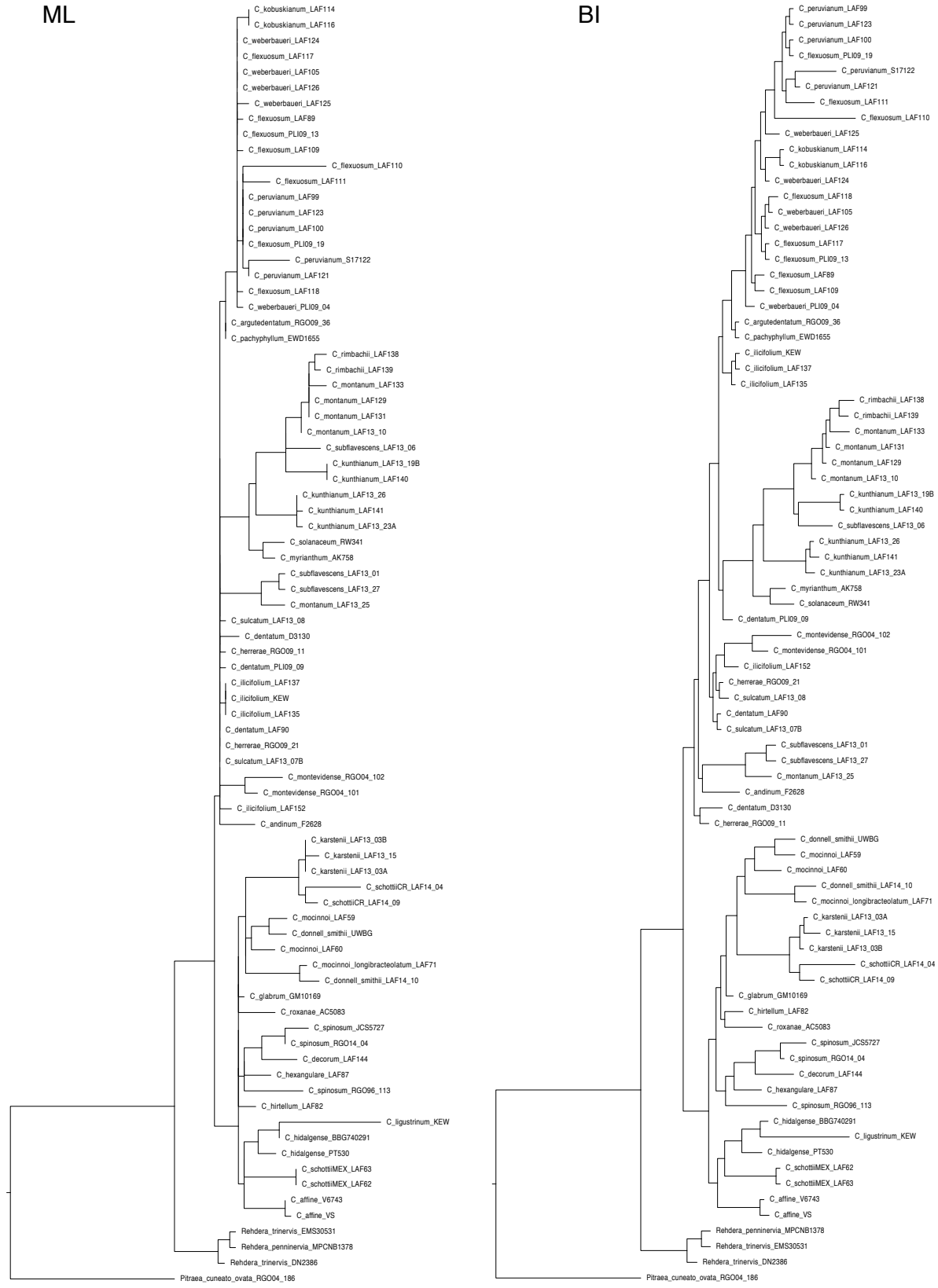


Fig. S7. waxy haplotype 1

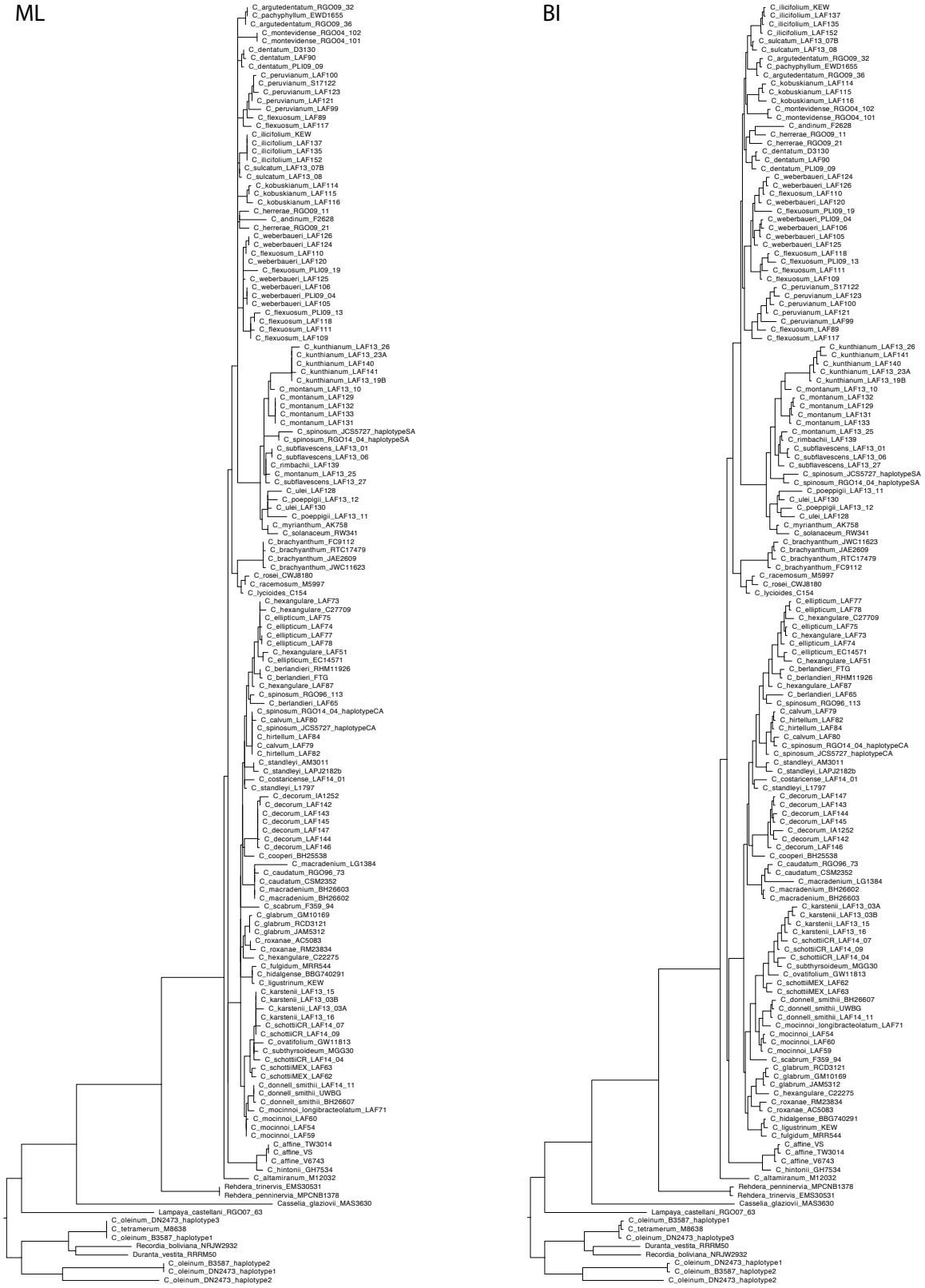


Fig. S8. waxy haplotype 2

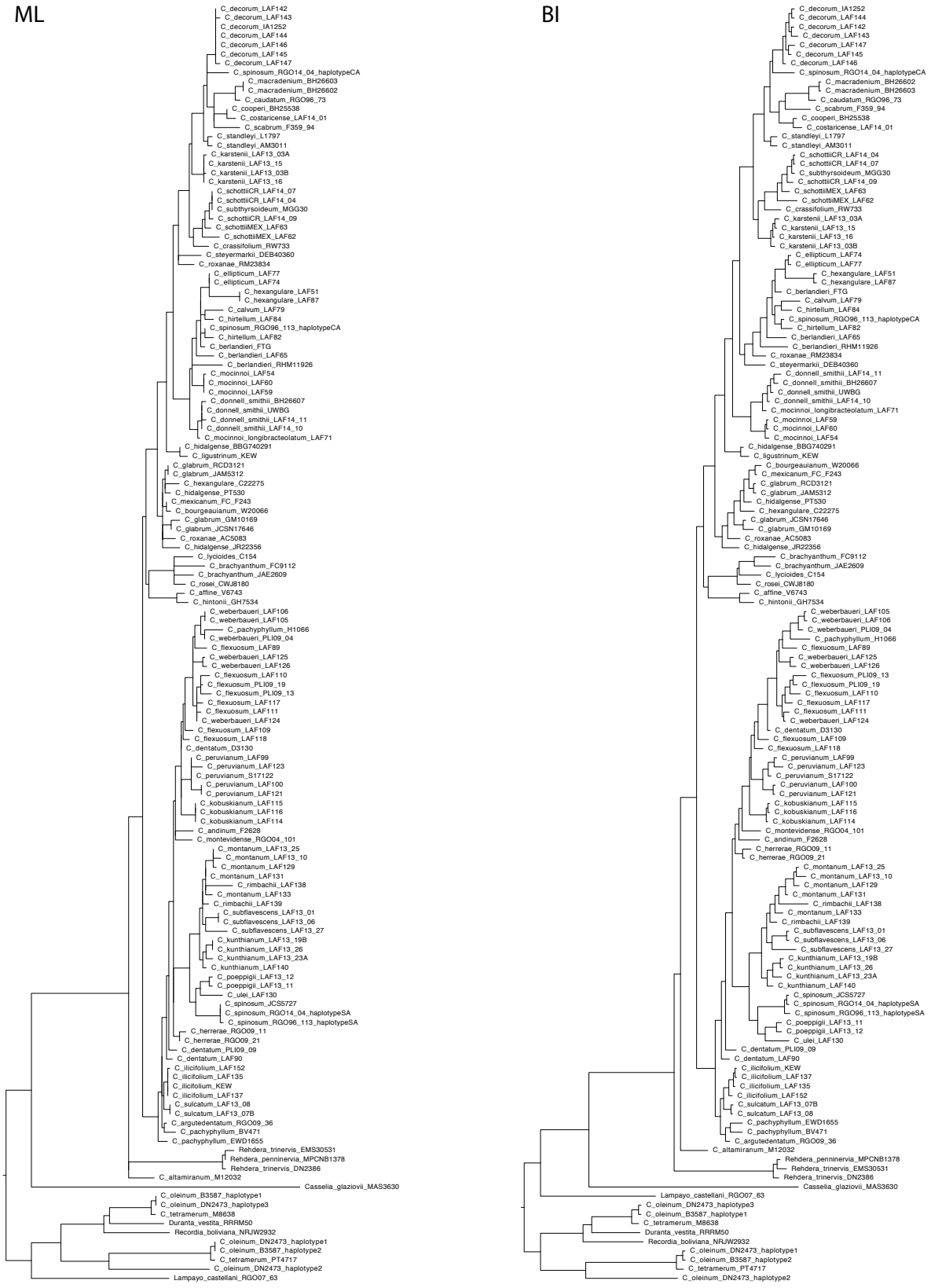


Fig. S9. Thorns

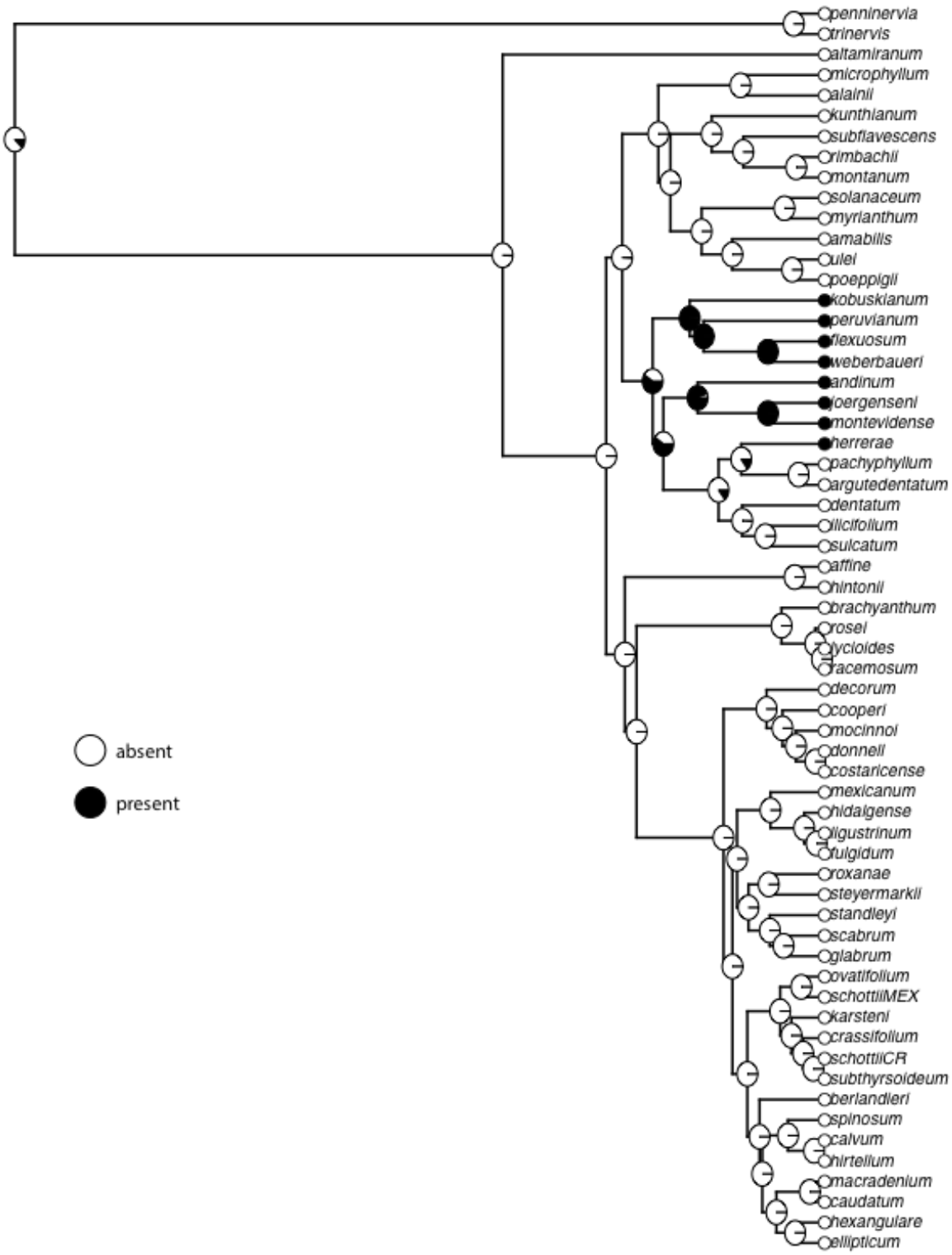


Fig. S10. Terminal inflorescences

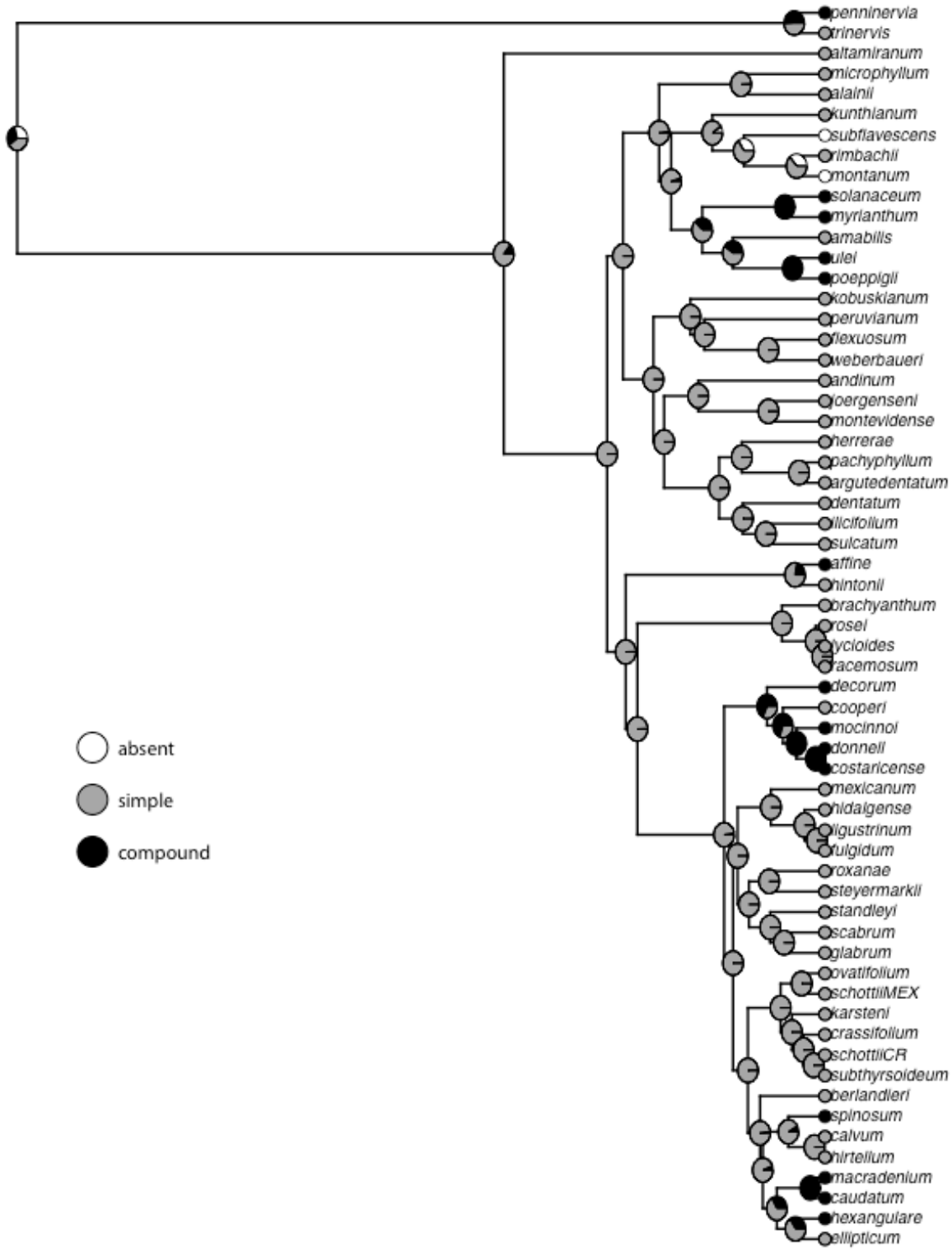


Fig. S11. Axillary inflorescences

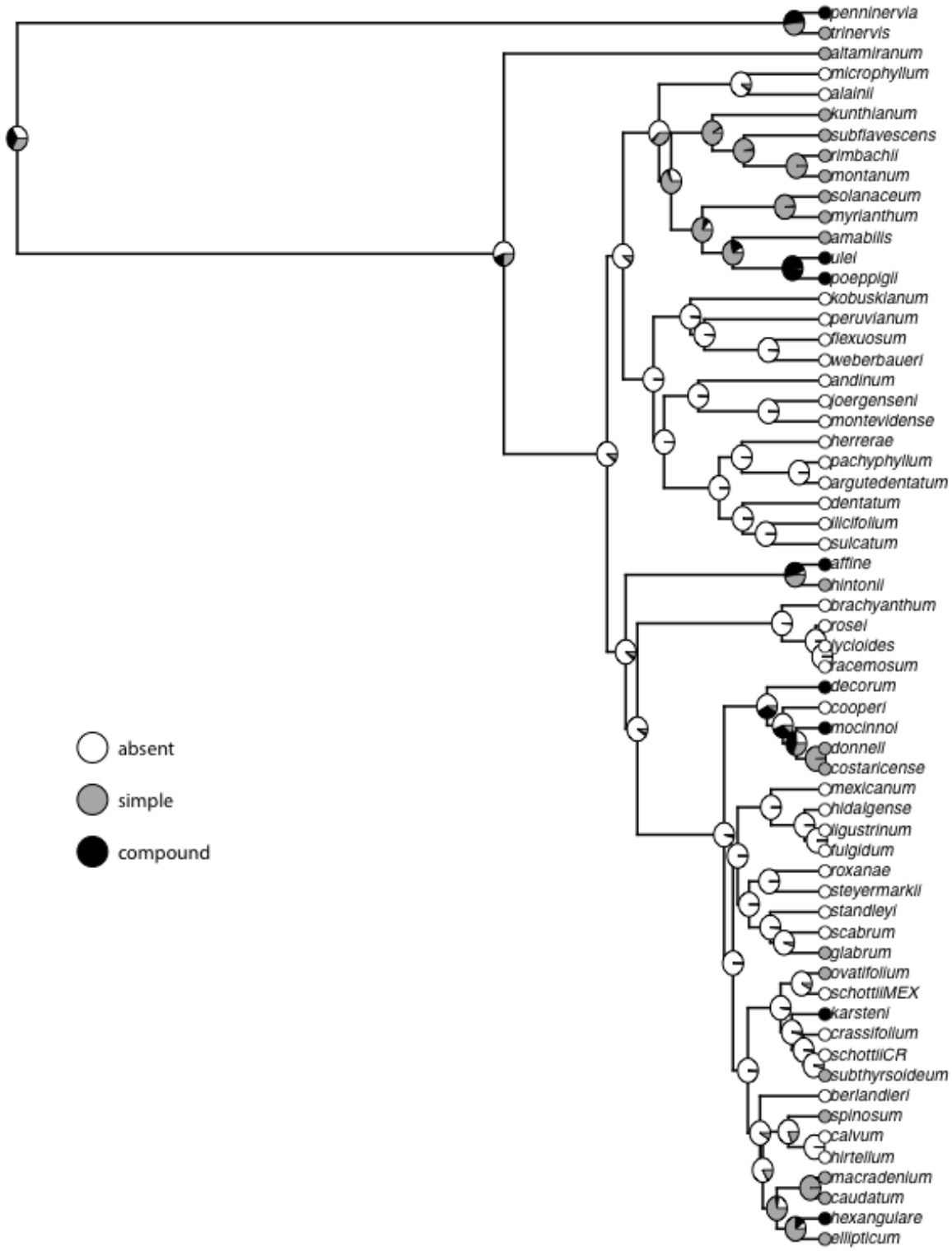


Fig. S12. Flower number

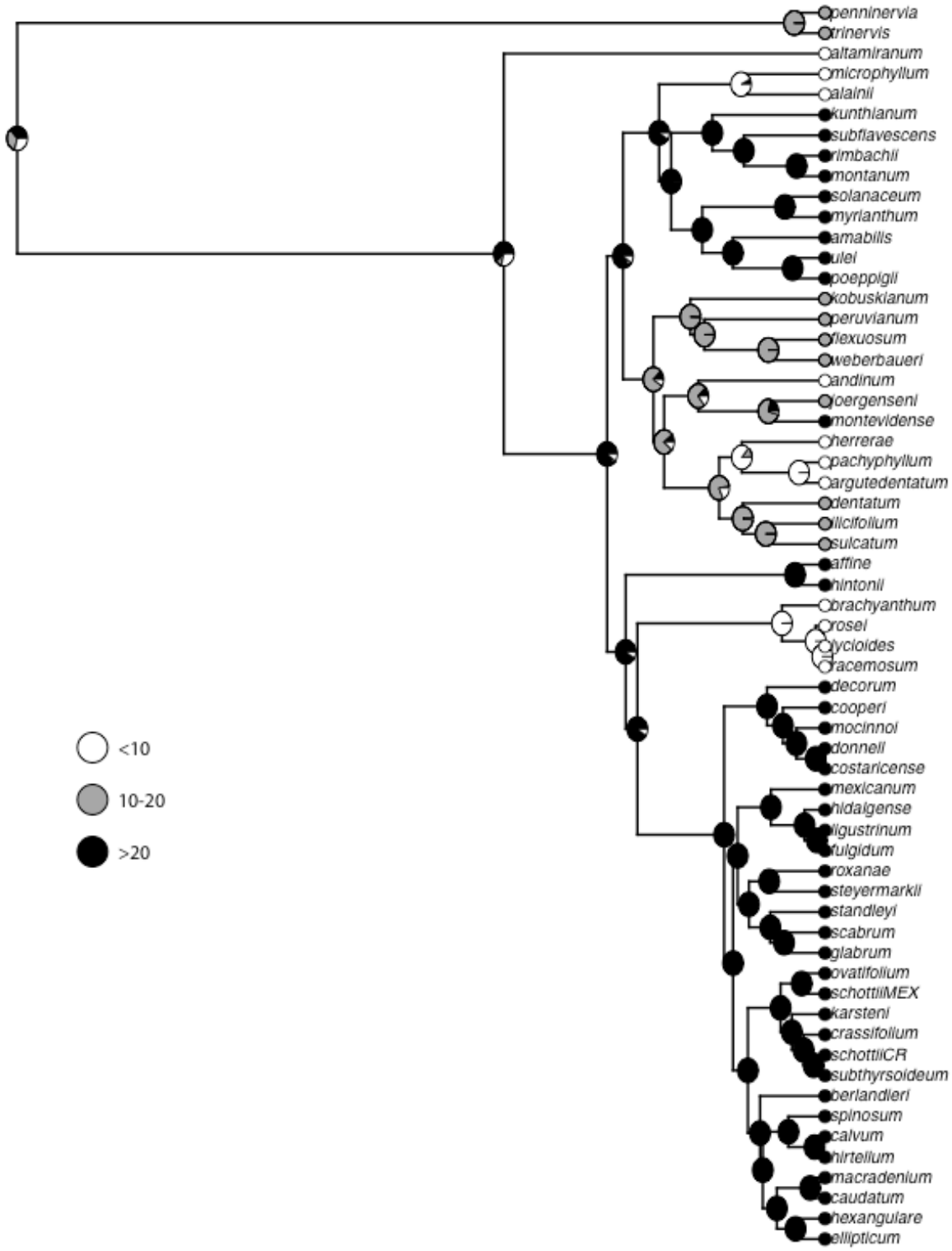


Fig. S13. Flower color

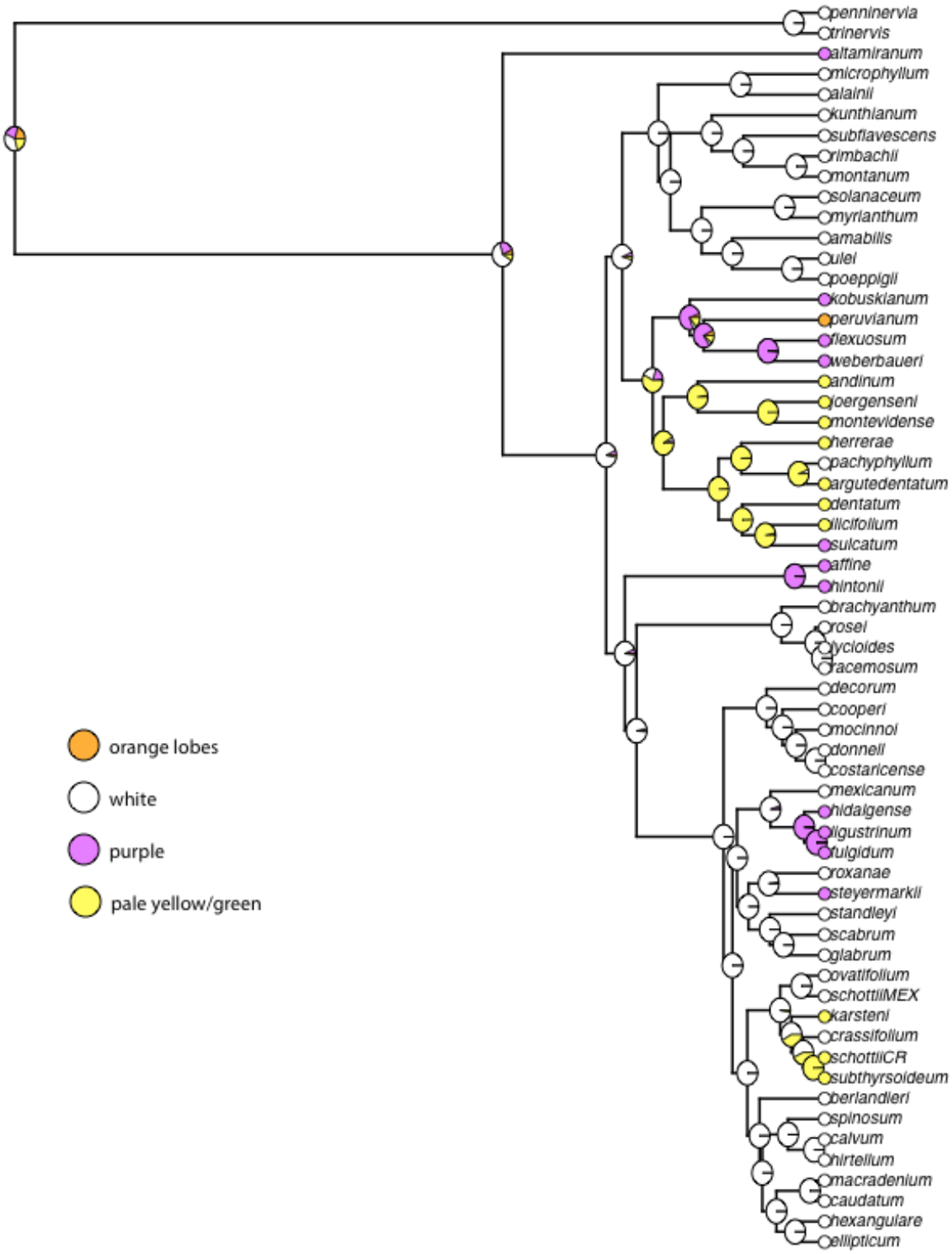
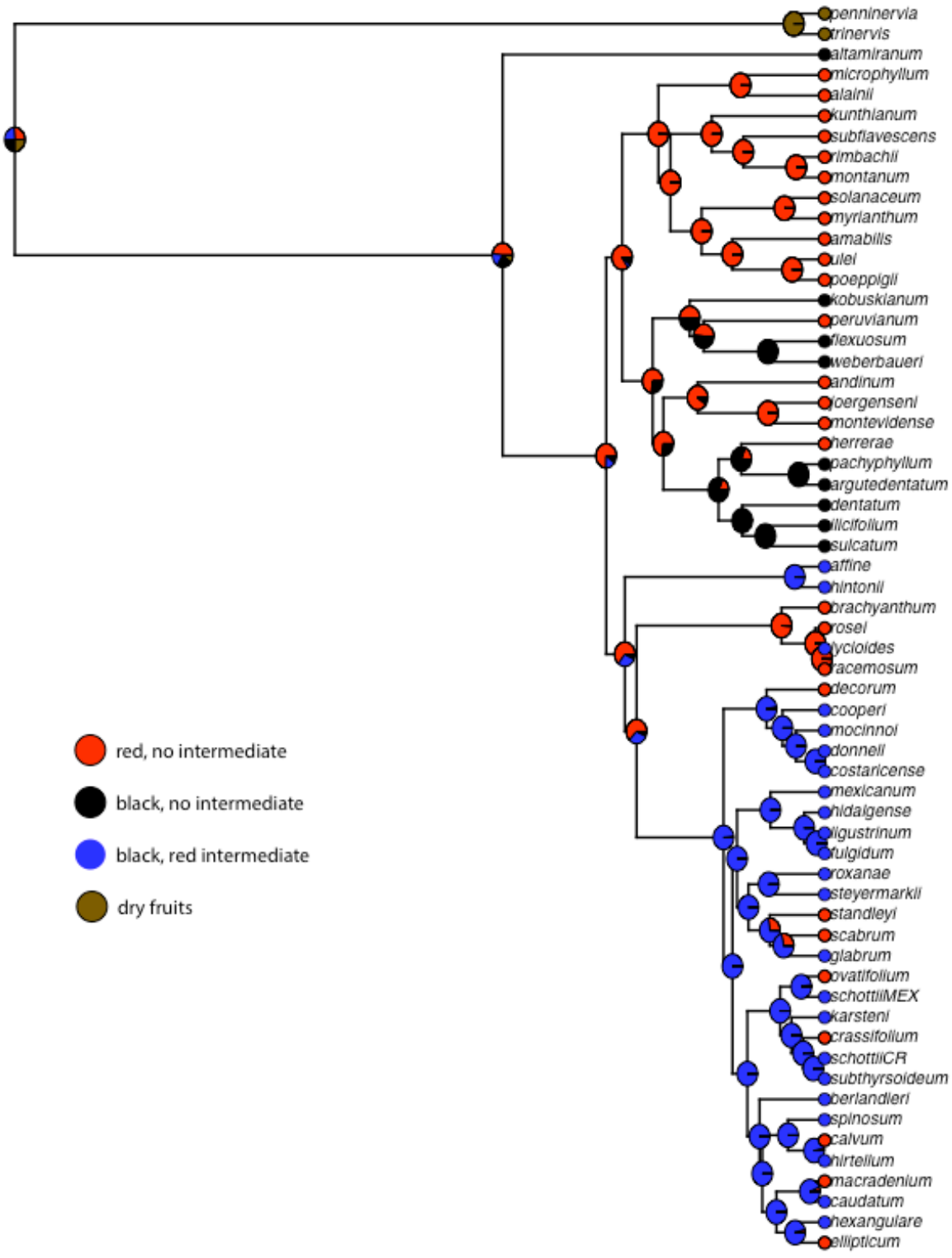


Fig. S14. Fruit color through maturation



Appendix S1. Sequence ID; Taxon (accepted); Locality; Voucher (Herbarium); GenBank accessions: ndhF, rpoC2, trnT-L-F, rbcL, matK, rpl32, ETS, ITS, PPR24, PPR62, PPR70, PPR123, waxy haplotype 1, waxy haplotype 2

Seq ID	Taxon (accepted)	Locality	Voucher (Herbarium)	ndhF	rpoC2	trnT-L-F	rbcL	matK	rpl32	ETS	ITS	PPR24	PPR62	PPR70	PPR123	waxy1	waxy2
Baillonia_amabilis_MC4522	*Baillonia amabilis* Bocq. Ex Baill.	Bolivia: Chiquitos; Feb 1950	M. Cardenas, 4522 (US)	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####					
C_affine_TW3014	*Citharexylum affine* D.Don	Herbarium specimen, Field Museum	T. Wendt et al. 3014 (F)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####					GB#####	
C_affine_V6743	*Citharexylum affine* D.Don	Mexico: Veracruz; 17 May 2010	Villanueva 6743 (MEXU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####	GB#####	GB#####
C_affine_VS	*Citharexylum affine* D.Don	Mexico: Veracruz; 05 April 2005	Velasco-Sinaca (MEXU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####	GB#####	
C_alainii_VSR2959A	*Citharexylum alainii* Moldenke	Dominican Republic: Salcedo; 18 June 2003	Veloz, Salazar, Reyes 2959-A (FTG)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			
C_altamiranum_EC2609	*Citharexylum altamiranum* Greenm.	Mexico: Bajío; 12 August 1990	E. Carranza 2609 (MEXU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####			
C_altamiranum_GJD9971	*Citharexylum altamiranum* Greenm.	Mexico: Zacatecas; 22 Aug 1975	G. and J. Davidse 9971 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####		GB#####			
C_altamiranum_M12032	*Citharexylum altamiranum* Greenm.	Mexico	Magaña 12032	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####				GB#####	GB#####
C_altamiranum_OH9864	*Citharexylum altamiranum* Greenm.	Mexico: Jardin Botanico Regional de Cadereyta; 22 M	Orozoco H. 9864 (MEXU)							GB#####	GB#####						
C_altamiranum_RH4704	*Citharexylum altamiranum* Greenm.	Mexico: Hidalgo; 13 Jul 1980	R. Hernandez 4704 (MO)	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####	GB#####					
C_andinum_F2628	*Citharexylum andinum* Moldenke	Bolivia: Santa Cruz; 02 Oct 2004	E. Fernandez et al 2628 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####	GB#####
C_argutedentatum_RGO09_32	*Citharexylum argutedentatum* Moldenke	Peru: Cusco; 6 Mar 2009	R.G. Olmstead 2009-32 (WTU)	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####	GB#####				GB#####	
C_argutedentatum_RGO09_36	*Citharexylum argutedentatum* Moldenke	Peru: Cusco; 7 Mar 2009	R.G. Olmstead 2009-36 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_berlandieri_JT674	*Citharexylum berlandieri* S.Watson	United States: Florida	J. Trusty 674 (FTG)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_berlandieri_LAF65	*Citharexylum berlandieri* S.Watson	Mexico: Chiapas; 10 Sept 2015	L.A. Frost 65 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####	GB#####
C_berlandieri_RHM11926	*Citharexylum berlandieri* S.Watson	Mexico: Querétaro; 15 July 2001	R.H. Magaña 11926 (MEXU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_bourgeauianum_W20066	*Citharexylum bourgeauianum* Greenm.	Mexico: Puebla; 21 Aug 1975	Webster et al 20066 (HUH)							GB#####	GB#####	GB#####		GB#####			GB#####
C_brachyanthum_FC9112	*Citharexylum brachyanthum* (A.Gray ex Hemsl.) A. Gray	Mexico: Coahuila; 02 Sept 1972	F. Chiang 9112 (F)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####	GB#####
C_brachyanthum_JAE2609	*Citharexylum brachyanthum* (A.Gray ex Hemsl.) A. Gray	Mexico: Nuevo León; 02 August 2008	J.A. Encina 2609 (MEXU)	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####				GB#####	GB#####
C_brachyanthum_JWC11623	*Citharexylum brachyanthum* (A.Gray ex Hemsl.) A. Gray	Mexico: Coahuila; 05 July 1973	Johnston, Wendt, Chiang 11623 (F)	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####		GB#####			GB#####	
C_brachyanthum_RTC17479	*Citharexylum brachyanthum* (A.Gray ex Hemsl.) A. Gray	Mexico: San Luis Potosí; 22 September 2012	R.T. Colín 17479 (MEXU)	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####			GB#####	
C_calvum_MSEC12309	*Citharexylum calvum* Moldenke	Mexico: Quintana Roo; 1 May 1982	M. Sousa S. & E.F. Cabrera C. 12309 (MO)							GB#####							
C_calvum_LAF79	*Citharexylum calvum* Moldenke	Mexico: Quintana Roo; 19 Sept 2015	L.A. Frost 79 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_calvum_LAF80	*Citharexylum calvum* Moldenke	Mexico: Quintana Roo; 19 Sept 2015	L.A. Frost 80 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	
C_caudatum_CSM2352	*Citharexylum caudatum* L.	Dominican Republic: San Juan de la Maguana; 3 Dec	Clase, Santana Montilla 2352 (FTG)	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####				GB#####	
C_caudatum_RGO96_73	*Citharexylum caudatum* L.	Topes de Collantes, Cuba, seed collected; 6 Jul 1996	R.G. Olmstead 1996-73A (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####	GB#####
C_cooperi_BH25538	*Citharexylum cooperi* Standl.	Costa Rica	B. Hammel 25538 (INB)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_costaricense_E626	*Citharexylum costaricense* Moldenke	Costa Rica	Espinoza 626	GB#####		GB#####											
C_costaricense_LAF14_01	*Citharexylum costaricense* Moldenke	Costa Rica: Limon; June 2014	L.A. Frost 14-01 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_costaricense_RL1136	*Citharexylum costaricense* Moldenke	Costa Rica: Puntarena; 16 Jan 1977	R. Lawton 1136 (F)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####						
C_crassifolium_RW733	*Citharexylum crassifolium* Greenm.	Honduras: San Marcos; 15 Aug 1971	R. Wanderlin et al. 733 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####					GB#####
C_decorum_IA1252	*Citharexylum decorum* Moldenke	Ecuador: Galapagos; 18 May 1999	I. Aldoz 1252 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####		GB#####	GB#####
C_decorum_LAF142	*Citharexylum decorum* Moldenke	Ecuador: Santo Domingo de los Tsachilas; 08 Oct 201	L.A. Frost 142 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_decorum_LAF143	*Citharexylum decorum* Moldenke	Ecuador: Manabi; 10 Oct 2016	L.A. Frost 143 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_decorum_LAF144	*Citharexylum decorum* Moldenke	Ecuador: Manabi; 10 Oct 2016	L.A. Frost 144 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####		GB#####	GB#####
C_decorum_LAF145	*Citharexylum decorum* Moldenke	Ecuador: Manabi; 10 Oct 2016	L.A. Frost 145 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_decorum_LAF146	*Citharexylum decorum* Moldenke	Ecuador: Manabi; 10 Oct 2016	L.A. Frost 146 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_decorum_LAF147	*Citharexylum decorum* Moldenke	Ecuador: Tungurahua; 13 Oct 2016	L.A. Frost 147 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####					GB#####	GB#####
C_dentatum_BS2014	*Citharexylum dentatum* D.Don	Peru: Ancash; 28 Jan 1985	B. Stein et al. 2014 (F)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####						
C_dentatum_D3130	*Citharexylum dentatum* D.Don	Peru: Ancash, Yungay; 28 Jan1983	Dillon et al. 3130 (F)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_dentatum_LAF90	*Citharexylum dentatum* D.Don	Peru: Ancash; 01 Sept 2016	L.A. Frost 090 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_dentatum_PLI09_09	*Citharexylum dentatum* D.Don	Peru	P. Lu-Irving 2009-09 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_donnel_smithii_RCD3121	*Citharexylum donnell-smithii* Greenm.	Mexico: Guerrero; 05 October 1998 (or May 10?)	R.C. Durán 3121 (MEXU)													GB#####	
C_donnell_smithii_BH26607	*Citharexylum donnell-smithii* Greenm.	Costa Rica	B. Hammel 26607 (INB)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####				GB#####	GB#####
C_donnell_smithii_LAF14_10	*Citharexylum donnell-smithii* Greenm.	Costa Rica: Alajuela; 19 July 2014	L.A. Frost 14-10 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####			GB#####		GB#####
C_donnell_smithii_LAF14_11	*Citharexylum donnell-smithii* Greenm.	Costa Rica: San Jose; 20 July 2014	L.A. Frost 14-11 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####	GB#####
C_donnell_smithii_UWBG	*Citharexylum donnell-smithii* Greenm.	United States: Washington (in cultivation)	cultivated UWBG, seed from CR	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####	GB#####
C_ellipticum_EC14571	*Citharexylum ellipticum* Moc. & Sessé ex D.Don	Mexico, Campeche; 22 Sept 1987	E. Cabrera 14571 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####			GB#####	
C_ellipticum_LAF74	*Citharexylum ellipticum* Moc. & Sessé ex D.Don	Mexico: Campeche; 18 Sept 2015	L.A. Frost 74 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####				GB#####	GB#####
C_ellipticum_LAF75	*Citharexylum ellipticum* Moc. & Sessé ex D.Don	Mexico: Campeche; 18 Sept 2015	L.A. Frost 75 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####				GB#####	
C_ellipticum_LAF77	*Citharexylum ellipticum* Moc. & Sessé ex D.Don	Mexico: Campeche; 18 Sept 2015	L.A. Frost 77 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####	GB#####
C_ellipticum_LAF78	*Citharexylum ellipticum* Moc. & Sessé ex D.Don	Mexico: Campeche; 18 Sept 2015	L.A. Frost 78 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####		GB#####		GB#####	
C_flabellifolium_FS6115	*Citharexylum flabellifolium* S.Watson	Mexico: Sonora; 27 Feb 1933	F. Shreve 6115 (F)								GB#####			GB#####			
C_flexuosum_LAF109	*Citharexylum flexuosum* (Ruiz & Pav.) D.Don	Peru: Cajamarca; 12 Sept 2016	L.A. Frost 109 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_flexuosum_LAF110	*Citharexylum flexuosum* (Ruiz & Pav.) D.Don	Peru: Cajamarca; 12 Sept 2016	L.A. Frost 110 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_flexuosum_LAF111	*Citharexylum flexuosum* (Ruiz & Pav.) D.Don	Peru: Cajamarca; 12 Sept 2016	L.A. Frost 111 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_flexuosum_LAF117	*Citharexylum flexuosum* (Ruiz & Pav.) D.Don	Peru: Cajamarca; 14 Sept 2016	L.A. Frost 117 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_flexuosum_LAF118	*Citharexylum flexuosum* (Ruiz & Pav.) D.Don	Peru: Cajamarca; 14 Sept 2016	L.A. Frost 118 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_flexuosum_LAF89	*Citharexylum flexuosum* (Ruiz & Pav.) D.Don	Peru: Ancash; 31 Aug 2016	L.A. Frost 089 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_flexuosum_PLI09_13	*Citharexylum flexuosum* (Ruiz & Pav.) D.Don	Peru	P. Lu-Irving 2009-13 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_flexuosum_PLI09_19	*Citharexylum flexuosum* (Ruiz & Pav.) D.Don	Peru	P. Lu-Irving 2009-19 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	
C_fulgidum_MRR544	*Citharexylum fulgidum* Moldenke	Mexico: Veracruz; 29 July 1967	M. Rosas R. 544 (HUH-Arnold Arboretum)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####					GB#####	GB#####
C_glabrum_GM10169	*Citharexylum glabrum* (S.Watson) Greenm.	Mexico: Oaxaca; 08 November 2012	Garcia-Mendoza 10169 (MEXU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####
C_glabrum_JAM5312	*Citharexylum glabrum* (S.Watson) Greenm.	Mexico, Jalisco; 26 Dec 1986	J.A. Machuca 5312 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####
C_glabrum_JCSN17646	*Citharexylum glabrum* (S.Watson) Greenm.	Mexico: Guerrero; 22 January 2010	J.C. Soto N. 17646 (MEXU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####
C_glabrum_RCD3121	*Citharexylum glabrum* (S.Watson) Greenm.	Mexico: Guerrero; 05 October 1998 (or May 10?)	R.C. Durán 3121 (MEXU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####			
C_guatemalense_IC1827	*Citharexylum guatemalense* (Moldenke) D.N.Gibson	Nicaragua: Jinotega; 27 June 2005	I. Coronado et al. 1827 (MO)	GB#####	GB#####		GB#####			GB#####	GB#####						
C_herrerae_RGO09_11	*Citharexylum herrerae* Mansf.	Peru: Apurimac; 3 Mar 2009	R.G. Olmstead 2009-11 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_herrerae_RGO09_21	*Citharexylum herrerae* Mansf.	Peru: Apurimac; 5 Mar 2009	R.G. Olmstead 2009-21 WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_hexangulare_C22275	*Citharexylum hexangulare* Greenm.	Mexico: Juxtlahuaca; 22 October 1997	Calzada 22275 (MEXU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####				GB#####	GB#####
C_hexangulare_C27709	*Citharexylum hexangulare* Greenm.	Mexico: Veracruz; 11 September 2011	Chagata 27709 (MEXU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####	
C_hexangulare_LAF51	*Citharexylum hexangulare* Greenm.	Mexico: Veracruz; 30 Aug 2015	L.A. Frost 51 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####
C_hexangulare_LAF73	*Citharexylum hexangulare* Greenm.	Mexico: Tabasco; 18 Sept 2015	L.A. Frost 73 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####				GB#####	
C_hexangulare_LAF87	*Citharexylum hexangulare* Greenm.	Mexico: Chiapas; 21 Sept 2015	L.A. Frost 87 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_hidalgense_A1971	*Citharexylum hidalgense* Moldenke	Mexico: Hidalgo, Eloxochitlán; 22 Apr 1995	Ayala 1971	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####				GB#####	GB#####
C_hidalgense_BBG740291	*Citharexylum hidalgense* Moldenke	United States: California (in cultivation)	BBG	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####	
C_hidalgense_JR22356	*Citharexylum hidalgense* Moldenke	Mexico: Mexico; 15 May 1966	J. Rzedowski 22356 (F)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####			GB#####
C_hidalgense_PT530	*Citharexylum hidalgense* Moldenke	Mexico, Hidalgo; 4 June 1982	P. Tenorio 530 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####
C_hidalgense_R2603	*Citharexylum hidalgense* Moldenke	Mexico	Rincon 2603							GB#####							
C_hintonii_GH7534	*Citharexylum hintonii* Moldenke	Mexico, Mexico; 19 March 7534	G. Hinton 7531 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####			GB#####
C_hirtellum_LAF82	*Citharexylum hirtellum* Standl.	Mexico: Quintana Roo; 20 Sept 2015	L.A. Frost 82 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_hirtellum_LAF84	*Citharexylum hirtellum* Standl.	Mexico: Quintana Roo; 20 Sept 2015	L.A. Frost 84 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_ilicifolium_KEW	*Citharexylum ilicifolium* Kunth	England: Royal Botanical Gardens Kew		GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_ilicifolium_LAF135	*Citharexylum ilicifolium* Kunth	Ecuador: Pichincha; 04 Oct 2016	L.A. Frost 135 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_ilicifolium_LAF137	*Citharexylum ilicifolium* Kunth	Ecuador: Pichincha; 04 Oct 2016	L.A. Frost 137 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_ilicifolium_LAF152	*Citharexylum ilicifolium* Kunth	Ecuador: Azuay; 18 Oct 2016	L.A. Frost 152 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_joergenseni_EF1711	*Citharexylum joergensenii* (Lillo) Moldenke	Bolivia: Cochabamba; 14 June 2003	E. Fernandez et al. 1711 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####						
C_karsteni_LAF13_03A	*Citharexylum karstenii* Moldenke	Colombia: Cundinamarca; 21 Sept 2013	L.A. Frost 13-03 A (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####
C_karsteni_LAF13_03B	*Citharexylum karstenii* Moldenke	Colombia: Cundinamarca; 21 Sept 2013	L.A. Frost 13-03 B (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####
C_karsteni_LAF13_15	*Citharexylum karstenii* Moldenke	Colombia: Cundinamarca; 25 Sept 2013	L.A. Frost 13-15 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####
C_karsteni_LAF13_16	*Citharexylum karstenii* Moldenke	Colombia: Cundinamarca; 25 Sept 2013	L.A. Frost 13-16 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####	GB#####
C_kobuskianum_LAF114	*Citharexylum kobuskianum* Moldenke	Peru: Cajamarca; 13 Sept 2016	L.A. Frost 114 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####	GB#####
C_kobuskianum_LAF115	*Citharexylum kobuskianum* Moldenke	Peru: Cajamarca; 13 Sept 2016	L.A. Frost 115 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####		GB#####	GB#####
C_kobuskianum_LAF116	*Citharexylum kobuskianum* Moldenke	Peru: Cajamarca; 13 Sept 2016	L.A. Frost 116 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####	GB#####
C_kunthianum_LAF13_19B	*Citharexylum kunthianum* Moldenke	Colombia: Antioquia; 30 Sept 2013	L.A. Frost 13-19 B (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_kunthianum_LAF13_23A	*Citharexylum kunthianum* Moldenke	Colombia: Valle del Cauca; 08 Oct 2013	L.A. Frost 13-23 A (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_kunthianum_LAF13_26	*Citharexylum kunthianum* Moldenke	Colombia: Valle del Cauca; 09 Oct 2013	L.A. Frost 13-26 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_kunthianum_LAF140	*Citharexylum kunthianum* Moldenke	Ecuador: Imbabura; 06 Oct 2016	L.A. Frost 140 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_kunthianum_LAF141	*Citharexylum kunthianum* Moldenke	Ecuador: Imbabura; 06 Oct 2016	L.A. Frost 141 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####	GB#####	
C_ligustrinum_KEW	*Citharexylum ligustrinum* Van Houtte	England: Royal Botanical Gardens Kew		GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####	GB#####
C_lycioides_C154	*Citharexylum lycioides* D.Don	Mexico: Querétaro; 28 Aug 2007	Chávez et al. rjchm 154	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####		GB#####	GB#####
C_lycioides_S955	*Citharexylum lycioides* D.Don	Herbarium specimen, Field Museum	Spellman et al. 955 (F)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####						
C_macradenium_H26602_209	*Citharexylum macradenium* Greenm.	Costa Rica	B. Hammel 26602 (INB)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_macradenium_H26602_210	*Citharexylum macradenium* Greenm.	Costa Rica	B. Hammel 26603 (INB)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_macradenium_LG1384	*Citharexylum macradenium* Greenm.	Costa Rica	L. Gonzalez 1384													GB#####	
C_mexicanum_FC_F243	*Citharexylum mexicanum* Moldenke	Mexico: Puebla; 31 Aug 1979	F. Chiang et al. F-243 (MO, MEXU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####				GB#####
C_mexicanum_GP2165	*Citharexylum mexicanum* Moldenke	Mexico: Veracruz; 28 July 1971	Gomez Pompa 2165 (MEXU)							GB#####							
C_mexicanum_S625	*Citharexylum mexicanum* Moldenke	Mexico	Salinas 625	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####						
C_microphyllum_ARSG8502	*Citharexylum microphyllum* (DC.) O.E.Schulz	Dominican Republic: Peravia; 22 Sept 1996	Acasado-Rdgz., Siaca, Garcia 8502 (FTG)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####					
C_microphyllum_RGNR4084	*Citharexylum microphyllum* (DC.) O.E.Schulz	Dominican Republic: Isla Saona; 24 July 1992	R. Garcia, N. Ramirez 4084 (F)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####						
C_mocinnoi_LAF54	*Citharexylum mocinnoi* D.Don	Mexico: Veracruz; 01 Sept 2015	L.A. Frost 54 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####
C_mocinnoi_LAF59	*Citharexylum mocinnoi* D.Don	Mexico: Oaxaca; 05 Sept 2015	L.A. Frost 59 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_mocinnoi_LAF60	*Citharexylum mocinnoi* D.Don	Mexico: Oaxaca; 05 Sept 2015	L.A. Frost 60 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_mocinnoi_longibracteolata_LAF71	*Citharexylum mocinnoi var. longibracteolatum* Moldenke	Mexico: Chiapas; 14 Sept 2015	L.A. Frost 71 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####
C_montanum_LAF129	*Citharexylum montanum* Moldenke	Ecuador: Napo; 02 Oct 2016	L.A. Frost 129 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_montanum_LAF13_10	*Citharexylum montanum* Moldenke	Colombia: Cundinamarca; 22 Sept 2013	L.A. Frost 13-10 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_montanum_LAF13_25	*Citharexylum montanum* Moldenke	Colombia: Valle del Cauca; 08 Oct 2013	L.A. Frost 13-25 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_montanum_LAF131	*Citharexylum montanum* Moldenke	Ecuador: Napo; 03 Oct 2016	L.A. Frost 131 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_montanum_LAF132	*Citharexylum montanum* Moldenke	Ecuador: Napo; 03 Oct 2016	L.A. Frost 132 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####				GB#####	
C_montanum_LAF133	*Citharexylum montanum* Moldenke	Ecuador: Napo; 03 Oct 2016	L.A. Frost 133 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_montevidense_RGO04_101	*Citharexylum montevidense* (Spreng.) Moldenke	Argentina: Buenos Aires; 1 Dec 2004	R.G. Olmstead 2004-101 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_montevidense_RGO04_102	*Citharexylum montevidense* (Spreng.) Moldenke	Argentina: Buenos Aires; 1 Dec 2004	R.G. Olmstead 2004-102 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	
C_myrianthum_AK758	*Citharexylum myrianthum* Cham.	Brazil: Rio Grande do Sul; 30 Jan 2000	A. Kegler 578 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	
C_oleinum_B3587	*Citharexylum oleinum* (Benth. ex Lindl.) Moldenke	Mexico: San Luis Potosi; 28 Oct 1985	Bartholomew et al. 3587 (HUH-Gray)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####	GB#####
C_oleinum_DN2473	*Citharexylum oleinum* (Benth. ex Lindl.) Moldenke	Mexico: San Luis Potosi; 22 June 1982	Diggs & Nee 2473 (F)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_ovatifolium_GW11813	*Citharexylum ovatifolium* Greenm.	Guatemala: Sacatepequez; 27 Jan 1962	G. Webster et al. 11813 (F)	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####	GB#####	GB#####			GB#####	
C_ovatifolium_MY00781	*Citharexylum ovatifolium* Greenm.	Mexico: Tamaulipas; 13 May 1986	M. Yánez 00781 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####							
C_pachyphyllum_BV471	*Citharexylum pachyphyllum* Moldenke	Bolivia: Cochabamba; 5 Jan 1968	B. Vuilleumier 471 (HUH)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####				GB#####
C_pachyphyllum_EWD1655	*Citharexylum pachyphyllum* Moldenke	Peru: Cusco; 20 Jan 1982	E.W. Davis et al. 1655 (F)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_pachyphyllum_H1066	*Citharexylum pachyphyllum* Moldenke	Peru: Cajamarca; 2 Dec 1974	Hudson 1066 (MO)							GB#####	GB#####						GB#####
C_peruvianum_LAF100	*Citharexylum peruvianum* N. O'Leary	Peru: La Libertad; 09 Sept 2016	L.A. Frost 100 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_peruvianum_LAF121	*Citharexylum peruvianum* N. O'Leary	Peru: Cajamarca; 17 Septt 2016	L.A. Frost 121 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_peruvianum_LAF123	*Citharexylum peruvianum* N. O'Leary	Peru: Cajamarca; 17 Septt 2016	L.A. Frost 123 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_peruvianum_LAF99	*Citharexylum peruvianum* N. O'Leary	Peru: La Libertad; 09 Sept 2016	L.A. Frost 99 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_peruvianum_S17122	*Citharexylum peruvianum* N. O'Leary	Peru: Cajamarca: Gran Chium; 7 Nov. 2002	Segasteguii 17122 (USM)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_poeppigii_LAF13_11	*Citharexylum poeppigii* Walp.	Colombia: Meta; 23 Sept 2013	L.A. Frost 13-11 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_poeppigii_LAF13_12	*Citharexylum poeppigii* Walp.	Colombia: Meta; 23 Sept 2013	L.A. Frost 13-12 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####
C_racemosum_M5997	*Citharexylum racemosum* Sessé & Moc.	Mexico	Martinez 5997	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####		GB#####			GB#####	
C_reticulatum_AA581	*Citharexylum reticulatum* Kunth	Bolivia: La Paz; 20 Jun 2003	A. Araijo et al. 581 (MO)							GB#####							
C_rimbachii_LAF138	*Citharexylum rimbachii* Moldenke	Ecuador: Imbabura; 05 Oct 2016	L.A. Frost 138 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####		GB#####
C_rimbachii_LAF139	*Citharexylum rimbachii* Moldenke	Ecuador: Imbabura; 05 Oct 2016	L.A. Frost 139 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_rosei_CWJ8180	*Citharexylum rosei* Greenm.	Mexico: San Luis Potosi; 30 June 1972	Chiang, Wendt, Johnston 8180 (F)	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####		GB#####		GB#####	GB#####
C_roxanae_AC5083	*Citharexylum roxanae* Moldenke	Mexico: Baja California Sur; 02 Oct 1965	A. Carter 5083 (F)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####	GB#####
C_roxanae_RM23834	*Citharexylum roxanae* Moldenke	Mexico: Baja California; 24 Nov 1976	R. Moran 23834 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####				GB#####	GB#####
C_scabrum_F359_94	*Citharexylum scabrum* Moc. & Sessé ex D.Don	Mexico	Friedman 359-94	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_schottiiCR_LAF14_04	*Citharexylum schottii* Greenm.	Costa Rica: Guanacaste; 18 Jul 2014	L.A. Frost 14-04 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####
C_schottiiCR_LAF14_07	*Citharexylum schottii* Greenm.	Costa Rica: Guanacaste; 18 Jul 2014	L.A. Frost 14-07 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####	GB#####
C_schottiiCR_LAF14_09	*Citharexylum schottii* Greenm.	Costa Rica: Guanacaste; 18 Jul 2014	L.A. Frost 14-09 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####
C_schottiiMEX_LAF62	*Citharexylum schottii* Greenm.	Mexico: Oaxaca; 08 Sept 2015	L.A. Frost 62 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_schottiiMEX_LAF63	*Citharexylum schottii* Greenm.	Mexico: Oaxaca; 08 Sept 2015	L.A. Frost 63 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####
C_solanaceum_RW341	*Citharexylum solanaceum* Cham.	Brazil: Rio Grande do Sul; 11 Dec 1999	R. Wasum 341 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	
C_spinosum_JCS5727	*Citharexylum spinosum* L.	USA: Puerto Rico, 31 Dec, 1980	J.C. Solomon 5727 (TEX)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_spinosum_RGO14_04	*Citharexylum spinosum* L.	Argentina: Buenos Aires?; 27 Mar 2014	R.G. Olmstead 2014-04 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####

Appendix 1 (cont.). Sequence ID; Taxon (accepted); Locality; Voucher (Herbarium); GenBank accessions: ndhF, rpoC2, trnT-L-F, rbcL, matK, rpl32, ETS, ITS, PPR24, PPR62, PPR70, PPR123, waxy haplotype 1, waxy haplotype 2

C_spinosum_RGO96_113	*Citharexylum spinosum* L.	Cuba: Pinar del Rio (from seed); 17 July 1996	R.G. Olmstead 1996-113 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_standleyi_AM3011	*Citharexylum standleyi* Moldenke	Mexico: Jalisco; 23 July 1981	A. Magallanes 3011 (F)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####				GB#####	GB#####
C_standleyi_L1797	*Citharexylum standleyi* Moldenke	Mexico: Jalisco; 08 Aug 1983	Lott 1796	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####		GB#####	GB#####
C_standleyi_LAPJ2182b	*Citharexylum standleyi* Moldenke	Mexico	LAPJ 2182b	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####		GB#####	
C_steyermarkii_DEB40360	*Citharexylum steyermarkii* Moldenke	Mexico: Chiapas; 19 Sept 1976	D.E. Breedlove 40360 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####
C_subflavescens_LAF13_01	*Citharexylum subflavescens* S.F.Blake	Colombia: Bogotá, D.C.; 20 Sept 2013	L.A. Frost 13-01 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_subflavescens_LAF13_06	*Citharexylum subflavescens* S.F.Blake	Colombia: Cundinamarca; 21 Sept 2013	L.A. Frost 13-06 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_subflavescens_LAF13_27	*Citharexylum subflavescens* S.F.Blake	Colombia: Valle del Cauca; 10 Oct 2013	L.A. Frost 13-27 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
C_subthyrsoideum_MGG30	*Citharexylum subthyrsoideum* Pittier	Venezuela: Libertador; 17 Jul 1990	M. Gabriela Gomez 30 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####		GB#####	GB#####
C_sulcatum_LAF13_07B	*Citharexylum sulcatum* Moldenke	Colombia: Bogotá, D.C.; 21 Sept 2013	L.A. Frost 13-07 B (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_sulcatum_LAF13_08	*Citharexylum sulcatum* Moldenke	Colombia: Cundinamarca; 22 Sept 2013	L.A. Frost 13-08 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####
C_tetramerum_M8638	*Citharexylum tetramerum* Moldenke	Mexico	Mendoza 8638	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####					GB#####	GB#####
C_tetramerum_PT4717	*Citharexylum tetramerum* Moldenke	Herbarium specimen, Field Museum	P. Teuoria 4717 (F)	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####		GB#####			GB#####
C_tristachyum_G11484	*Citharexylum tristachyum* Turcz.	United States: Florida; 17 Aug 1971	Gillis 11484 (FTG)								GB#####			GB#####			
C_ulei_LAF128	*Citharexylum ulei* Moldenke	Ecuador: Napo; 02 Oct 2016	L.A. Frost 128 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			
C_ulei_LAF130	*Citharexylum ulei* Moldenke	Ecuador: Sucumbios; 03 Oct 2016	L.A. Frost 130 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####
C_weberbaueri_LAF103	*Citharexylum weberbaueri* Hayek	Peru: La Libertad; 10 Sept 2016	L.A. Frost 103 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####						
C_weberbaueri_LAF105	*Citharexylum weberbaueri* Hayek	Peru: La Libertad; 11 Sept 2016	L.A. Frost 105 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####	GB#####
C_weberbaueri_LAF106	*Citharexylum weberbaueri* Hayek	Peru: La Libertad; 11 Sept 2016	L.A. Frost 106 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####
C_weberbaueri_LAF120	*Citharexylum weberbaueri* Hayek	Peru: Cajamarca; 16 Sept 2016	L.A. Frost 120 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####					GB#####	
C_weberbaueri_LAF124	*Citharexylum weberbaueri* Hayek	Peru: Cajamarca; 17 Sept 2016	L.A. Frost 124 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####
C_weberbaueri_LAF125	*Citharexylum weberbaueri* Hayek	Peru: Cajamarca; 17 Sept 2016	L.A. Frost 125 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####
C_weberbaueri_LAF126	*Citharexylum weberbaueri* Hayek	Peru: Cajamarca; 17 Sept 2016	L.A. Frost 126 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####
C_weberbaueri_PL09_04	*Citharexylum weberbaueri* Hayek	Peru	P. Lu-Irving 2009-04 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####		GB#####	GB#####	GB#####
Cassella_glazioviI_MAS3630	*Cassella glazioviI* (Briq. & Moldenke) Moldenke	Brazil: Minas Gerais, 30 Nov. 1997	M. Aparecidada Silena et al. 3630 (US)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####					GB#####	GB#####
Duranta_vestita_RRRM50	*Duranta vestita* Cham.	Brazil: Mun. de Bom Jesus; 19 Mar 1999	M. Rossato & R. Wasum 50 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####				GB#####	GB#####
Hierbotana_inflata_JH6199	*Hierbotana inflata* (Kunth) Briq.	Ecuador: Pichincha	J. Humbles 6199 (TEX)	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####						
Lampaya_castellani_RGO07_63	*Lampayo castellani* Moldenke	Argentina: Jujuy; 23 Feb. 2007	R.G. Olmstead 2007-63 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####					GB#####	GB#####
Lantana_depressa_PL12_01	*Lantana depressa* Small	United States: Washington (in cultivation)	P. Lu-Irving 2012-01 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####					
Nashia_inaguensis_WTG13097	*Nashia inaguensis* Millsp.	Bahamas: Inagua; 11 Mar 1976	William T. Gillis 13097 (TEX)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####			
Petrea_maynensis_RB1765	*Petrea maynensis* Huber	Ecuador: Orellana; 25 Oct 1998	R. Burnham 1765 (MO)							GB#####	GB#####						
Pitraea_cuneato_ovata_RGO04_186	*Pitraea cuneato ovata* (Cav.) Caro	Argentina: Mendoza; 16 Dec 2004	R.G. Olmstead 2004-186 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####		
Recordia_boliviana_NRJW2932	*Recordia boliviana* Moldenke	Bolivia: Florida; 4 Feb 1996	N. Ritter, J. Wood 2932 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####		GB#####	GB#####
Rehdera_penninervia_CLEC19938	*Rehdera penninervia* Moldenke	Guatemala: Peten; 21 Jan 1976	C.L. Lundell, Elias Contreras 19938 (TEX)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####					
Rehdera_penninervia_MPCNB1378	*Rehdera penninervia* Moldenke	Guatemala: Peten; 17 Mar 2002	M. Pena-Chocarro & N. Bonilla 1378 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####	GB#####	GB#####	GB#####
Rehdera_trinervis_DN2386	*Rehdera trinervis* Moldenke	Nicaragua: Matagalpa; 14 Aug 1977	D. Neil 2386 (MO)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####		GB#####
Rehdera_trinervis_EMS30531	*Rehdera trinervis* Moldenke	MX: Campeche;, 05 Apr 1998	E.Martinez S. 30531 (TEX)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####			GB#####	GB#####	GB#####
Rehdera_trinervis_MG11320	*Rehdera trinervis* Moldenke	Costa Rica; Guanacaste; 12 Jan 2003	M. Grayum et.al. 11320 (MO)							GB#####							
Verbena_officinalis_RGO03_156	*Verbena officinalis* L.	United States: Washington (in cultivation)	R.G. Olmstead 2003-156 (WTU)	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####	GB#####		GB#####				

CHAPTER 2:

Is it faster to move or evolve? Comparing effects of niche conservatism and niche evolution on diversification rate in the Neotropical plant genus Citharexylum (Verbenaceae)

ABSTRACT

Aim: Niche evolution has emerged as an important biogeographical pattern in plant lineages, but we are only beginning to understand how these events contribute to diversification. These patterns are poorly understood in the Neotropics, the area of highest plant diversity in the world. We use the fiddlewoods (Citharexylum) a flowering plant genus of Neotropical origin and with a broad distribution in multiple biomes through the Neotropics to assess the direction and frequency of biome shift and how radiation within a biome versus colonization of new biomes effects diversification.

Location: The New World tropics, from northern Mexico to southern Brazil and Argentina

Taxon: *Citharexylum* (Verbenaceae)

Methods: Phylogenetic relationships were reconstructed with near-complete taxonomic sampling of tribe Citharexyleae and representative sampling of the geographic range and biome diversity. Divergence times were estimated, and the dated phylogeny was used to infer biogeographical history through reconstruction of discrete ancestral areas in BioGeoBEARS and fitting of multi-optima Ornstein-Uhlenbeck models to climate variables.

Results: Citharexylum originated in Mesoamerica in the mid-Miocene, but the crown radiation occurred in the late Miocene to early Pliocene. Early diversification occurred in mid-elevation montane forests with some temperate elements. Dispersal to South America was followed by separate radiations in open, Andean biomes and low to mid-elevation forest. In Mesoamerica, species repeatedly colonized xeric, seasonally dry, and moist forest biomes. Diversification rates were highest in Mesoamerica and lowest in open biomes in South America.

Main Conclusions: Both niche conservatism and niche evolution have played important roles in the diversification of *Citharexylum*. The Mesoamerican clade exhibits a pattern of radiation within the ancestral biome with more recent, independent colonization events, whereas the South American clade exhibits a pattern of early colonization and radiation within biomes. Despite differences in biogeographic patterns and diversification rates, both clades have produced similar taxonomic diversity in the same amount of time.

1 INTRODUCTION

Understanding patterns of global biodiversity is a primary goal of historical biogeography. The disparity in plant species richness between New World and Old World tropics has fueled interest in how plant lineages diversify in the Neotrpics (Antonelli and Sanmartín, 2011). Biogeographic studies at global scales have often revealed patterns of phylogenetic niche conservatism (PNC), the tendency for descendants to occupy similar climatic environments as their ancestors due to evolutionary constraints (Peterson et al. 1999, Prinzing et al. 2001, Wiens and Graham, 2005; Edwards and Donoghue 2006, Qian and Ricklefs 2004; Crisp et al. 2009; Kerkhoff et al. 2014). Early case studies of diversification in Neotropical groups found patterns consistent with phylogenetic niche conservatism (Hughes and Eastwood, 2006; Pennington et al., 2009, 2010; others about niche conservatism), lending support to the idea that physiological adaptations may be difficult to acquire and, therefore, uncommon. Overall, it might be easier to move than to evolve (Moore & Donoghue, 2007; Donoghue, 2008).

However, support for PNC may be biased by the fact that many case studies of Neotropical diversification have focused on large radiations within a single biome including rain forest (Richardson et al., 2001; Davis et al. 2004; mid-elevation), montane forest (Ornelas and González, 2014; Schwery et al., 2015; Lagomarsino et al., 2016), seasonally dry tropical forest (Pennington et al., 2004; Pennington et al., 2010; Werneck et al. 2011; Särkinen et al., 2012), paramo (Hughes and Eastwood, 2006; Madriñan et al., 2013; Nürk et al., 2013; Hughes and Atchisnon, 2015; Nürk et al., 2015; but see Nürk et al., 2018). With an increased body of literature and improved phylogenetic resolution, niche evolution (also called biome shift), the

ability of descendants to adapt to climatic environments different from that of their ancestors, has emerged as a prominent pattern in plant lineages (Spriggs et al., 2015, Givnish et al., 2014, Weeks et al., 2014; Cardillo et al., 2017).

Seemingly difficult evolutionary events are common in some lineages (e.g., evolution of C4 photosynthesis in PACMAD grasses; Edwards and Smith, 2010), leading to a revision of Donoghue's earlier statement: it is easy both to move and to evolve (Edwards and Donoghue, 2013). Edwards and Donoghue (2013) hypothesize that niche evolution may serve as a driver of speciation and niche conservatism is a deeper phylogenetic pattern. It seems that niche evolution could impact diversification in two ways: (1) high diversification rates in a colonized biome leading to a single, large radiation within an otherwise niche-conserved lineage, or (2) speciation through repeated and frequent colonization of different biomes—but possibly with high extinction—so that the majority of shifts occur near the tips, from ancestors inhabiting the original niche. Donoghue and Edwards (2014) go on to develop hypotheses of niche evolution based on emergent patterns: (1) biome shifts will occur more frequently into/out of the larger biomes and less frequently into/out of smaller ones; (2) more shifts will take place between adjacent and broadly connected biome than between geographically disjunct or narrowly connected biomes; (3) older, once more expansive biomes will more often be donors (e.g., tropical rainforest), and younger, once more restricted biomes will more often be receivers (e.g. deserts, savannas, alpine habitats); (4) if a lineage has shifted between a pair of biomes, it has likely done so multiple times.

Indeed, repeated biome shifts within many tropical lineages have been documented, including multiple colonizations of savannas within tropical moist forest lineages (Simon et al.,

2009; Bouchenak-Khelladi et al. 2010; Edwards and Smith, 2010; Lohmann et al., 2013), tropical dry forest within tropical moist forest lineages (Crayn et al., 2006; Weeks et al., 2014), arid biomes within mesic lineages (Arrigo et al. 2013), Andean-alpine, or paramo, within mid-elevation montane forest lineages (Sklenar et al., 2010), and alpine biomes within arid-adapted lineages (Heibl & Renner, 2012). Beyond the repeated colonization of one biome, studies have found shifts into/out of multiple different biomes within a lineage (Cardillo et al., 2017; Weeks et al. 2014; De-Nova et al. 2012; Heibl and Renner, 2012; Schrire et al., 2009; Olmstead, 2013; Arrigo et al., 2013; Holstein and Renner, 2011; Tölpel et al., 2012). While niche evolution has been documented in many lineages, few studies have investigated how frequency of niche evolution impacts diversification (Cardillo et al., 2017; Kozak and Weins, 2010; Weeks et al., 2014) or the effects shift directionality/occupation of different biomes has on diversification rates within lineages (Cardillo et al., 2017). Even fewer have investigated this in a Neotropical lineage (Weeks et al., 2014).

The verbena family (Verbenaceae) is a family of ca. 800 species that originated in the Neotropics during the Paleogene (Marx et al., 2010; Magallón et al., 2015; Tank and Olmstead, unpublished). Centers of diversity are in arid to semi-arid habitats, but some groups are abundant in more mesic habitats. The fiddlewoods (*Citharexylum*) is a genus of about 70 spp. of trees and shrubs with a generalist insect pollination syndrome and colorful, fleshy fruits (Frost et al., in prep.). The genus is distributed throughout the Neotropics from northern Mexico to southern Brazil and Argentina. Species inhabit moist broadleaf forest, seasonally dry tropical forests, savannas, midelevation montane forest, montane grasslands and shrublands (subparamo), and xeric shrublands in both Mesoamerica and South America (Fig. 1). A previous phylogenetic

study of *Citharexylum* (Frost et al., in prep.) revealed that the early diverging lineages of *Citharexylum* are distributed in Mesoamerica and that most of the diversity is evenly divided between two clades defined by geography: a Mesoamerican (MA) clade and South American (SA) clade. *Citharexylum* is well-suited for the study of the impact of biome shift on diversification, because it occupies multiple biomes on two continents, it includes clades of equal age and diversity in Mesoamerica and South America, and it has no obvious morphological traits or innovations that might otherwise influence diversification rates within the genus (e.g. pollinator specialization, changes in fruit type, etc.). With *Citharexylum* we will address the following questions:

1. What are the crown and stem ages of *Citharexylum*? When did dispersals between continents and colonizations of different biomes occur and how do those events relate to geologic history (e.g. closure of the Isthmus of Panama or Andean orogeny)?
2. Which biome did the most recent common ancestor of *Citharexlum* occupy?
3. What is frequency and directionality of biome shift? How do inferred patterns compare to hypotheses of niche evolution?
4. Do some biomes incur higher or lower diversification rates than others?
5. Is one pattern—niche conservatism or niche evolution—associated with higher diversification rates: Is it faster to move or evolve?

To do this, we used the multi-locus, 61-species dataset for tribe Citharexyleae from Frost et al. (in prep.) to infer a dated phylogeny for the group. We analyzed niche evolution by assigning species to biomes and performing ancestral area reconstructions and by locating shifts in climatic optima for species/clades through fitting a multi-optima Ornstein-Uhlenbeck process

to individual climate variables and climate scores. The second approach provides some indication of how climatically distinct some biomes are from the ancestral, and that shifts inferred from discrete ancestral area reconstructions are, in fact, climatically significant. Finally, the number and location of diversification rate shifts were inferred.

2 METHODS

2.1 Divergence Time Estimation

Bayesian multi-species coalescent (MSC) analyses were performed in BEAST v1.8.4 (Drummond et al., 2012) using the Citharexyleae dataset and partitioning scheme from Frost et al. (in prep.). Four species from the broader Verbenaceae were added to provide nodes for secondary calibration: *Verbena officinalis*, *Stachtarpheta cayennensis*, *Duranta vestita*, and *Petra volubis* (*Stachytarpheta* and *Duranta* will be referred to as a group by the name of the inclusive tribe Duranteae). Inclusion of these species follows sampling of a fossil-calibrated phylogeny of the Lamiales (Tank and Olmstead, unpublished). Species sets for all taxa, Citharexyleae + Verbena + Duranteae, and Citharexyleae + Verbena were constrained as monophyletic and normal priors with SD +/- 1 were set at 43.1 (44.8-41.4), 33.8 (35.4-32.2), and 28.7 (30.3-27.06) Ma, respectively (Tank and Olmstead, unpublished). Three independent chains of mcmc were run for 500 million generations, sampling every 50,000 generations. Results were visualized in Tracer v1.6 (Rambaut et al., 2014) to determine appropriate burn-in and to verify that effect sample size (ESS) values were adequate (> 200), individual runs had reached stationarity, and separate runs had converged. The runs were combined with LogCombiner v1.8.4 (Drummond et al., 2012), and a maximum clade credibility tree was summarized and annotated with

TreeAnnotator v1.8.4 (Drummond et al., 2012). Output was visualized in FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

2.2 Climate Data

Climatic variables were used to approximate niche/biome. Data for the 19 BioClim variables were extracted from WorldClim global climate layers (Fick and Hijmans, 2017) for species of Citharexyleae. Data were extracted for each species using the R packages raster (Hijmans, 2017) and dismo (Hijmans et al., 2017) with GPS coordinates obtained from geo-referenced, databased specimens in GBIF (gbif.org, 27-29 March 2108) and voucher specimens (Supplementary table). Individuals were manually geo-referenced if coordinates for databased specimens were not available (Supplementary table). Elevation data were also collected for each species from those records. Coordinates were processed so that only unique occurrence points were retained. Additionally, points outside of the natural range (e.g. collections of individuals in botanical gardens or invasives) as well as specimens with georeferencing errors (e.g. points that mapped in the ocean or the center of a country when locality data was limited to the country level) were removed from analyses. For each species the average of extracted data was taken for all 19 climate variables and elevation (from now on referred to as the average dataset). The range (max - min) of extracted data for each species was also taken for all 19 climate variables as a proxy for niche breadth (from now on referred to as the range dataset). A phylogenetic principal component analysis (pPCA) was performed on the average datasets using the R package phytools (Revell, 2012) to obtain climatic niche scores for species for subsequent analyses and to determine which climatic variables were important to niche.

2.3 Ancestral Reconstruction

Ancestral area reconstructions were performed for geographic area and biome with the BioGeoBEARS (Matzke, 2013; Matzke, 2014; Massana et al., 2015) package implemented in RASP v4.0 (Yu et al., 2015). To more accurately reconstruct early nodes, analyses were performed using the Citharexyleae portion of the MSC tree plus one branch representing the sister lineage Casselieae. Geographic areas were defined as Mesoamerica, the Caribbean, and South America. The DIVAlike + j model (Matzke, 2014) was selected from six models as the best fitting model.

For ancestral biome reconstructions, species were assigned to one of the following biomes: moist broadleaf forest, seasonally dry tropical forest, mid-elevation montane forest (1000-2500 m elevation), xeric shrublands, or montane grasslands and shrublands (subparamo; >2500 m elevation). Biomes were adapted from Olson et al. (2001). Some terrestrial ecoregions were combined to better detect broad patterns: seasonally dry forest encompassed tropical/ subtropical dry broadleaf forest and tropical/subtropical grasslands, savannas and shrublands; montane forest included tropical coniferous forest and moist broadleaf forest between 1000-2500 m. Maximum likelihood estimations of ancestral characters were performed for all climate variables in the average and range datasets using the R package phytools (Revell, 2012). Reconstructions for continuous data were performed using the MSC tree topology for Citharexyleae. Outgroup taxa were trimmed using the R package ape (Paradis et al., 2004)

2.4 Niche evolution

To detect major shifts in climatic optima within lineages of *Citharexylum*, PC scores and individual climate variables from the average dataset were treated as continuous characters and analyzed using the R package bayou (Uyeda et al., 2013; Uyeda & Harmon, 2014). Because bayou fits multi-optima OU models to the data, the R package geiger (Pennell et al., 2014) was used to ensure that an OU model was the best fit compared to Brownian motion or white noise. Precipitation variables were log normalized to correct for edge effects. PC scores for the first two principal components and individual variables were analyzed with a standard error of 0.5. The maximum number of shifts per branch was limited to one with equal probability of a shift occurring across all branches. A half-cauchy distribution was set as the prior for rate of adaptation (alpha) and magnitude of uncorrelated diffusion (sigma^2), a normal prior was set for the optimum (theta), and a conditional Poisson was set for the number of shifts (k) [make.prior(tree, dists = list(dalpha="dhalfcauchy", dsig2="dhalfcauchy", dsb="dsb", dk="cdpois", dtheta="dnorm"), param = list(dalpha=list(scale=1), dsig2=list(scale=1), dk=list(lambda=8, kmax=120), dsb=list(bmax=1, prob=1), dtheta=list(mean=mean(dat), sd=2*sd(dat))))]. The mcmc was run for 1 million generations and the first 30% of samples were discarded as burn-in.

2.5 Diversification rate analysis

The number and location of rate shifts were estimated using BAMM (bayesian analysis of macroevolutionary mixtures; Rabosky et al., 2013; Rabosky, 2014). Analyses were performed on the Citharexyleae portion of the MSC. To account for incomplete and biased sampling, a sampling fraction was applied to each of the six subgenera of *Citharexylum* and to *Rehdera*. The

lambda initial prior, lambda shift prior, mu initial prior, and the prior for expected number of shifts were set using the setBAMMpriors() function in the R package BAMMtools (Rabosky et al., 2014). One analysis was run with default settings for the remaining priors and another was run with a "MEDUSA-like" model (updateRateLambdaShift=0). The mcmc was run for 5 million generations, sampling every 2,000 generations. The first 10% of samples were discarded as burnin. The R package coda (Plummer et al., 2006) was used to verify that the run had converged and ESS values were adequate. Results were summarized and visualized with BAMMtools.

3 RESULTS

3.1 Divergence Time Estimation

Topology and support for major clades in the dated tree were concordant with previous studies of Citharexyleae. Divergence time estimations for calibrated nodes were 41.3 (45.3-37.8), 32.7 (35.9-29.4), and 30.1 (33.3-26.3) Ma for all taxa, Citharexyleae + Verbena + Duranteae, and Citharexyleae + Verbena, respectively (Fig. 1). These estimates are congruent with clade ages from the fossil-calibrated phylogeny of the Lamiales. The crown age for the Citharexyleae was estimated as 14.8 (19.2-11.4) Ma and the crown age for *Citharexylum* 5.9 (8.4-3.5) Ma. The divergence of MA and SA clades was estimated to be 4.0 (5.2-3.4) Ma, and the crown ages of the MA clade and the SA clade 3.7 (4.5-2.8) Ma and 3.7 (4.4-2.4) Ma, respectively. See Table 1 for divergence times of subgenera.

3.2 Climate Data

The number of unique occurrences for species ranged from three (*C. fulgidum*) to 439 (*C. caudatum*). In total, 3,679 unique occurrence points were used. For the average dataset, the first three principal components explained 84.8% of the variation (PC1: 41.5%, PC2: 31.8%, and PC3: 11.5%). Variables that retained most variance in PC1 were minimum temperature of coldest month (bio 6), mean temperature of coldest quarter (bio 11), and mean temperature of driest quarter (bio 9). Twelve of the 20 variables contributed more than average to the variability of the first principal component (Table ?). Variables that retained most variance in PC2 were maximum temperature of warmest month (bio 5), mean temperature of wettest quarter (bio 8), and mean temperature of warmest quarter (bio 10). Eight of the 20 variables contributed more than average to the variability of the second principal component.

3.3 Ancestral Reconstructions

3.3.1 *Geographic area*

Mesoamerica was reconstructed as the ancestral area of the MRCA for both Citharexyleae and *Citharexylum* (Fig. 2). Dispersal to South America occurred five times. The first dispersal, establishing the SA clade, was estimated to have occurred 4.0 (5.2-3.4) to 3.7 (4.4-2.4) Ma. Four subsequent dispersals to South America have occurred within the last million years, each resulting in a single South American species. Three species (*C. decorum*, *C. karstenii*, and *C. subthyrsoideum*) have ancestors in Mesoamerica; *C. spinosum* is found in the Caribbean and northern South America from ancestors in Mesoamerica. *Citharexylum caudatum* has extended its range from Mesoamerica to include the Caribbean.

3.3.2 *Biome*

Montane forest was reconstructed as the ancestral biome of the most recent common ancestor (MRCA) of *Citharexylum* (Fig. 3). This was also the most probable ancestral biome for early nodes of the MA clade and the ancestor of the SA clade. In *Citharexylum*, eleven shifts from montane forest to other biomes were inferred (six to broadleaf moist forest, two to seasonally dry forest, and three to xeric shrublands; Fig. 2; Table 2). Three shifts back to montane forest were inferred (two from broadleaf moist forest and one from seasonally dry forest), but two of them with low support. Six additional shifts between colonized biomes were inferred: four from moist broadleaf forest to seasonally dry forest, one from xeric shrublands to seasonally dry forest, and one from seasonally dry forest to montane grasslands and shrublands (Fig. 2; Table 2).

Climate variables—Results for ancestral character estimations of both averages and ranges of individual climate variables are available in Supplementary #-#. Precipitation variables (annual precipitation, precipitation of driest quarter, and precipitation of wettest quarter), reconstruct values for the MRCA of the SA clade (Table 3) more similar to the MRCA of MA and SA clades, which was reconstructed to be montane forest in discrete character analyses, than the MRCA of *C.* subg. *Andinum*, which was reconstructed to be xeric shrubland in discrete character analyses.

3.4 Niche evolution

Eleven shifts within Citharexylum were detected by the bayou analysis of climate scores (PC1) from the PCA (Fig. 3a; Fig. S1). Shifts were detected along branches leading to five of the six subgenera. A shift was not detected at the base of *C.* subg. *Citharexylum*, but four shifts were found within the subgenus. An additional shift was found in *C.* subg. *Andinum* leading to *C.*

montevidense, and another in *C.* subg. *Sylvaticum* on the branch leading to *C. poeppigii* and *C. ulei*. Five of the shift locations found by analyses of climate scores were concordant with biome shifts inferred by the BioGeoBEARS analysis (Fig. 4a,c), Nine shifts were detected in the analysis of PC2: six in subg. *Citharexylum*, one in subg. *Andinum*, and two in sugb. *Sylvaticum*. Six of the shift locations for PC2 corresponded with shifts inferred by the BioGeoBEARS analysis (Fig. 4a,c; Fig. S2).

Analyses of individual climate variables detected shifts in 30 locations on the tree (Fig. 4b; Figs. S3-S21). Shifts in multiple variables were found on the branches leading to subg. *Mexicanum*, subg. *Andinum*, and subg. *Sylvaticum* (Fig. 4b). Eight subsequent shifts were found in subg. *Andinum*, five by multiple variables and three by a single climate variable. Four more shift locations were recovered in subg. *Sylvaticum*, three by multiple and one by a single variable. In subg. *Citharexylum*, shifts were found in twelve locations, four by multiple variables and eight by a single variable. Six of the shift locations found by analyses of individual variables corresponded with the analysis of climate scores (Fig. 4a,b) and ten were concordant with biome shifts inferred by the BioGEOBEARS analysis (Fig. 4b,c).

3.5 Diversification rate analysis

In both the default-setting and the MEDUSA-like model, one shift on the branch leading to the ancestor of the MA and SA clades was detected with strong support (Fig. S21-S22). A second shift on the branch leading to subg. *Citharexylum* was also detected in both with weak support (Figs. S21-S22). Rates varied throughout the tree at intermediate levels.

DISCUSSION

The stem age for *Citharexylum* is about 15 (19.2-11.4) Ma; the crown age is 6 (8.4-3.5) Ma. Dispersals to South America occurred within the last 4 Ma, when the Isthmus of Panama had more or less reached its current conformation (Montes et al., 2012). Similarly, biomes that are currently occupied by *Citharexylum* would have been present at the time of radiation (i.e., species were not colonizing biomes when the niche space was new/uninhabited) with the possible exceptions of mid-elevation montane biomes and montane grasslands in the northern Andes, which only reached their full height in the last 3 Ma. The MRCA of *Citharexylum* was reconstructed as inhabiting mid-elevation montane forests of Mesoamerica. Repeated colonizations of other biomes from the ancestral biome were reconstructed, the most frequent from mid-elevation montane forest to moist broadleaf forest. Multiple shifts from mid-elevation montane forest to xeric shrublands and from mid-elevation montane forest to seasonally dry forests and savannas were also found. Re-entry into mid-elevation montane forest occurred at least once from moist broadleaf forest (*C. macradenium*), possibly a second time in subg. *Sylvaticum*, though support is weak for moist broadleaf forest as the ancestral biome in this case. Another possible re-entry to mid-elevation montane forest from seasonally dry forest was found for *C. glabrum*, again, with weak support.

Following initial colonization of alternate biomes, shifts occurred multiple times from moist broadleaf forest to seasonally dry forest, in fact, more shifts than occurred from mid-elevation montane forest to seasonally dry forest. Single shifts from xeric shrublands to seasonally dry forest and from xeric shrublands to montane grasslands and shrublands were recovered. Shifts did not occur between moist broadleaf forest and montane grasslands and

shrublands, moist broadleaf forest and xeric shrublands, mid-elevation montane forest and montane grasslands and shrublands, or from seasonally dry forest to moist broadleaf forest, seasonally dry forest to xeric shrublands, montane grasslands and shrublands to xeric shrublands, xeric shrublands to mid-elevation montane forest.

A single shift in diversification rate with high support was placed near the base of *Citharexylum*, but rate variation along the branches revealed higher rates in the MA clade and overall lower rates in the SA clade. The lowest rates were found in subg. *Andinum*, which inhabits inter-Andean dry valleys and subparamo. The most shifts in climate optima were inferred at the base of this clade. Diversification rates were highest in subg. *Citharexylum*, which has repeatedly colonized multiple, adjacent biomes, but these colonizations did not show marked differences in many aspects of climate.

4.1 Origin and early-diverging lineages of *Citharexylum*

The ancestor of Citharexyleae originated in Mesoamerica ca. 15 (19.2-11.4) Ma, around the mid-Miocene climatic optimum. The crown group radiation started ca. 6 (8.4-3.5) Ma. The ancestral biome is inferred to be montane forest, which included coniferous, oak, and pine-oak forests of Mexico and mesic mid-elevation forest in this study. The origin in Mesoamerica and the distribution of early-diverging Mesoamerican lineages (*C. altamiranum* and subg. *Affine*) in pine-oak forest of Mexico, suggests that this is likely the ancestral biome for *Citharexylum*. Pollen collected from the upper Miocene Paraje Solo formation in Veracruz, Mexico reflect temperate coniferous and deciduous elements in the flora (Graham, 1976), elements that remain present in contemporary coniferous-oak forests of Mexico. Other elements of this late Miocene

flora suggest that temperatures would have been slightly cooler and precipitation more evenly distributed throughout the year than at present (Graham, 1976). There is no direct evidence of *Citharexylum* in this flora, but it does establish paleocommunities similar to the reconstructed ancestral biome concurrent with the crown age of the genus and a precursor to modern biomes in which species are distributed.

Except for a single, early-diverging species, *Citharexylum* comprises two major clades, one distributed primarily in Mesoamerica and one distributed primarily in South America. The dispersal to South America establishing the SA clade is estimated to have occurred approximately 4.0 (5.2-2.3) Ma, within younger, Pliocene estimates of the closure of the Isthmus of Panama (Keigwin, 1978; Coates et al., 2004). The ancestral biome of the SA clade is unresolved; both xeric grassland and montane forest biomes were reconstructed with similar probabilities in the discrete character analysis. Continuous character analyses on individual climate variables estimated values more similar to species inhabiting mid-elevation montane forest than xeric grasslands. Additionally, multi-optima fitting analyses for climate scores and individual variables located shifts along the daughter branches of the MRCA of the SA clade rather than the branch leading to it, suggesting that shifts in climatic optima occurred after establishment in South America.

4.2 The Mesoamerican clade

Mid-elevation montane forest is confidently reconstructed along the backbone of the MA clade. The clade exhibits the second of the two hypothesized patterns for niche evolution and niche conservatism when it is easy to move and to evolve: the ancestral biome for the genus is

conserved at deeper nodes and shifts to novel biomes occur more frequently near the tips. Additionally, shifts in fewer climate variables per shift location on the phylogeny are found in the MA clade compared to shift events in the SA clade (i.e., more elements of the ancestral biome are retained). Two radiations within a colonized biome are inferred in the MA clade: one in xeric shrublands bordering the Chihuahuan Desert of Central Mexico *(C.* subg. *Mexicanum*) and one in broadleaf mesic forests along the Gulf Coast and Yucatan Peninsula (*C. ellipticum*-*C. spinosum*). Tropical wet forests often act as a source biome, but transitions into them are relatively rare (Donoghue and Edwards, 2014; Antonelli et al., 2018). However, in Mesoamerica, wet tropical forests were restricted during late Miocene and Pliocene, and tropical elements are poorly represented in paleofloras near the Gulf Coast during the Pliocene (Graham and Dilcher, 1998). If cool-temperate forests like those of the Paraje Solo formation were widespread, this would fit the hypothesis that shifts occur from a more expansive source biome (e.g., mid-elevation montane forest) into a more restricted biome (e.g., moist broadleaf forest).

The Gulf Coast/Caribbean clade of subg. *Citharexylum* is the only radiation within BMF in Mesoamerica, but the species have repeatedly colonized broadleaf moist forest in Mesoamerica. Four additional colonizations of broadleaf moist forest from mid-elevation montane forest by single species are found, two from mid-elevation, pine-oak forest by *C. schottii* (Costa Rica and Mexico populations, which are not each other's closest relative) and two from cloud forests in Costa Rica by *C. cooperi* and *C. costaricense*. Repeated colonizations of open biomes have also occurred. Two of these shifts also correspond with dispersals to South America: one by *C. decorum*, which is distributed in Ecuadorian dry forests west of the Andes, and one by *C. subthyrsoideum*, which has a narrow distribution in the Lower Catuche wood and

shrublands near Caracas, Venezuela. Colonization of xeric biomes has occurred three times in the MA clade: by subg. *Mexicanum*, distributed in shrublands bordering the Chihuahuan desert, and separately by *C. roxanae* and *C. flabellifolium* (not sampled in this study but see Frost et al., 2017 and Frost et al., in prep. for discussion of this species), distributed in Baja California Sur and Sonora.

The divergence of xeric-adapted sugb. *Mexicanum* occurred ca. 3.5 (4.3-2.5) Ma, within the timeframe that the ancestor of subg. *Andinum* colonized inter-Andean dry valleys (4.3-1.9). However, extant diversity in subg. *Mexicanum* radiated within the last million years, whereas divergence times in subg. *Andinum* are older. Each of the subgenera in the MA clade was established in the Pliocence, but did not radiate until the Pleistocene. Species diversity in some plant groups has been attributed to recent periods of unstable climate during Pleistocene glaciation cycles promoting allopatric speciation through fractured populations in refugia. These cycles may have promoted speciation within biomes, such as within xeric biomes in subg. *Mexicanum*, but may have also promoted shifts between biomes. Mesoamerica is hypothesized to have fluctuated between dry and wet cycles during Pleistocene (cite), these different cycles could have altered the connectivity of different biomes at different times, making shifts between them more probable.

4.3 The South American clade

In South America, biome shifts occurred early in lineages, and shifts were followed by radiation within those biomes. The two daughter lineages of the MRCA of the SA clade, subg. *Andinum* and subg. *Sylvaticum*, exhibit shifts in climatic optima in opposite directions. subg.

Andinum represents a radiation in cooler, drier, and higher biomes in the Andes. Species diversified in inter-Andean dry valleys of Peru (*C. flexuosum, C. weberbaueri, C. peruvianum,* and *C. kobuskianum*) and seasonally dry forest of Bolivia and northern Argentina (*C. andinum* and *C. joergensenii*) before colonizing montane grasslands and shrublands, or subparamo (>3,000 m a.s.l.). In the present distribution of Neotropical biomes, montane grasslands and shrublands have their greatest expanse in southern Peru, Bolivia, and northern Argentina. Seasonally dry forests flank the eastern slopes of the Andes in Bolivia and northern Argentina where xeric grasslands flank the western slopes in Peru and northern Chile. Both xeric shrublands and seasonally dry biomes are present in inter-Andean valleys in these countries and have persisted since the late Miocene, so there is potentially a history of connectivity between these biomes. This is the only region in which an exchange between xeric grasslands and seasonally dry tropical forest was found, and the only colonization of high-elevation montane grassland and shrublands. High alpine species show a history of northward dispersal in the Andes and allopatric speciation: *C. herrerae, C. argutedentatum,* and *C. pachyphyllum* are endemic to southern Peru, *C. dentatum* to central Peru, *C. iIlicifolium* to northern Peru and Ecuador, and *C. sulcatum* to Colombia.

Subgenus *Sylvaticum*, radiated in warmer, wetter, lower elevation biomes. The subgenus includes the only dispersal out of South America to the Caribbean, resulting a small clade of seasonal to humid-forest-adapted shrubs. Most of the diversity in subg. *Sylvaticum* is distributed in a clade of Amazonian and Atlantic forest trees and a clade of mid-elevation, cloud forest trees in the Northern Andes. One shift occurred in the Amazonian clade from moist broadleaf forest to tropical savanna in *C. amabilis*. This species, which was previously classified in a different

genus (*Baillonia*), is the only species of *Citharexylum* distributed in the Cerrado. *Citharexylum montevidense*, is distributed in the adjacent humid chaco and Uruguayan savanna. Aside from a couple of recent colonizations by single species, South American groups show a history of early colonization followed by a period of diversification within that biome.

4.4 Diversification

The MA and SA clades of *Citharexylum* provide a natural experiment for patterns of niche evolution and diversification on separate continents in the Neotropics. They are the same age and comprise similar species diversity, but exhibit distinct patterns. The only well-supported shift in diversification rate was located near the MRCA of the MA and SA clades. Variation of rates among branches reveals higher rates in the MA clade and lower rates in the SA clade; a weakly supported shift toward higher rates was found at the base of sugb. *Citharexylum*. Frequent biome shifts were found in subg. *Citharexylum* by the analysis of discrete biomes, but these shifts corresponded to shifts in few climate variables, often only one variable.

In the SA clade, rates are lower in subg. *Andinum* and higher in subg. *Sylvaticum*. Both clades are preceded by shifts in optima for multiple climatic variables, but rates seem marginally higher in cloud forest species (*C. montanum*, *C. subflavescens*, *C. rimbachii*, and *C. kunthianum*) and Atlantic forest species (*C. myrianthum* and *C. solanaceum*) of subg. *Sylvaticum*. In each of these groups, elements of ancestral environment are restored. The cloud forest species reenter mid-elevation montane forest and the Atlantic coast forest, though characterized as moist broadleaf forest, is not as warm and wet as the Amazon forest occupied by other members of this clade. The Pleistocene glaciation and refugia model has also been invoked for speciation in

Atlantic forest (cite) and the northern Andean biomes (van der Hammen, 1974, Gentry 1982); this could explain higher rates of diversification in those environments. It could also explain lower rates in inter-Andean dry valleys and seasonally dry forests in subg. *Andinum.* Pennington et al. (2004) found that Pleistocene glaciation cycles did not influence speciation in woody, South American plants inhabiting those biomes.

Another interpretation of lower diversification rates in subg. *Andinum* is that properties of biomes themselves influence rates in lineages. Hypotheses for Neotropical biomes are most developed for Andean habitats. Species poor and slowly diversifying, but highly endemic groups are characteristic of inter-Andean valley/seasonally dry forest biomes (Pennington et al., 2010; Särkinen et al., 2012). Somewhat more recent and rapid diversification is characteristic of mid-elevation montane forest (Lagomarsino et al., 2016; Givnish et al., 2014). Some of the highest diversification rates in plants have been reported from high-elevation, Andean grasslands, or paramo. Lowest rates would be expected in seasonally dry forest and xeric shrublands and higher rates in mid-elevation montane forest; both of these patterns are observed in *Citharexylum*. The highest rates would be expected in montane grasslands and shrublands, which is not observed. This could be due to the fact that *Citharexylum* does not reach the paramo, but only the subparamo, and perhaps there is a marked difference in speciation between the two.

Subgenus *Andinum* also exhibited the greatest climatic divergence of any clade of *Citharexylum*. The colonization of xeric shrublands inferred at the base of subg. *Andinum* corresponded to shifts in 11 out of 20 climate variables assessed. To give perspective, shifts to xeric grasslands in Mesoamerica corresponded with shifts in 6 variables for subg. *Mexicanum* and 5 variables for C. *roxannae*. The lowest diversification rates were found in the group that is

most distinct from the ancestor. Likewise, the highest rates were found in sugb. *Citharexylum* in which shifts were common, but only in one or two variables. Retention of elements of the ancestral niche combined with some lability could promote parapatric speciation. If colonized biomes are climatically more similar to the ancestral biome, this may also reduce extinction rate in those biomes versus colonization of more dissimilar biomes.

Ultimately, the Mesoamerican and South American radiations have produced similar species diversity in the same amount of time. In Mesoamerica, lineages did not radiate until the Pleistocene; there is a lag between when the Mesoamerican subgenera diverge and when they radiate. South American lineages have older, steadier speciation events. Though each clade displays different biogeographic patterns and different patterns of diversification, the result are, more or less, the same.

Conclusions

Niche evolution has occurred frequently in the evolutionary history of *Citharexylum*. Both repeated colonizations of biomes and radiations within biomes have occurred. Shifts occurred most commonly to and from the largest biomes and in areas of high connectivity between biomes, which is consistent with hypothesized patterns of biome shift. Lowest diversification rates were recovered for open biomes in South America, with rates somewhat higher in forested biomes of South America and higher overall in Mesoamerica. Observed patterns of diversification may be the result of Pleistocene glaciation, slower speciation rates in general in open biomes of South America, or how much of the ancestral niche is retained in a

colonization event. Overall, there is not an observable difference in the effect of niche conservatism or niche evolution on species diversity in this group.

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REFERENCES

Antonelli, A., & Sanmartin, I. (2011) Why are there so many plant species in the Neotropics? Taxon, 60 (2), 403-414.

Arrigo, N., Therrien, J., Anderson, C.L., Windham, M.D., Haufler, C.H., Barker, M.S. (2013) A total evidence approach to understanding phylogenetic relationships and ecological diversity in Selaginella subg. Tetrgonostachys. American Journal of Botany, 100(8), 1672-1682.

Bouchenak-Khelladi, Y., Maurin, O., Hurter, J., van der Bank, M. (2010) The evolutionary history and biogeography of Mimosoideae (Leguminosae); an emphasis on African acacias. Molecular Phylogenetics and Evolution, 57, 495-508.

Coates, A.G., Collins, L.S., Aubry, M.-P., and Berggren, W.A. 2004. The geology of the Darien, Panama, and the late Miocene–Pliocene collision of the Panama arc with northwestern South America: Geological Society of America Bulletin, 116, 1327–1344. doi:10.1130/B25275.1

Crayn, D.M., Rossetto, M., Maynard, D.J. (2006) Molecualr phylogeny and dating reveals on Oligo-Miocene radiation of dry-adapted shrubs (former Tremandraceae) from rainforest tree progenitors (Elaeocarpaceae) in Australia. American Journal of Botany, 93(9), 1328-1342.

Crisp, M.D., Arroyo, M.T.K., Cook, L.G., Gandalfo, M.A., Jordan, G.J., et al. (2009) Phylogenetic biome conservatism on a global scale. Nature, 458, 754-756.

Davis, C.C., Webb, C.O., Wurdack, K.J., Jaramillo, C.A., Donoghue, M.J. (2005) Explosive radiation of Malpighiales supports a mid-Cretaceous origin of modern tropical rain forests. American Naturalist, 165, E36-E65

De-Nova, J.A., Medina, R., Montero, J.C., Weeks, A., Rosell, J.A. et al. (2012) Insights into the historical construction of species-rich Mesoamerican seasonally dry tropical forest: the diversification of Bursera (Buseraceae, Sapindales). New Phytologist, 193(1), 276-287

Donoghue, M.J. (2008) A phylogenetic perspective on the distribution of plant diversity. Proceedings of the National Academy of Sciences, 105, 11549-11555

Donoghue, M.J., & Edwards, E.J. (2104) Biome shifts and niche evolution in plants. Annual Review of Ecology, Evolution, and Systematics, 45, 547-572.

Drummond, A.J., Suchard, M.A., Dong Xie & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution, 29, 1969-1973.

Edwards, E.J., & Donoghue, M.J. (2006) Pereskia and the origin of the cactus life-form. American Naturalist, 167(6), 777-793

Edwards, E.J., & Donoghue, M.J. (2013) Is it easy to move and easy to evolve? Evolutionary accessibility and adaptation. Journal of Experimental Botany, 64, 4047-4052

Edwards, E.J., & Smith, S.A. (2010) Phylogenetic analyses reveal the shady history of C4 grasses. Proceedings of the National Academy of Sciences, 107(6), 2532-2538

Fick, S.E., and Hijmans, R.J. (2017) Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology. DOI: 10.1002/joc.5086

Frost, L. A., Tyson, S. M., Lu−Irving, P., O'Leary, N., & Olmstead, R. G. (2017). Origins of North American arid−land Verbenaceae: More than one way to skin a cat. American Journal of Botany, 104(11), 1708-1716.

GBIF.org (2018), GBIF Home Page. Available from: http://gbif.org [27-29 March 2018].

GBIF.org (27th March 2018) GBIF Occurrence Download. https://doi.org/10.15468/dl.ldyuv1

GBIF.org (28th March 2018) GBIF Occurrence Download. https://doi.org/10.15468/dl.pjcdof

GBIF.org (29th March 2018) GBIF Occurrence Download. https://doi.org/10.15468/dl.7ahgxl

Gentry, A.H. (1982) Neotropical Floristic Diversity: Phytogeographical Connections Between Central and South America, Pleistocene Climatic Fluctuations, or an Accident of the Andean Orogeny? Annals of the Missouri Botanical Garden, 69 (3), 557-593.

Givnish, T.J., Barfuss, M.H.J., Van Es, B., Riina, R., Schulte, K., Horres, R. … Sytsma, K.J. (2014) Adaptive radiations, correlated and contingent evolution, and net species diversification in Bromeliaceae. Molecular Phylogenetics and Evolution, 71, 55-78. doi: 10.1016/j.ympev. 2013.10.010

Graham, A. (1976) Studies in Neotropical Paleobotany. II. The Miocene Communities of Veracruz, Mexico. Missouri Botanical Garden Press, 63 (4), 787-842.

Graham, A., & Dilcher, D.A. (1998) Studies in Neotropical paleobotany. XII. A palynoflora from the Pliocene Rio Banano Formation of Costa Rica and the Neogene vegetation of Mesoamerica. American Journal of Botany, 85(10), 1426-1438.

Hiebl, C., Renner, S.S. (2011) Distribution models and a dated phylogeny for Chilean Oxalis species reveal occupation of new habitats by different lineages, not rapid adaptive radiation. Systematic Biology, 61(5), 823-834

Hijmans, R.J. (2017) raster: Geographic Data Analysis and Modeling. R package version 2.6-7. https://CRAN.R-project.org/package=raster

Hijmans, R.J., Phillips, S., Leathwick, J., and Elith, J. (2017) dismo: Species Distribution Modeling. R package version 1.1-4. https://CRAN.R-project.org/package=dismo

Holstein, N., Renner, S.S. (2011) A dated phylogeny and collection records reveal repeated biome shifts in the African genus Coccinia (Cucurbitaceae). BMC Evolution, 11, 28.

Hughes, C., & Eastwood, R. (2006) Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the Andes. Proceedings of the National Academy of Sciences, 103 (27), 10334-10339.

Hughes, C.E., Atchison, G.W. (2015) The ubiquity of alpine plant radiations: from the Andes to the Hengduan Mountains. New Phytologist, 207, 275–282.

Keigwin, L.D. 1978. Pliocene closing of the Isthmus of Panama, based on biostratigraphic evidence from nearby Pacifi c Ocean and Caribbean Sea cores: Geology, 6(10), 630–634.

Kozak, K.H., and Wiens, J.J. (2010) Accelerated rates of climatic-niche evolution underlie rapid species diversification. Ecology Letters, 13, 1378-1389. doi: 10.1111/j.1461-0248.2010.01530.x

Lagomarsino, L.P., Condamine, F.L., Antonelli, A., Mulch, A., Davis, C.C. (2016) The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). New Phytologist, 210, 1430–1442.

Lohmann, L. G., Bell, C. D., Calió, M. F., & Winkworth, R. C. (2013) Pattern and timing of biogeographical history in the Neotropical tribe Bignonieae (Bignoniaceae). Botanical Journal of the Linnean Society, 171(1), 154-170.

Madriñán, S., Cortés, A. J., & Richardson, J. E. (2013) Páramo is the world's fastest evolving and coolest biodiversity hotspot. Frontiers in genetics, 4, 192.

Marx, H. E., O'Leary, N., Yuan, Y. W., Lu−Irving, P., Tank, D. C., Múlgura, M. E., & Olmstead, R. G. (2010). A molecular phylogeny and classification of Verbenaceae. American journal of Botany, 97(10), 1647-1663.

Magallón, S., Gómez−Acevedo, S., Sánchez−Reyes, L. L., & Hernández−Hernández, T. (2015). A metacalibrated time−tree documents the early rise of flowering plant phylogenetic diversity. New Phytologist, 207(2), 437-453.

Matzke N.J. (2013) BioGeoBEARS: BioGeography with Bayesian (and likelihood) evolutionary analysis in R Scripts, CRAN: The Comprehensive R Archive Network, Vienna, Austria. http://cran.r-project.org/package=BioGeoBEARS

Matzke, N.J. (2014). "Model Selection in Historical Biogeography Reveals that Founder-event Speciation is a Crucial Process in Island Clades." Systematic Biology. 63(6): 951-970. http://dx.doi.org/10.1093/sysbio/syu056

Montes, C., Cardona, A., McFadden, R., Morón, S. E., Silva, C. A., Restrepo-Moreno, S., ... & Bayona, G. A. 2012. Evidence for middle Eocene and younger land emergence in central Panama: implications for Isthmus closure. Bulletin, 124(5-6), 780-799.

Moore, B. R., & Donoghue, M. J. (2007) Correlates of diversification in the plant clade Dipsacales: geographic movement and evolutionary innovations. the american naturalist, 170(S2), S28-S55.

Nürk, N., Scheriau, & C., Madriñán S. (2013) Explosive radiation in high Andean Hypericum—rates of diversification among New World lineages. Frontiers in Genetics, 4, 175. doi:10.3389/fgene.2013.00175

Nürk, N., Uribe-Convers, S., Gehrke, B., Tank, D.C., & Blattner, F.R. (2015) Oligocene niche shift, Miocene diversification – cold tolerance and accelerated speciation rates in the St. John's Worts (Hypericum, Hypericaceae). BMC Evolutionary Biology, 15, 80. doi: 10.1186/s12862-015-0359-4

Nürk, N.M., Michling, F., & Linder, H.P. (2018) Are the radiations of temperate lineages in tropical alpine ecosystems pre−adapted? Global Ecology and Biogeography, 27, 334–345. https://doi.org/10.1111/geb.12699

Olmstead, R. G. (2013). Phylogeny and biogeography in Solanaceae, Verbenaceae and Bignoniaceae: a comparison of continental and intercontinental diversification patterns. Botanical Journal of the Linnean Society, 171(1), 80-102.

Ornelas, J. F. & González, C. (2014) Interglacial genetic diversification of Moussonia deppeana (Gesneriaceae), a hummingbird−pollinated, cloud forest shrub in northern Mesoamerica. Molecular Ecology, 23, 4119-4136. doi:10.1111/mec.12841

Paradis E., Claude J. & Strimmer K. (2004) APE: analyses of phylogenetics and evolution in R language. Bioinformatics, 20, 289-290.

Pennington, R.T., Lavin, M., Prado, D.E., Pendry, C.A., Pell, S.K. & Butterworth, C.A. (2004) Historical climate change and speciation: Neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. Philosophical Transactions of the Royal Society B: Biological Sciences, 359, 515-537. DOI: 10.1098/rstb.2003.1435

Pennington, R.T., Lavin, M., Särkinen, T., Lewis, G.P., Klitgaard, B.B., & Hughes, C.E. (2010) Contrasting plant diversification histories within the Andean biodiversity hotspot. Proceedings of the National Academy of Sciences, 107 (31), 13783-13787. DOI: 10.1073/pnas.1001317107

Peterson, A.T., Soberon, J., Sanchex-Cordero, V. (1999) Conservatism of Ecological Niches in Evolutionary Time. Science, 285, 1265-1267. DOI: 10.1126/science.285.5431.1265

Plummer, M., Best, N., Cowles, K., & Vines, K. (2006) CODA: Convergence Diagnosis and Output Analysis for MCMC. R News, 6, 7-11.

Rabosky, D.L., Santini, F., Eastman, J.M., Smith, S.A., Sidlauskas, B., Chang, J. & Alfaro, M.E. (2013) Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. Nature Communications, 4, 1958. doi:10.1038/ncomms2958.

Rabosky, D.L. (2014) Automatic detection of key innovations, rate shifts, and diversity−dependence on phylogenetic trees. PLoS One, 9, e89543.

Rabosky, D.L., Grundler, M.C., Anderson, C.J., Title, P.O., Shi, J.J., Brown, J.W., Huang, H., & Larson, J.G. (2014) BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. Methods in Ecology and Evolution, 5, 701-707.

Revell, L. J. (2012) phytools: An R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution. 3 217-223. doi:10.1111/j.2041-210X.2011.00169.x

Richardson, J.E., Pennington, R.T., Pennington, T.D., Hollingsworth, P.M. (2001) Rapid Diversification of a species-rich genus of Neotropical rain forest trees. Science, 293, 2242-2245.

Ricklefs, R.E. (2004) A comprehensive framework for global patterns in biodiversity. Ecology Letters, 7(1):1-15

Särkinen, T., Pennington, R.T., Lavin, M., Simon, M.F., and Hughes, C.E. (2012) Evolutionary islands in the Andes: persistence and isolation explain high endemism in Andean dry tropical forests. Journal of Biogeography, 39, 884-900

Schnitzler, J., Graham, C.H., Dormann, C.F., Schiffers, K., Linder, H.P. (2012) Climatic niche evolution and species diversification in the Cape flora, South Africa. Journal of Biogeograpy, 39(12), 2201-2211

Schrire, B.D., Lavin, M., Barker, N.P., Forest, F. 2009. Phylogeny of the tribe Indigofereae (Leguminosae—Papilionoideae): geographically structured more in succulent-rich and temperate settings than in grass-rich environments. American Journal of Botany, 96(4), 816-852

Schwery, O., Onstein, R.E., Bouchenak-Khelladi, Y., Xing, Y., Carter, R.J., Linder, H.P. (2015) As old as the mountains: the radiations of the Ericaceae. New Phytologist, 207, 355-367. doi: 10.1111/nph.13234

Simon, M.F, Grether, R., de Queiroz, L.P., Skema, C., Pennington, R.T., Hughes, C.E. (2009) Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. Proceeding son the National Academy of Sciences, 106(48), 816-852

Sklenář, P., Dušková, E. & Balslev, H. (2011) Tropical and Temperate: Evolutionary History of Páramo Flora. The Botanical Review, 77(2): 71-108. https://doi.org/10.1007/s12229-010-9061-9

Spriggs, E. L., Clement, W. L., Sweeney, P. W., Madriñán, S., Edwards, E. J., & Donoghue, M. J. (2015) Temperate radiations and dying embers of a tropical past: the diversification of Viburnum. New Phytologist, 207(2), 340-354.

Tiede, Y., Homeier, J., Cumbicus, N., Peña, J., Albrecht, J., Ziegenhagen, B., . . . Farwig, N. (2016) Phylogenetic niche conservatism does not explain elevational patterns of species

richeness, phylodiversity and family age of tree assemblages in Andean rainforest. Erdkunde, 70(1), 83-106.

Töpel, M., Antonelli, A. Yesson, C., Eriksen, B. (2012) Past climate change and plant evolution in western North America: a case study in Rosaceae. PLOS ONE, 7(12), e50358

Uyeda, J.C., Eastman, J., & Harmon, L. (2013) bayou: Bayesian fitting of Ornstein-Uhlenbeck models to phylogenies. R package version 1.0.

Uyeda, J.C., & Harmon, L. (2014) A Novel Bayesian Method for Inferring and Interpreting the Dynamics of Adaptive Landscapes from Phylogenetic Comparative Data. Systematic Biology, 63 (6), 902-918. DOI:10.1093/sysbio/syu057

van der Hammen, T. (1974). The Pleistocene changes of vegetation and climate in tropical South America. Journal of Biogeography, 3-26.

Weeks, A., Zapata, F., Pell, S. K., Daly, D. C., Mitchell, J. D., & Fine, P. V. 2014. To move or to evolve: contrasting patterns of intercontinental connectivity and climatic niche evolution in "Terebinthaceae"(Anacardiaceae and Burseraceae). Frontiers in Genetics, 5, 409.

Werneck, F.P. (2011) The diversification of eastern South American open vegetation biomes: historical biogeography and perspectives. Quaternary Science Reviews, 30, 1630-1648. doi: 10.1016/j.quascirev.2011.03.2009

Wiens, J.J., & Graham, C.H. (2005) Niche conservatism: integrating evolution, ecology, and conservation biology. Annual Review of Ecology, Evolution, and Systematics, 36, 519-539.

Yu, Y., Harris, A.J., Blair, C., & He, X.J. (2015) RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Molecular Phylogenetics and Evolution,* 87, 46-49.

TABLES

Table 1. Divergence times for major clades of *Citharexylum*.

	Stem age (error)	Crown age (error)
Citharexylum	**14.84** (19.25-11.44)	**5.90** (8.42-3.54)
subg. Altamiranum	**5.90** (8.42-3.54)	**5.90** (8.42-3.54)
- SA clade	**4.00** (5.21-3.39)	**3.71** (4.38-2.35)
subg. Sylvaticum	**3.71** (4.38-2.35)	**3.05** (4.19-2.16)
subg. Andinum	**3.71** (4.38-2.35)	**3.15** (4.07-1.96)
- MA clade	**4.00** (5.21-3.39)	**3.66** (4.47-2.76)
subg. Affine	**3.66** (4.47-2.76)	**0.55** (1.63-0.0022)
subg. Mexicanum	**3.45** (4.31-2.49)	**0.79** (2.14-0.11)
sugb. Citharexylum	**3.45** (4.31-2.49)	**1.85** (3.16-1.33)

Table 2. Inferred biome shifts and their locations from discrete biome reconstructions in BioGeoBEARS and analysis of PC1 and PC2 scores and individual climate variables in bayou with support values and biological interpretation.

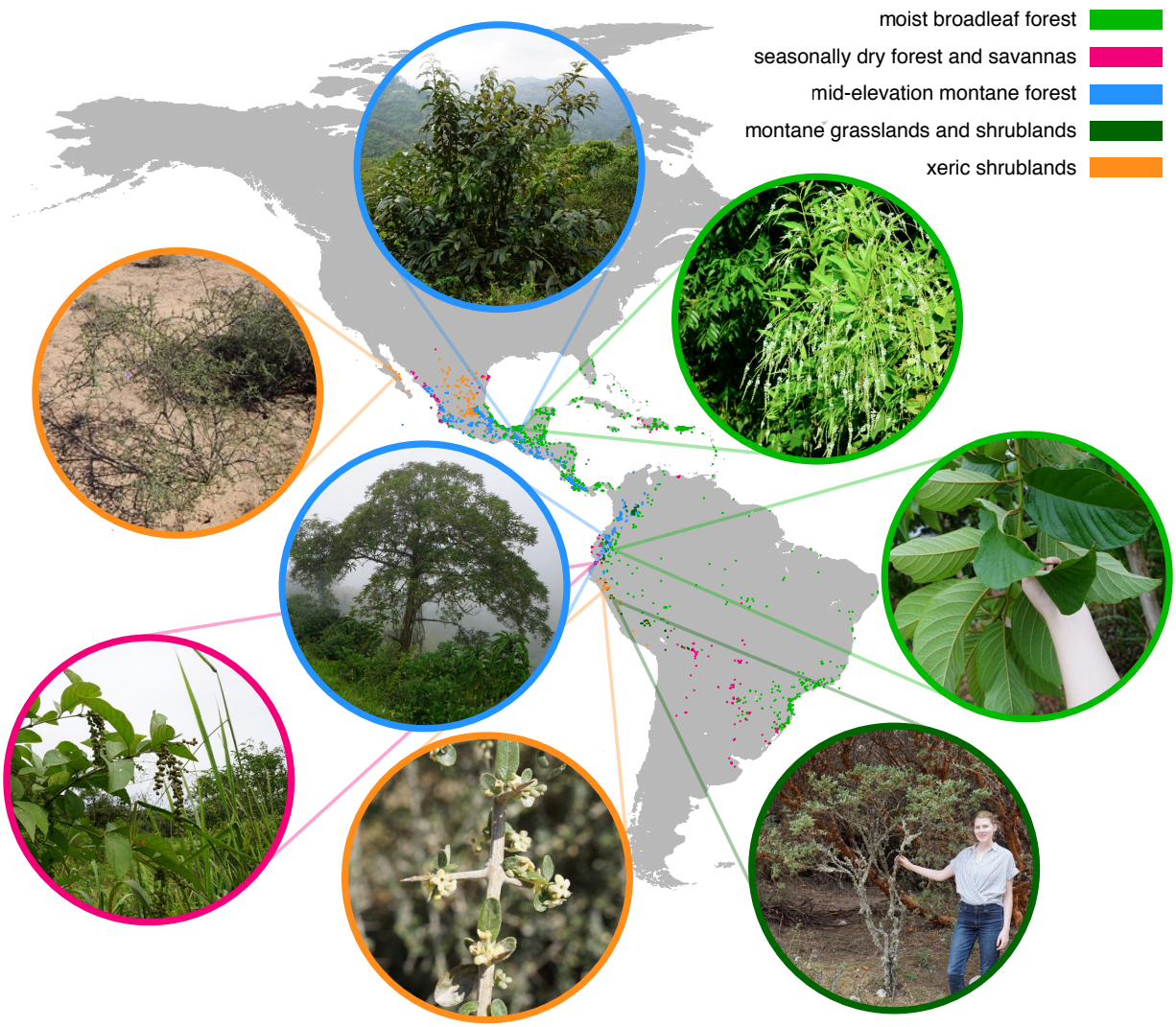
FIGURE LEGENDS

Fig. 1 Distribution map of *Citharexylum*. Points indicate individual occurrences, and points are colored according to the biome assignment for that given species. Photos of representative species for each biome include:

Fig. 2 Multispecies coalescent tree with divergence time estimations from *BEAST and ancestral area reconstructions for geographic area. Bars at nodes indicate error surrounding age estimates. Colored squares at branch tips indicate the current distribution of each species; colors and assignments correspond to the map in the top left corner. Pie charts on nodes display the probability of the reconstructed ancestral biome and indicate locations of dispersal events on the tree.

Fig. 3 Biome reconstructions mapped on the MSC tree, trimmed to tribe Citharexyleae. Pie charts on nodes indicate the probability of a given biome at that node. Colors correspond to biome assignments, noted by the legend in the lower left. The map in the center displays the extent of each Neotropical biome in the New World; it does not represent the distribution of *Citharexylum*. The graph on the left provides the relative size of biomes (km^2) and relative placement of biomes in relation to annual temperature and annual precipitation. Arrows indicate the direction and number of shifts between biomes; heavier weighted arrows indicate more shifts from one biome to another.

Fig. 4 Niche evolution and diversification rate analysis results. Heated colors on branches represent relative rates inferred from the MEDUSA-like model depicted using quantile breaks to highlight variation between lineages. Circles mapped onto to trees represent: (a) shifts detected in PC scores for PC1 (closed circles) and PC2 (open circles), size represents the posterior probability of the shift; (b) summary results of individual climate variables, size of circles reflects the number of variables that recovered a shift on that branch (smallest circles represent a shift in one variable, the largest circle represents shifts in eleven variables); and (c) locations of shifts inferred in the discrete biome analysis for comparison, the size of the circles is arbitrary.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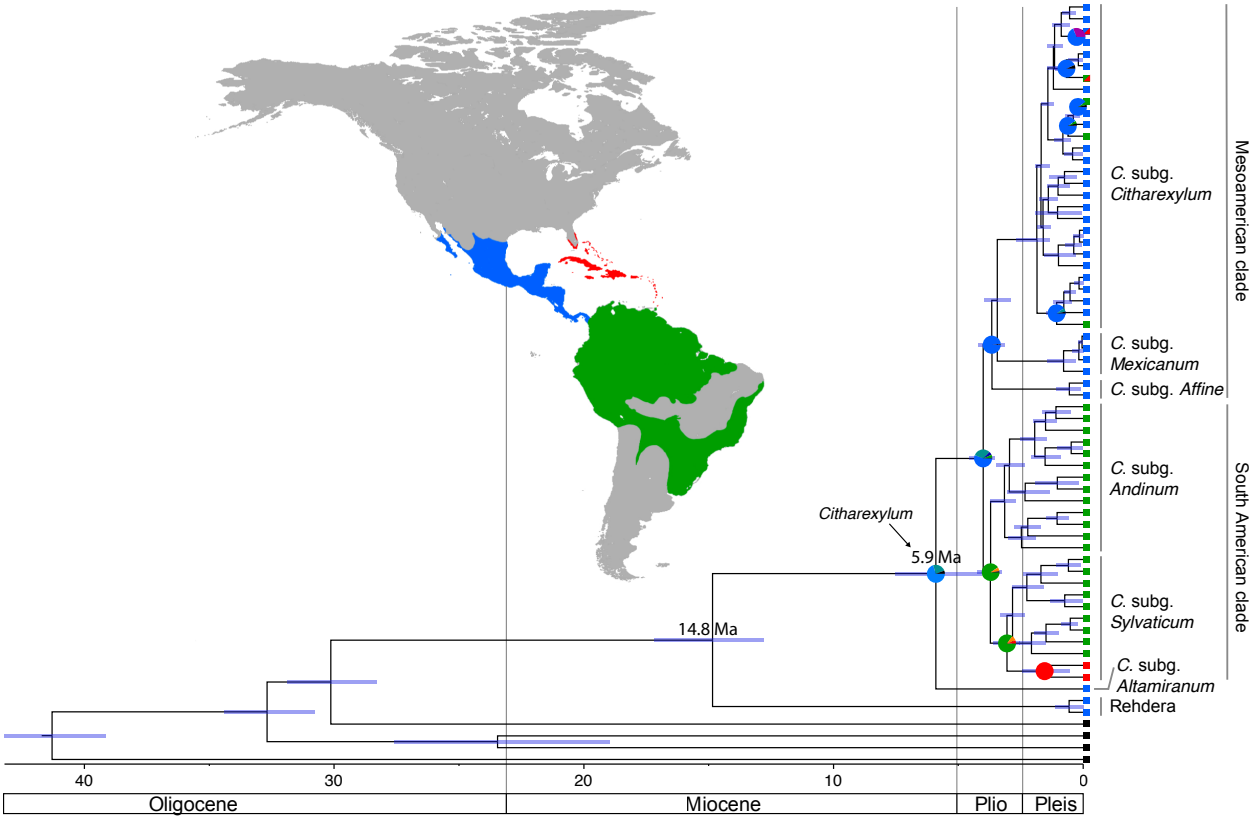
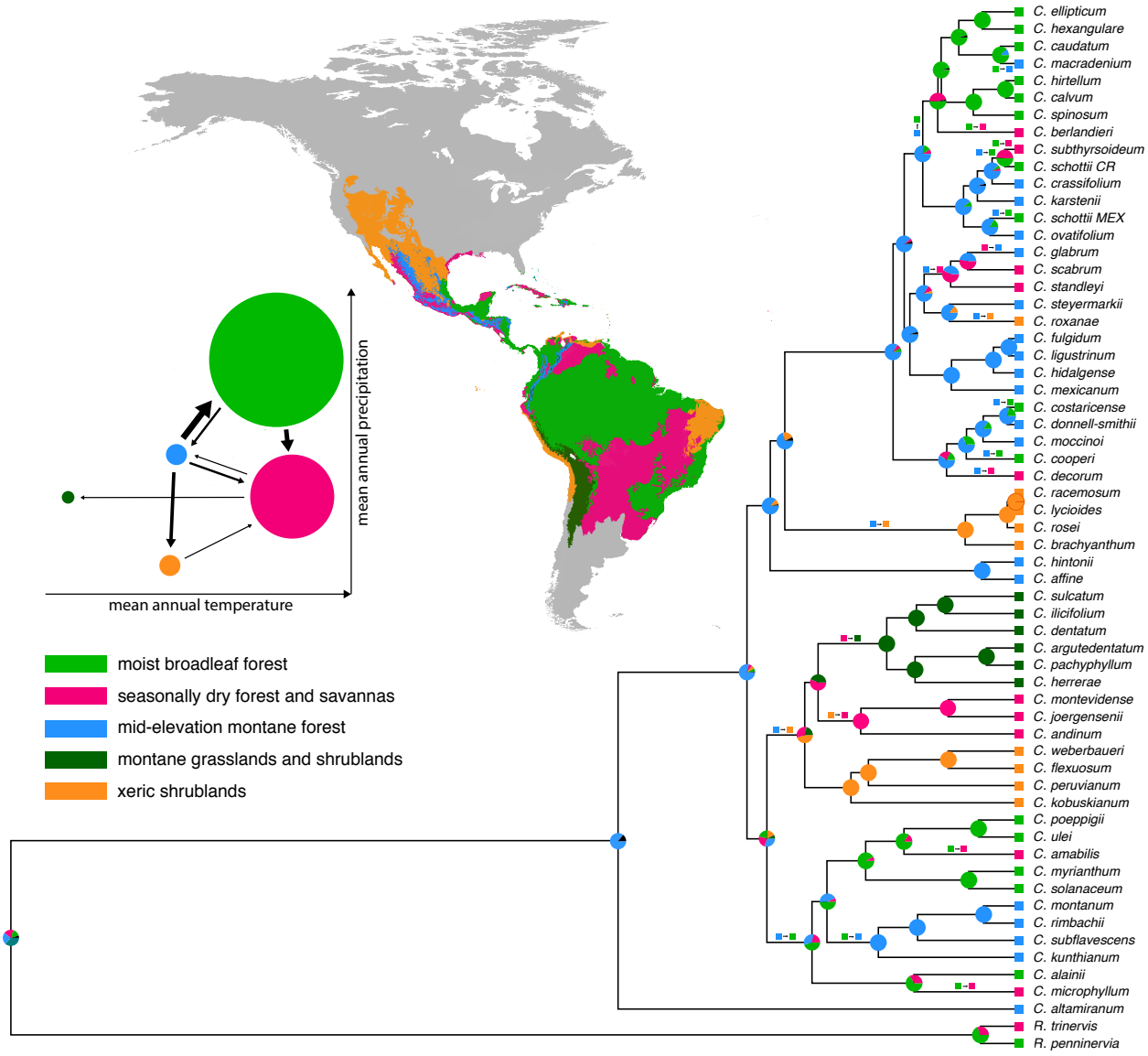
seasonally dry forest and savannas
mid-elevation montane forest
montane grasslands and shrublands
xeric shrublands


C. subg. Citharexylum
C. subg. Mexicanum
C. subg. Affine
C. subg. Andinum
C. subg. Sylvaticum
C. subg. Altamiranum
Rehdera
Mesoamerican clade
South American clade
Citharexylum
5.9 Ma
14.8 Ma
40
30
20
10
0
Oligocene
Miocene
Plio
Pleis


mean annual precipitation
mean annual temperature
moist broadleaf forest
seasonally dry forest and savannas
mid-elevation montane forest
montane grasslands and shrublands
xeric shrublands
C. ellipticum
C. hexangulare
C. caudatum
C. macradenium
C. hirtellum
C. calvum
C. spinosum
C. berlandieri
C. subthyrsoideum
C. schottii CR
C. crassifolium
C. karstenii
C. schottii MEX
C. ovatifolium
C. glabrum
C. scabrum
C. standleyi
C. steyermarkii
C. roxanae
C. fulgidum
C. ligustrinum
C. hidalgense
C. mexicanum
C. costaricense
C. donnell-smithii
C. moccinoi
C. cooperi
C. decorum
C. racemosum
C. lycioides
C. rosei
C. brachyanthum
C. hintonii
C. affine
C. sulcatum
C. ilicifolium
C. dentatum
C. argutedentatum
C. pachyphyllum
C. herrerae
C. montevidense
C. joergensenii
C. andinum
C. weberbaueri
C. flexuosum
C. peruvianum
C. kobuskianum
C. poeppigii
C. ulei
C. amabilis
C. myrianthum
C. solanaceum
C. montanum
C. rimbachii
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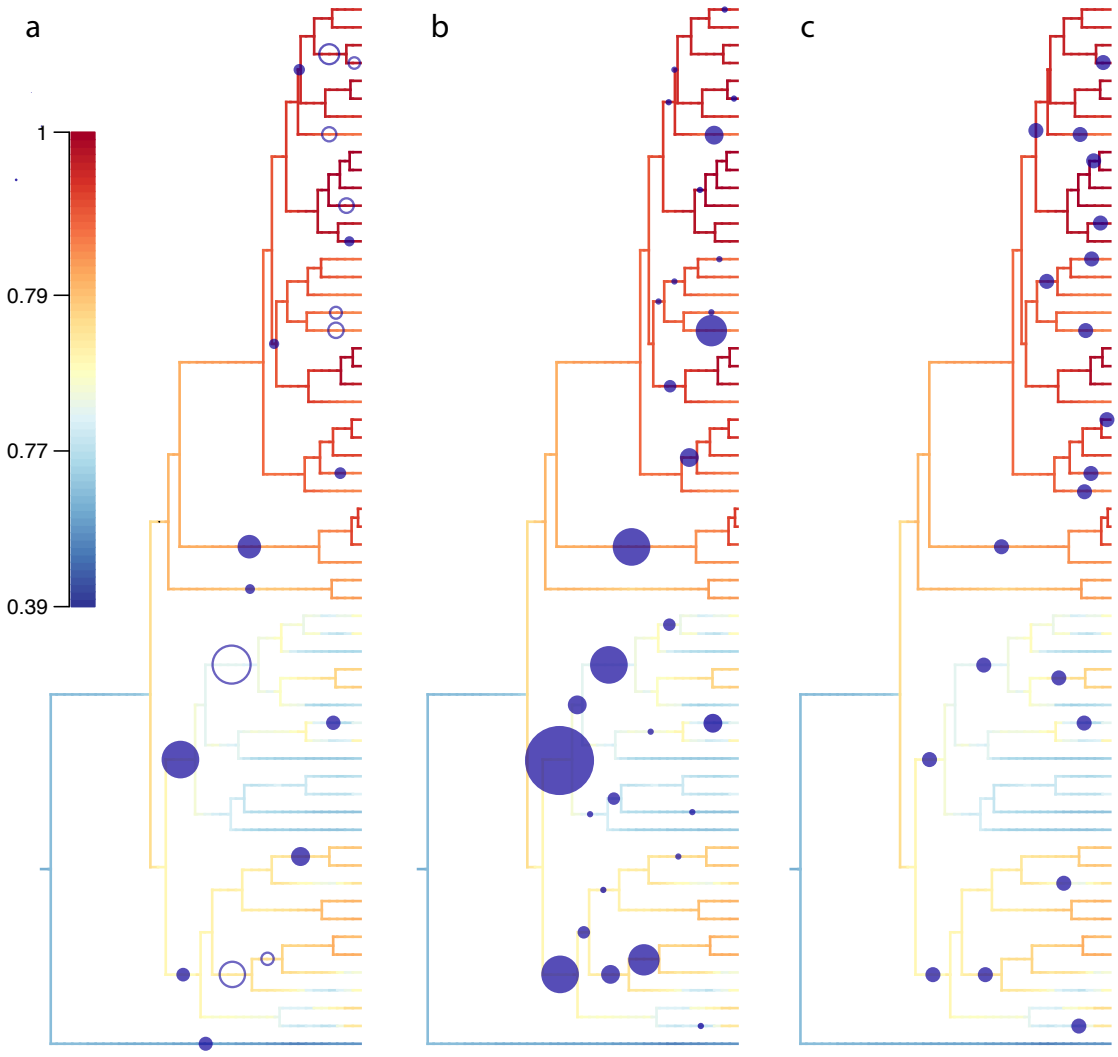
C. microphyllum
C. altamiranum
R. trinervis
R. penninervia


a
b
c
1
0.79
0.77
0.39

SUPPLEMENTARY FIGURES

Fig. S1. bayou output for analysis of PC1 scores (posterior probability cutoff = 0.##) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S2. bayou output for analysis of PC2 scores (posterior probability cutoff = 0.12) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S3. bayou output for analysis of mean annual temperature (WorldClim BioClim variable Bio 1; posterior probability cutoff = 0.1) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S4. bayou output for analysis of mean diurnal range (WorldClim BioClim variable Bio 2; posterior probability cutoff = 0.14) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S5. bayou output for analysis of isothermality (WorldClim BioClim variable Bio 3; posterior probability cutoff = 0.2) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S6. bayou output for analysis of temperature seasonality (WorldClim BioClim variable Bio 4; posterior probability cutoff = 0.3) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on

phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S7. bayou output for analysis of maximum temperature of warmest month (WorldClim BioClim variable Bio 5; posterior probability cutoff = 0.2) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S8. bayou output for analysis of minimum temperature of coldest month (WorldClim BioClim variable Bio 6; posterior probability cutoff = 0.11) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S9. bayou output for analysis of annual temperature range (WorldClim BioClim variable Bio 7; posterior probability cutoff = 0.12) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S10. bayou output for analysis of mean temperature of wettest quarter (WorldClim BioClim variable Bio 8; posterior probability cutoff = 0.15) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S11. bayou output for analysis of mean temperature of driest quarter (WorldClim BioClim variable Bio 9; posterior probability cutoff = 0.12) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S12. bayou output for analysis of mean temperature of warmest quarter (WorldClim BioClim variable Bio 10; posterior probability cutoff = 0.2) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S13. bayou output for analysis of mean temperature of coldest quarter (WorldClim BioClim variable Bio 11; posterior probability cutoff = 0.13) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S14. bayou output for analysis of mean annual precipitation (WorldClim BioClim variable Bio 12; posterior probability cutoff = 0.15) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S15. bayou output for analysis of precipitation of wettest month (WorldClim BioClim variable Bio 13; posterior probability cutoff = 0.14) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S16. bayou output for analysis of precipitation of driest month (WorldClim BioClim variable Bio 14; posterior probability cutoff = 0.3) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S17. bayou output for analysis of precipitation seasonality (WorldClim BioClim variable Bio 15; posterior probability cutoff = 0.2) including plots for (a) phylogenetic tree with posterior

probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S18. bayou output for analysis of precipitation of wettest quarter (WorldClim BioClim variable Bio 16; posterior probability cutoff = 0.13) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S19. bayou output for analysis of precipitation of driest quarter (WorldClim BioClim variable Bio 17; posterior probability cutoff = 0.3) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

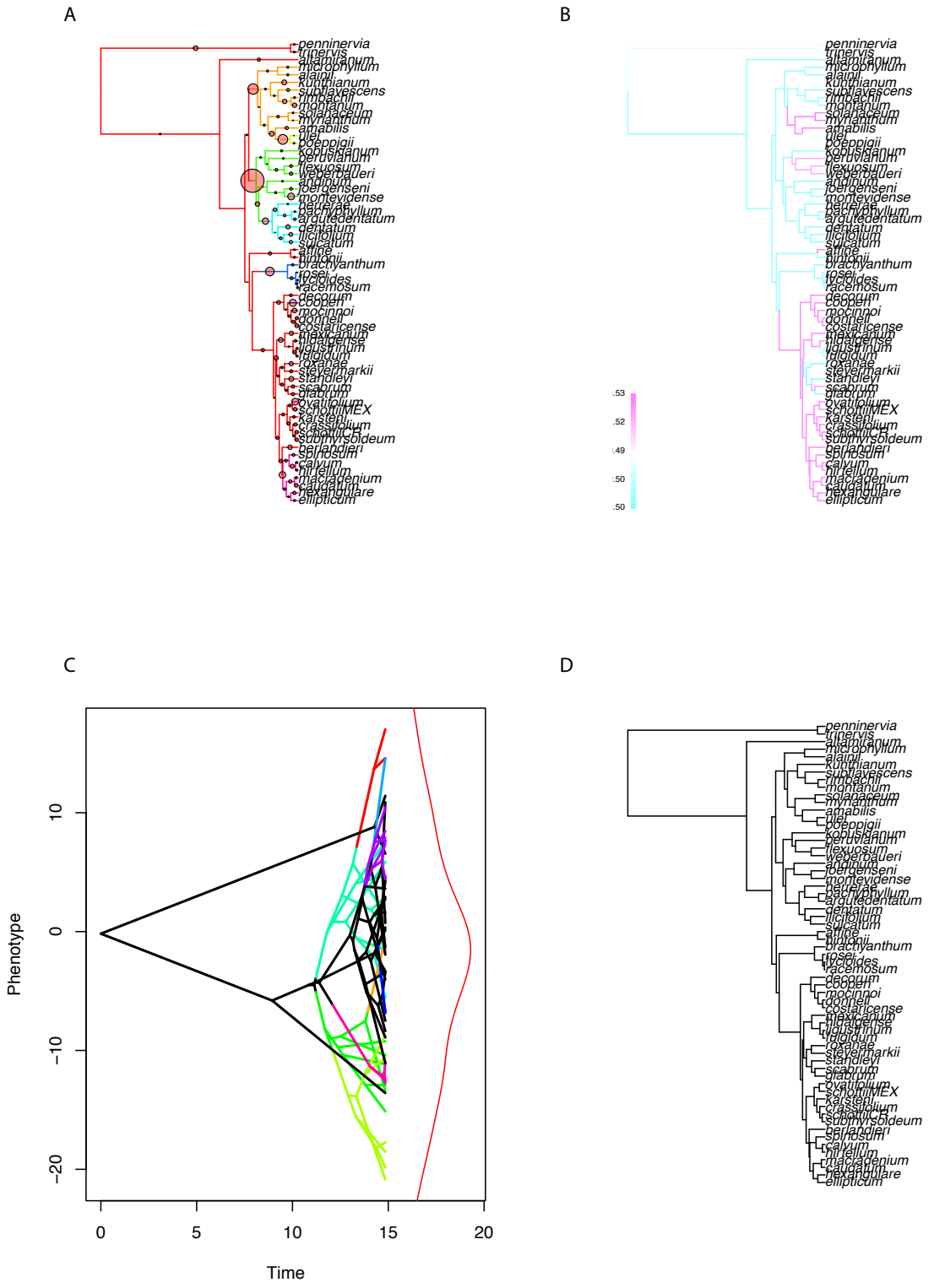
Fig. S20. bayou output for analysis of precipitation of warmest quarter (WorldClim BioClim variable Bio 18; posterior probability cutoff = 0.2) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S21. bayou output for analysis of precipitation of coldest quarter (WorldClim BioClim variable Bio 19; posterior probability cutoff = 0.18) including plots for (a) phylogenetic tree with posterior probabilities from the bayouMCMC chain (b) heat map of reconstructed parameter values on phylogeny (c) phenogram and posterior density for optima values (d) topology of input phylogeny.

Fig. S22. 95% confidence set of diversification rate shift configurations inferred in BAMM using the default model.

Fig. S23. 95% confidence set of diversification rate shift configurations inferred in BAMM using the MEDUSA-like model.

Fig. S1. PC1


A
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C
D
penninervia
trinervis
altamiranum
microphyllum
alainii
kuntzianum
subtiavescens
rimbachii
montanum
solanaceum
myrianthum
amabilis
ulei
poeppigii
kobuskianum
peruvianum
flexuosum
weberbaueri
andinum
bergenseni
montevidense
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dentatum
ilicifolium
sulcatum
alnifolia
burtonii
brachyanthum
rosei
ilicoides
racemosum
decorum
cooperi
mocinnoi
donnell
costaricense
mexicanum
nitidense
uliginosum
triplicium
roxanae
stevenmarkii
standleyi
scabrum
glabrum
ovatifolium
schottiMEX
karstenii
crassifolium
schottii
subintrisoideum
berlandieri
spinosum
calvum
nitellum
macfadenium
hexangulare
elliptica
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.52
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Phenotype
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−20
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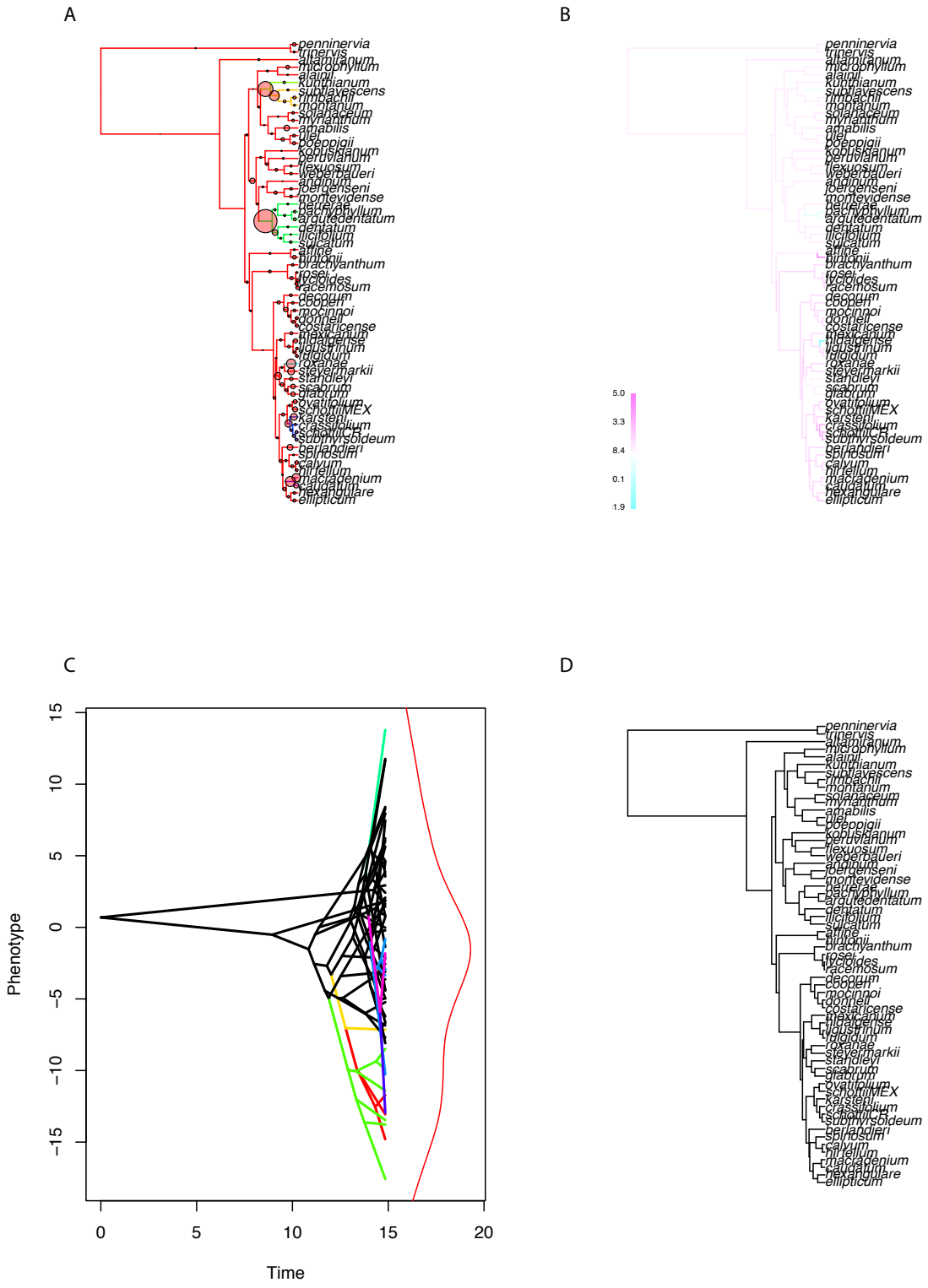
5
10
15
20
Time

Fig. S2. PC2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B
C
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penninervia
trinervis
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subtiavescens
rimbachii
montanum
solanaceum
myrianthum
amabilis
ulei
poeppigii
kobuskianum
peruvianum
flexuosum
weberbaueri
arginum
roergenseni
montevidense
hederae
pachyphyllum
ardutedentatum
dentatum
ilicifolium
sulcatum
aiphe
hintonii
brachyanthum
rosei
ilicoides
racemosum
decorum
cooperi
mocinnoi
donnell
costaricense
mexicanum
nitidense
ligustrinum
riparium
roxanae
stevenmarkii
standleyi
scabrum
glabrum
ovatifolium
schottiMEX
karstenii
crassifolium
schottii
submyrsinoideum
berlandieri
spinosum
calvum
hirtellum
macfadenium
cauliflorum
hexangulare
ellipticum
5.0
3.3
8.4
0.1
1.9
Phenotype
Time
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10
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0
−5
−10
−15
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5
10
15
20

Fig. S3. Bio 1: mean annual temperature

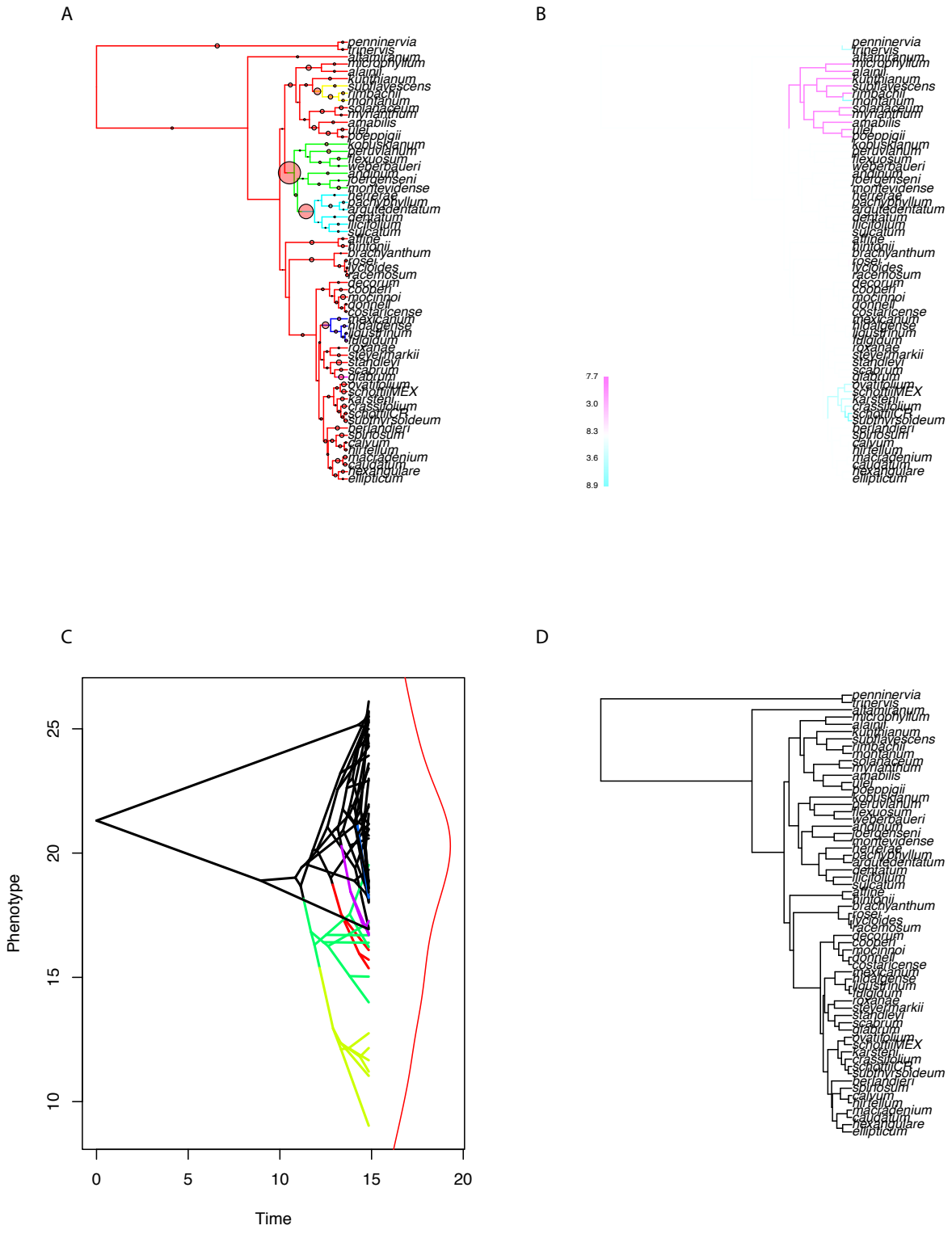


Fig. S4. Bio 2: mean diurnal range

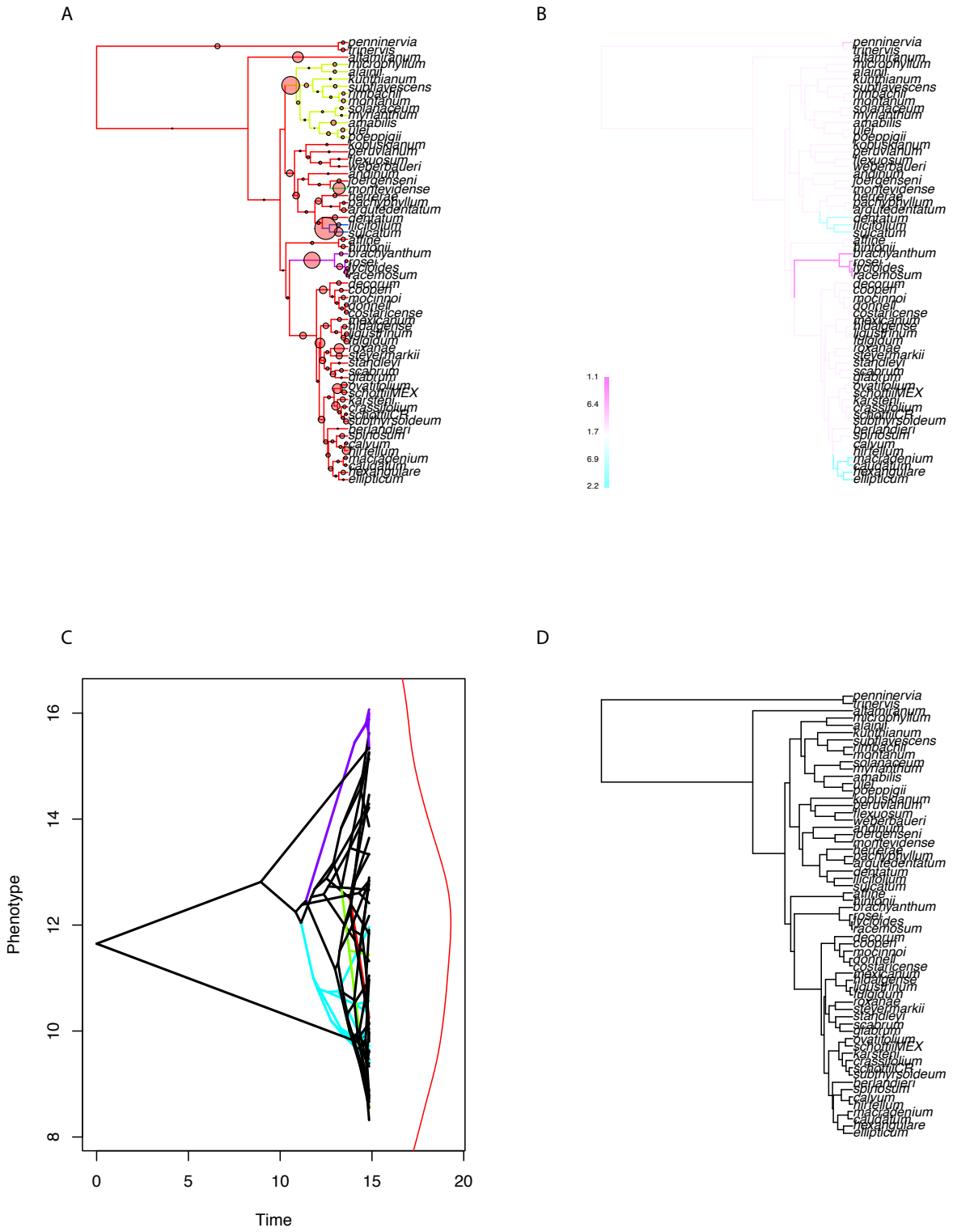


Fig. S5. Bio 3: isothermality

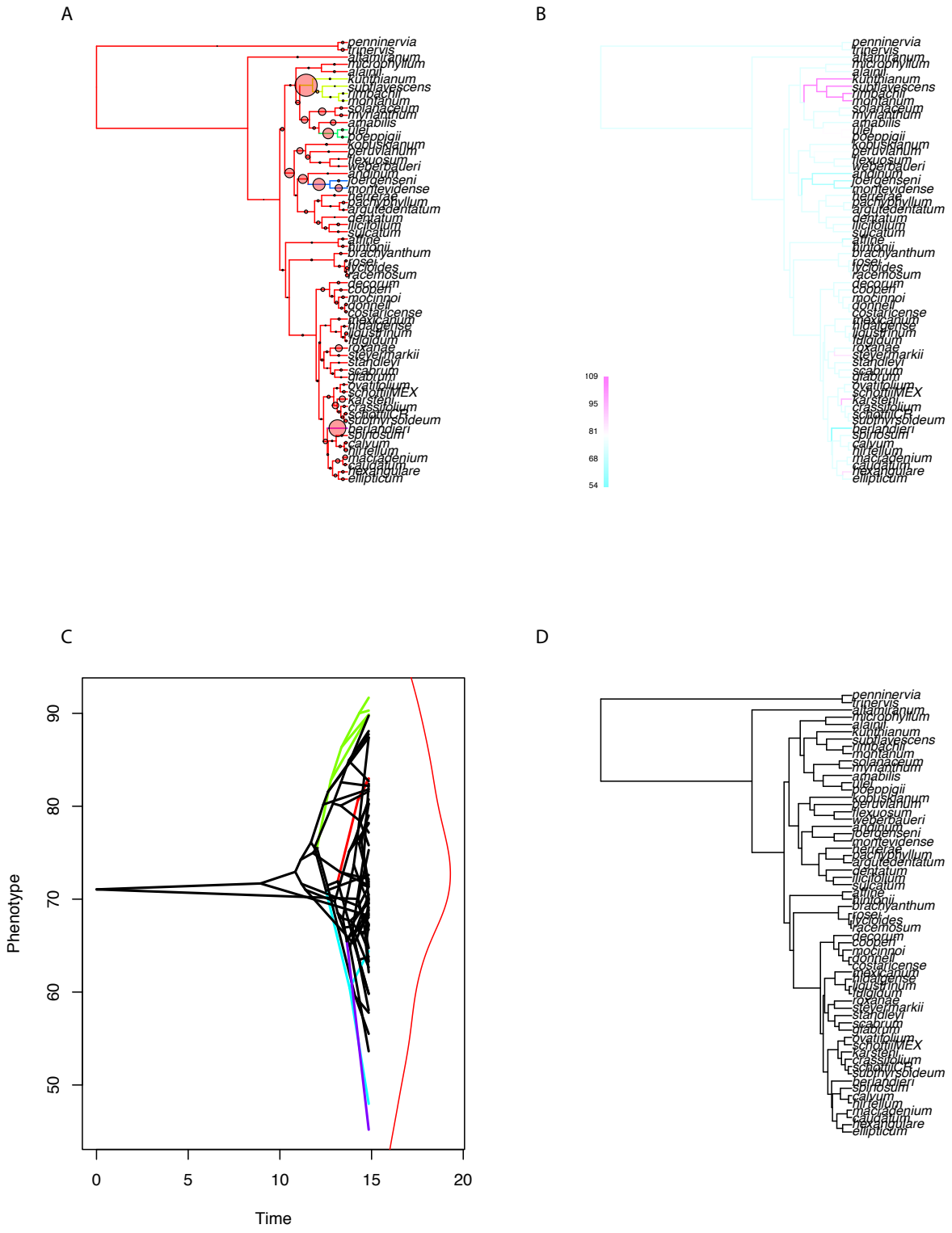


Fig. S6. Bio 4: temperature seasonality

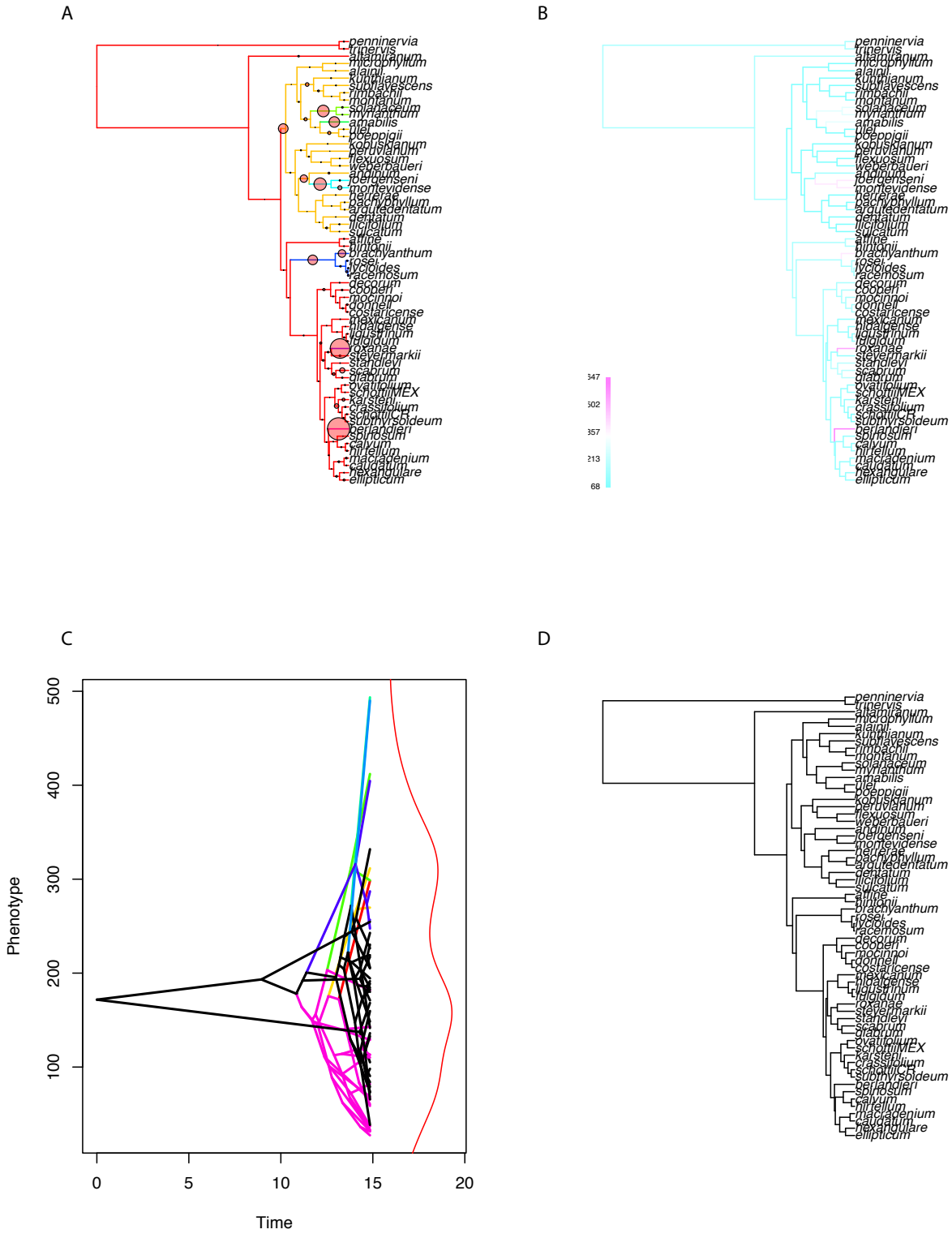


Fig. S7. Bio 5: maximum temperature of warmest month

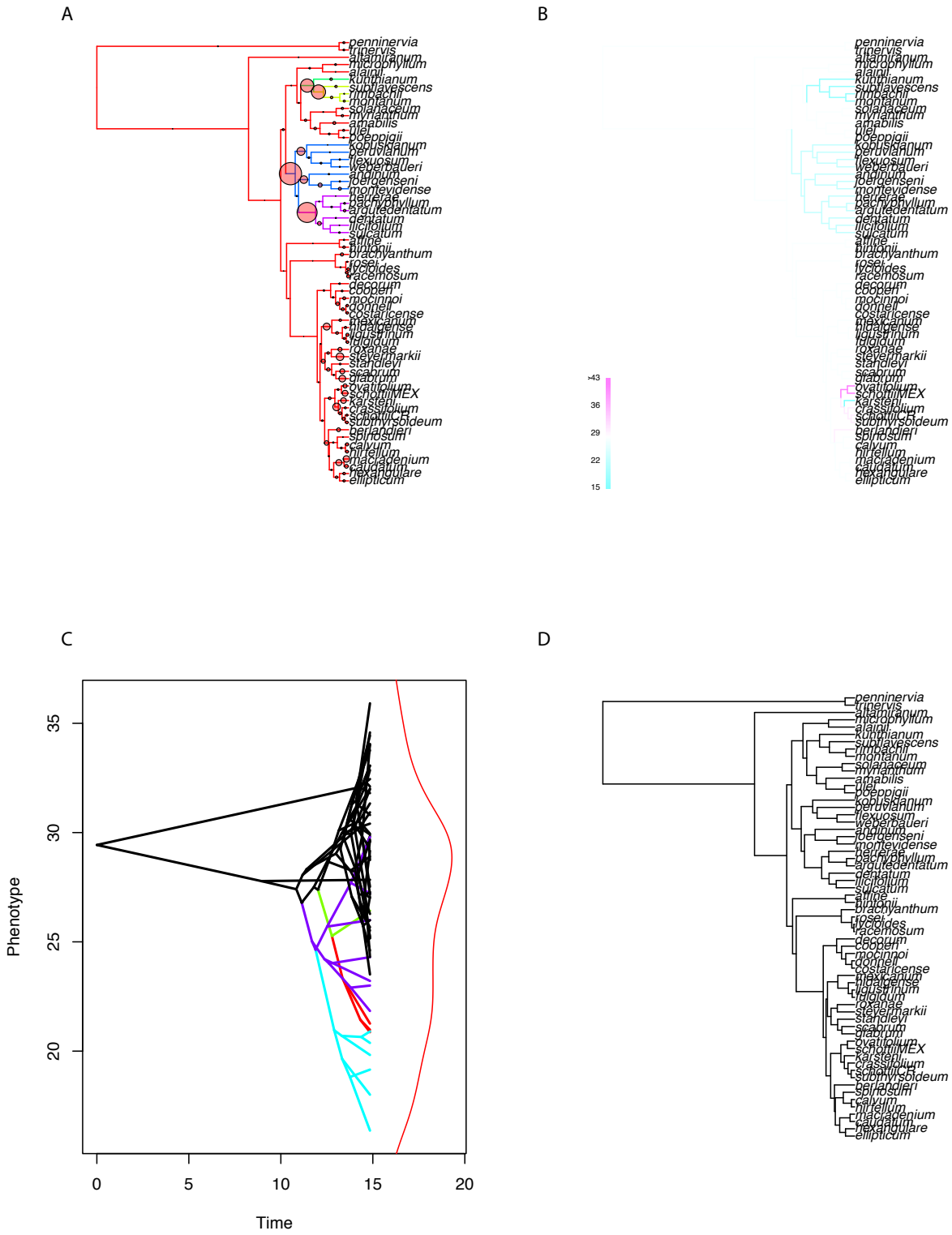


Fig. S8. Bio 6: minimum temperature of coldest month

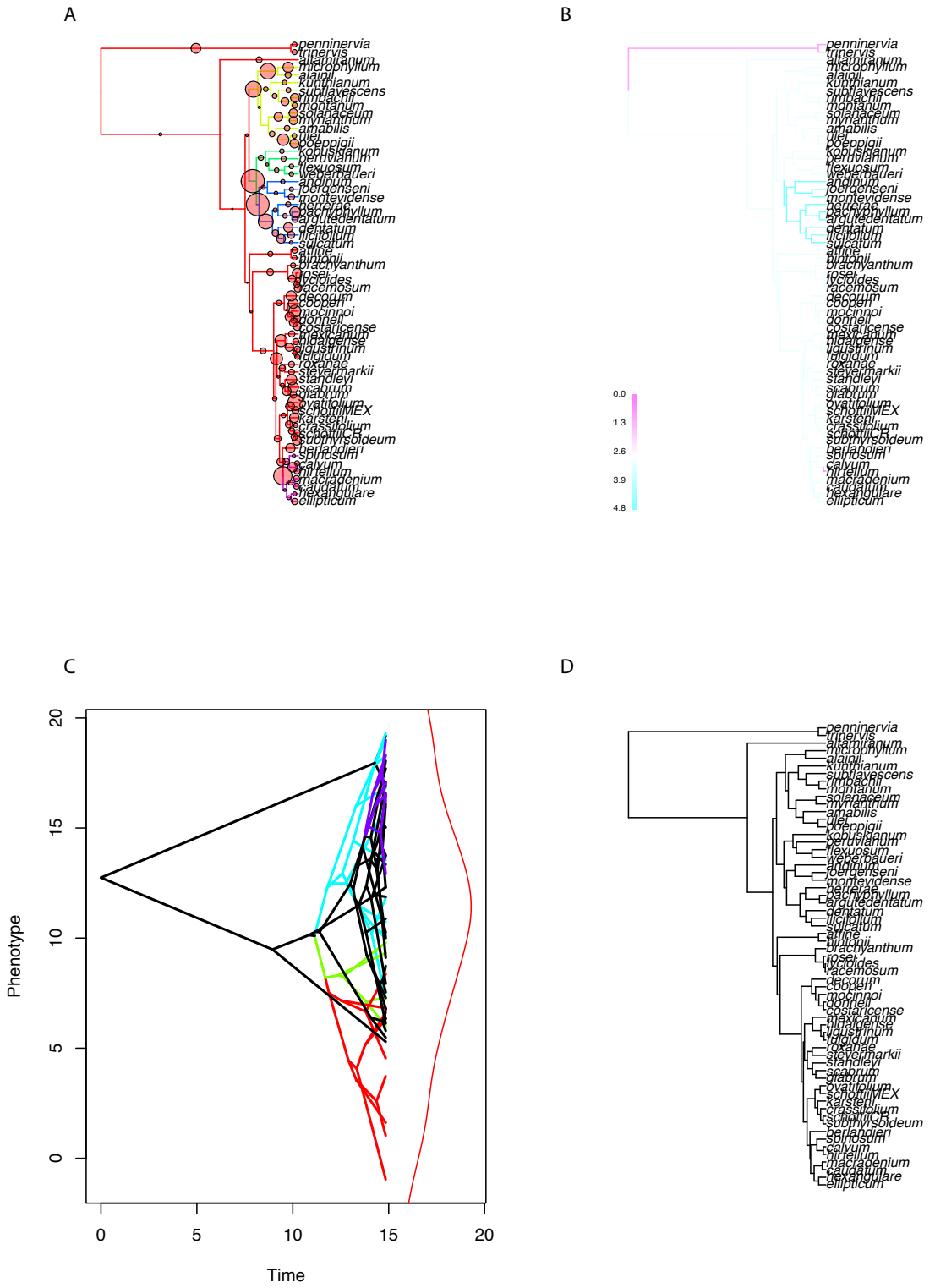


Fig. S9. Bio 7: temperature annual range

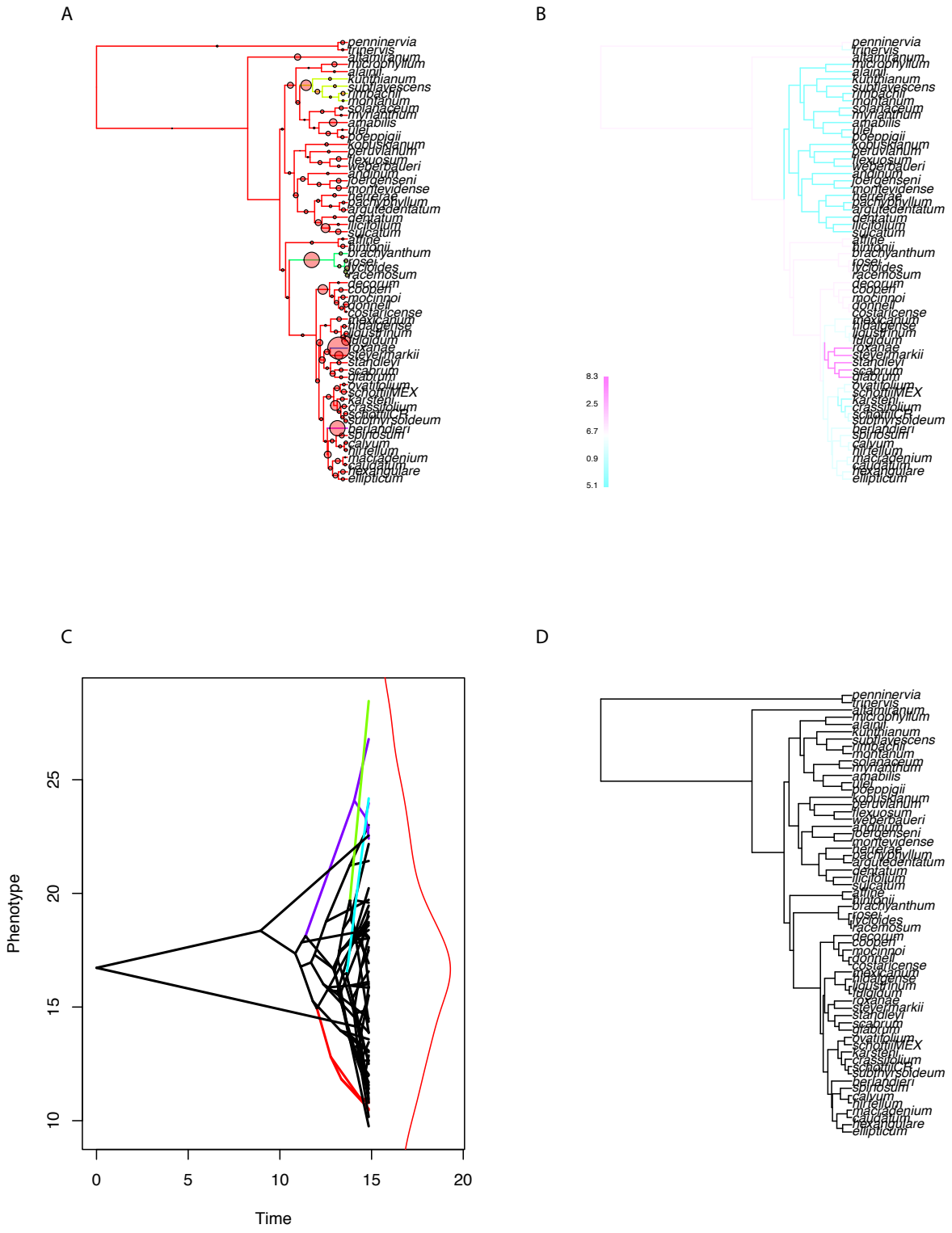


Fig. S10. Bio 8: mean temperature of wettest quarter

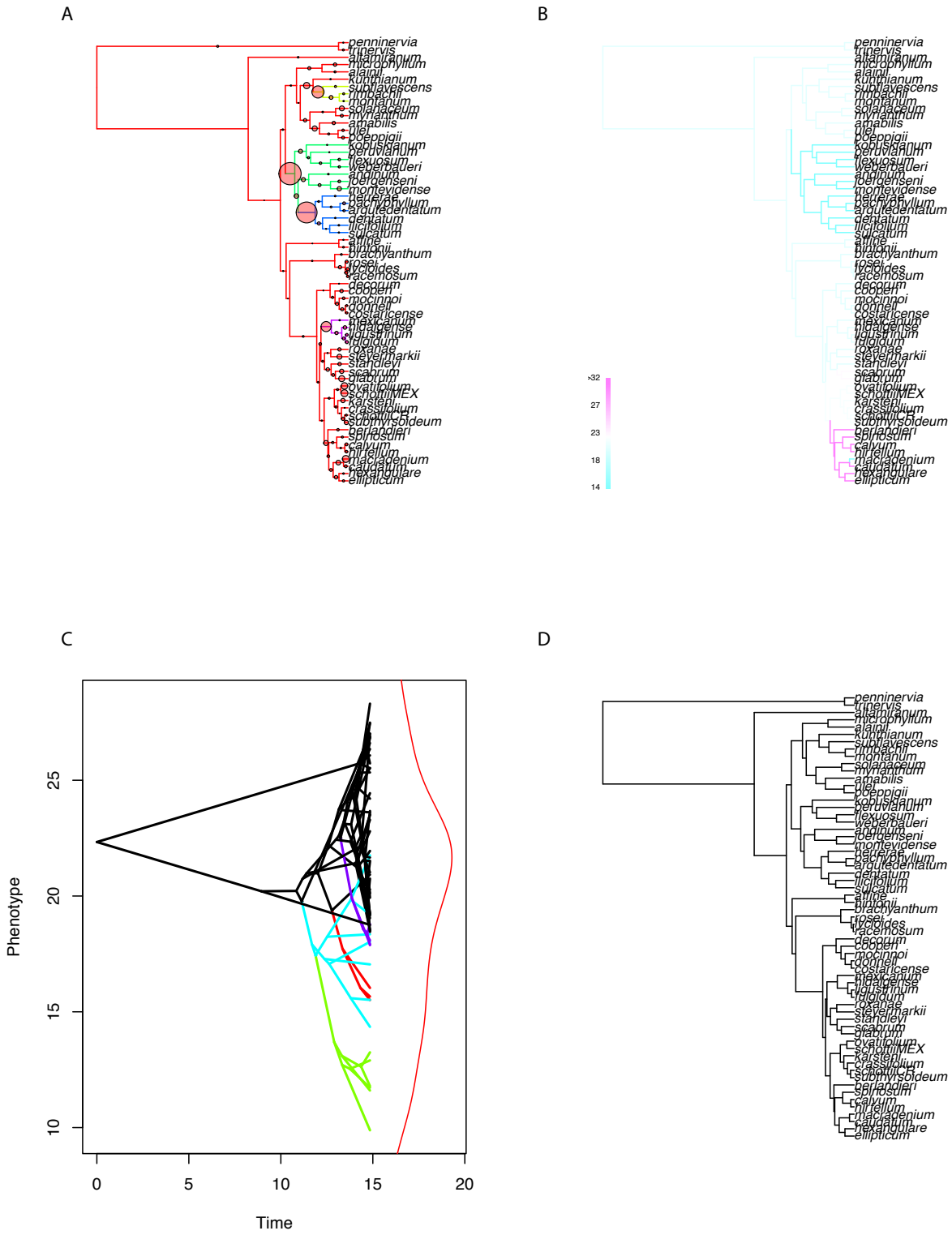


Fig. S11. Bio 9: mean temperature of driest quarter

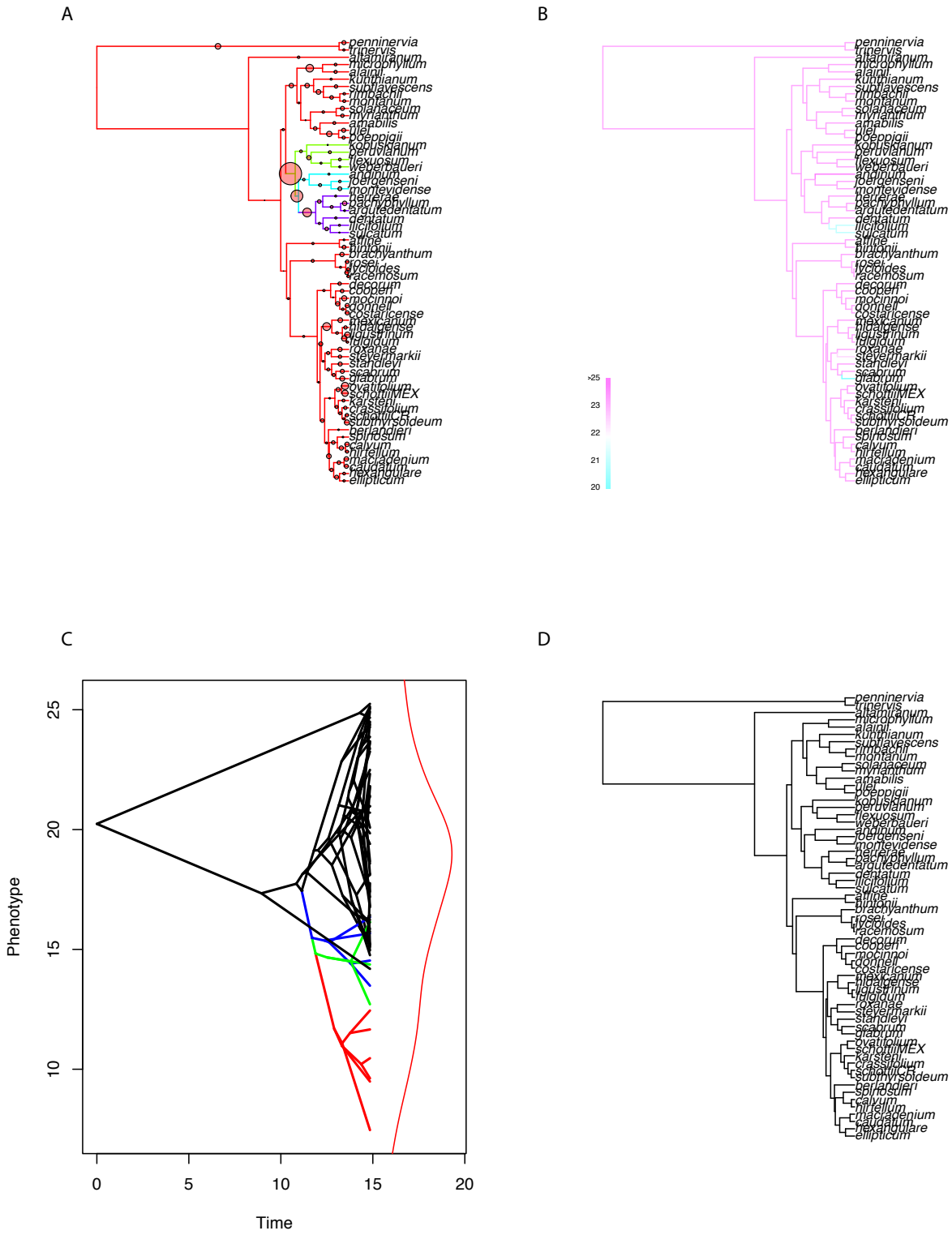


Fig. S12. Bio 10: mean temperature of warmest quarter

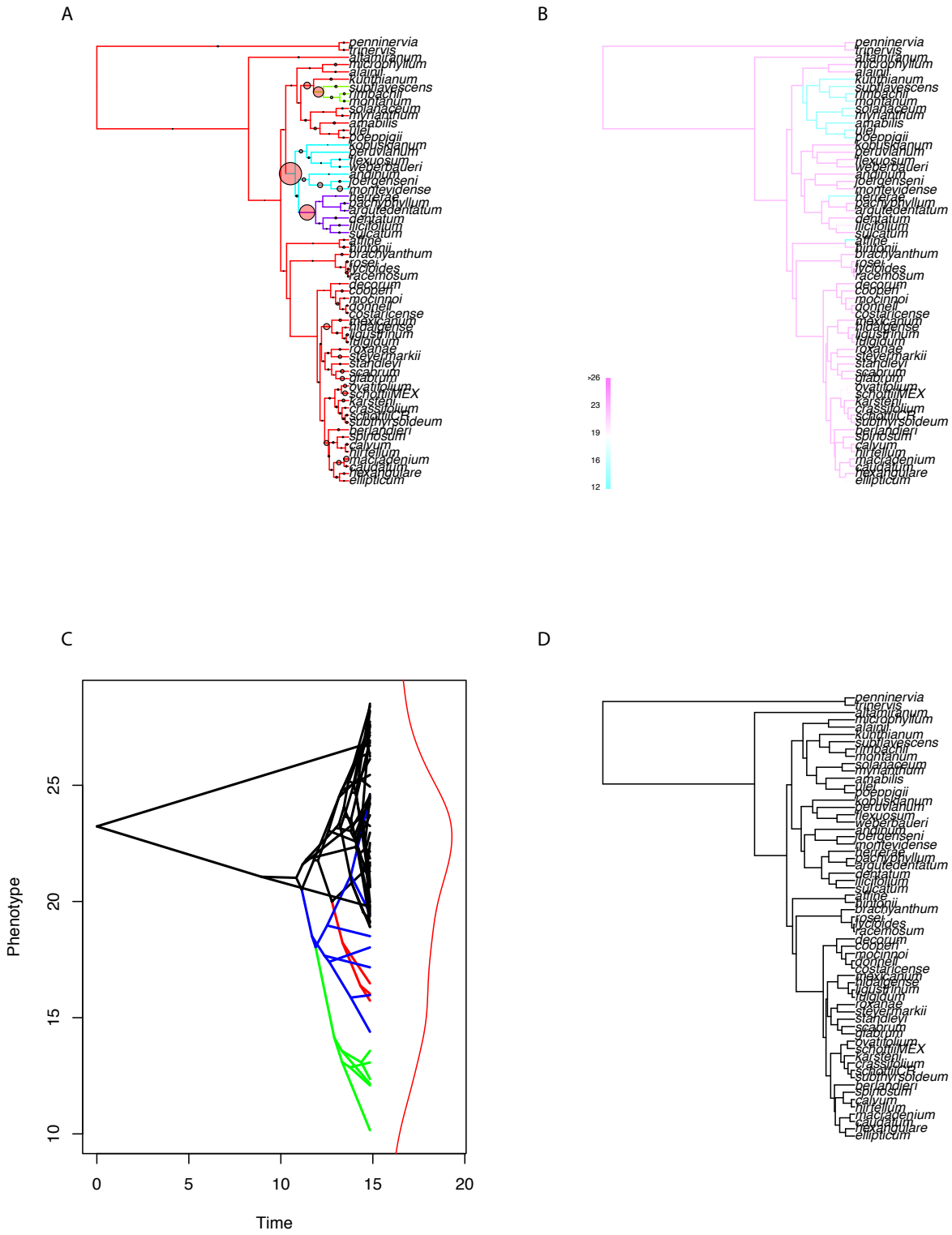


Fig. S13. Bio 11: mean temperature of coldest quarter

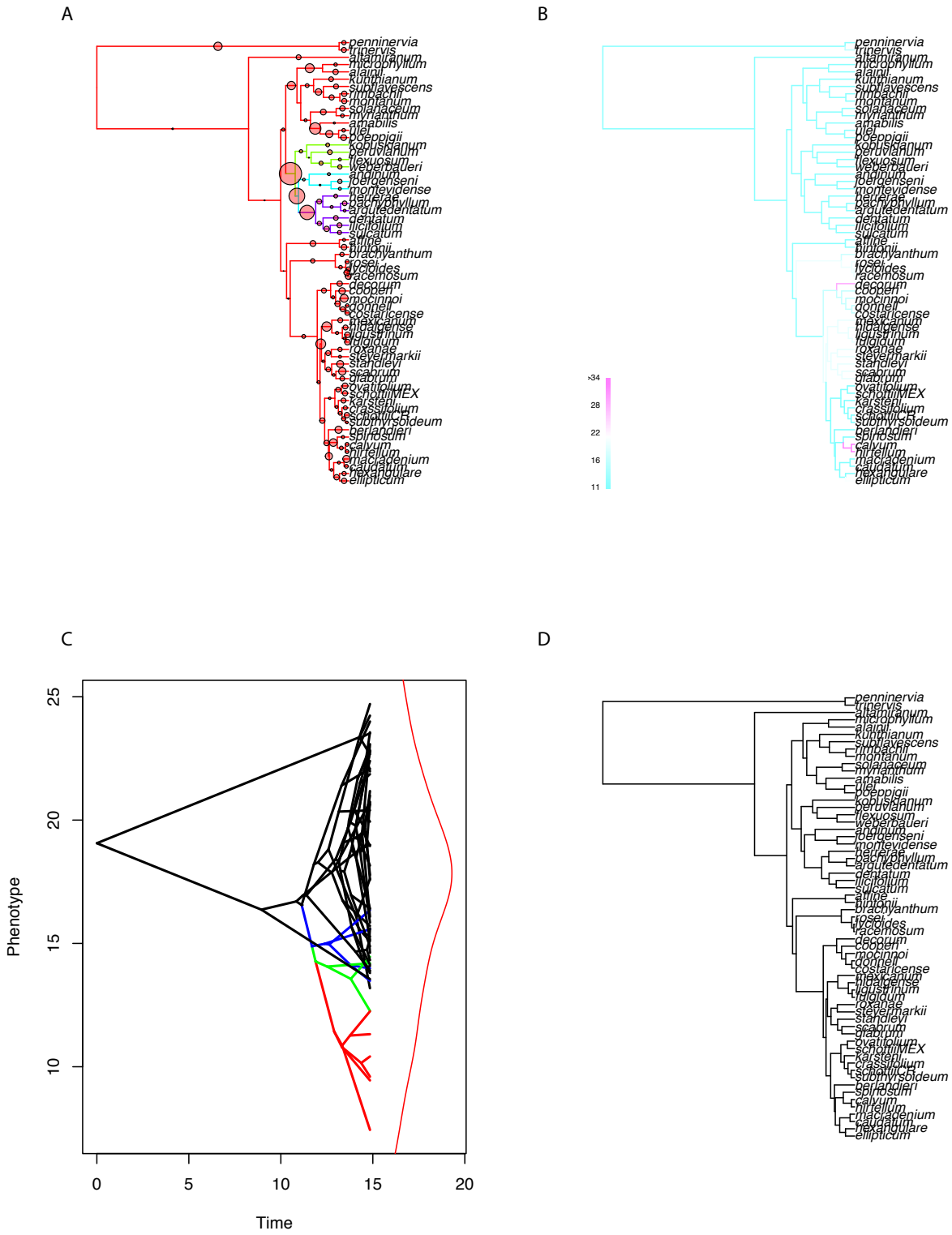


Fig. S14. Bio 12: mean annual precipitation

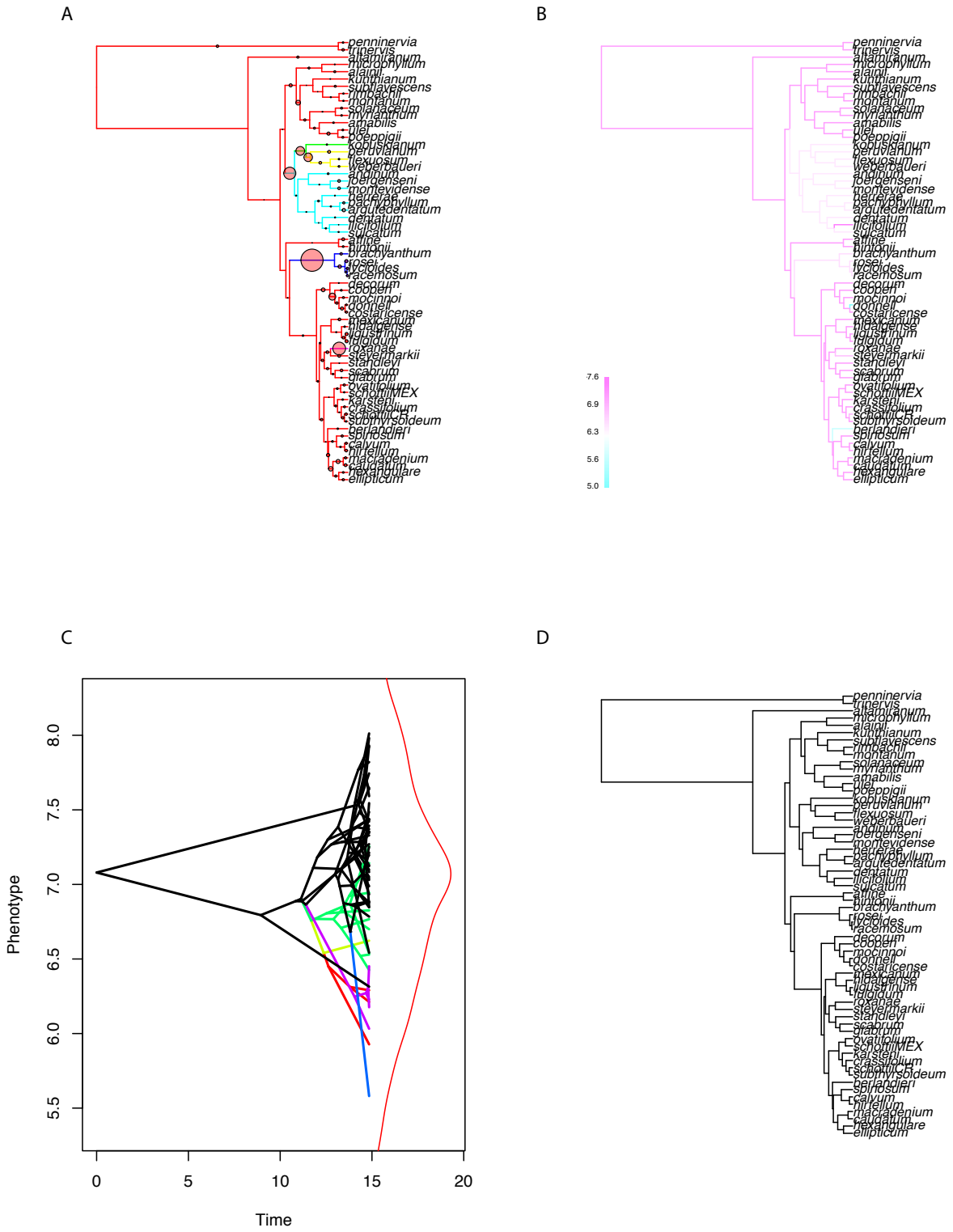


Fig. S15. Bio 13: precipitation of wettest month

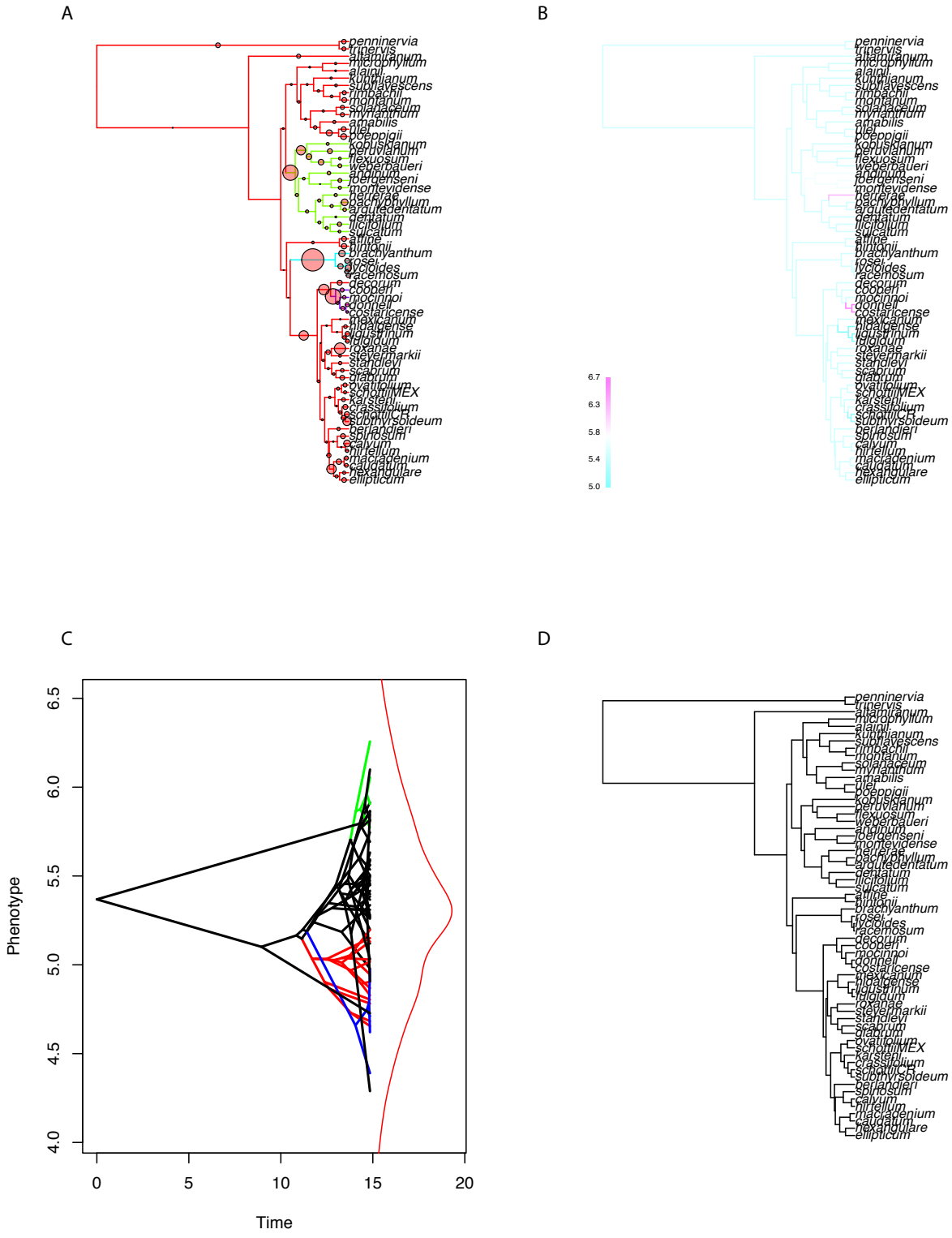


Fig. S16. Bio 14: precipitation of driest month

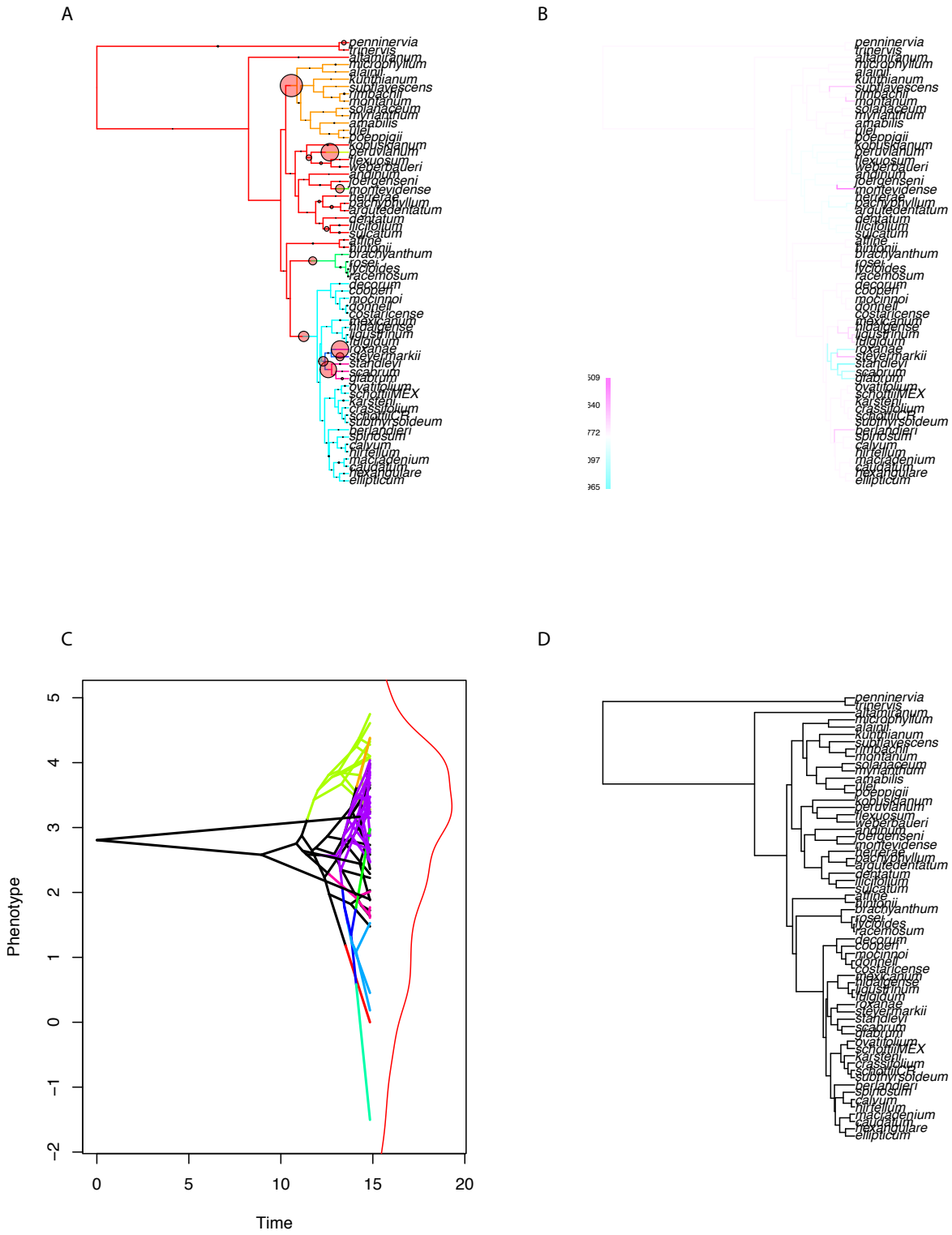


Fig. S17. Bio 15: precipitation seasonality

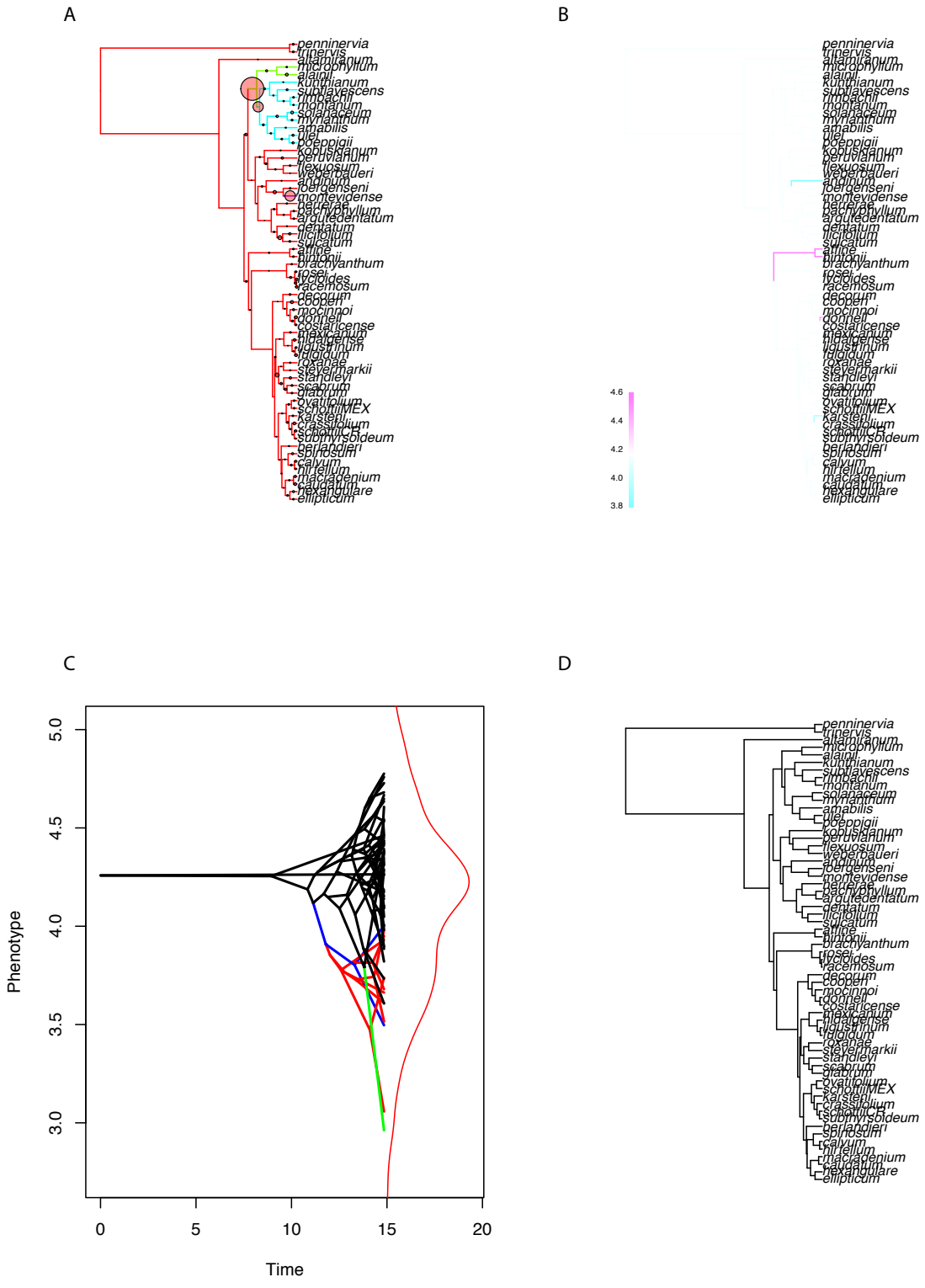


Fig. S18. Bio 16: precipitation of wettest quarter

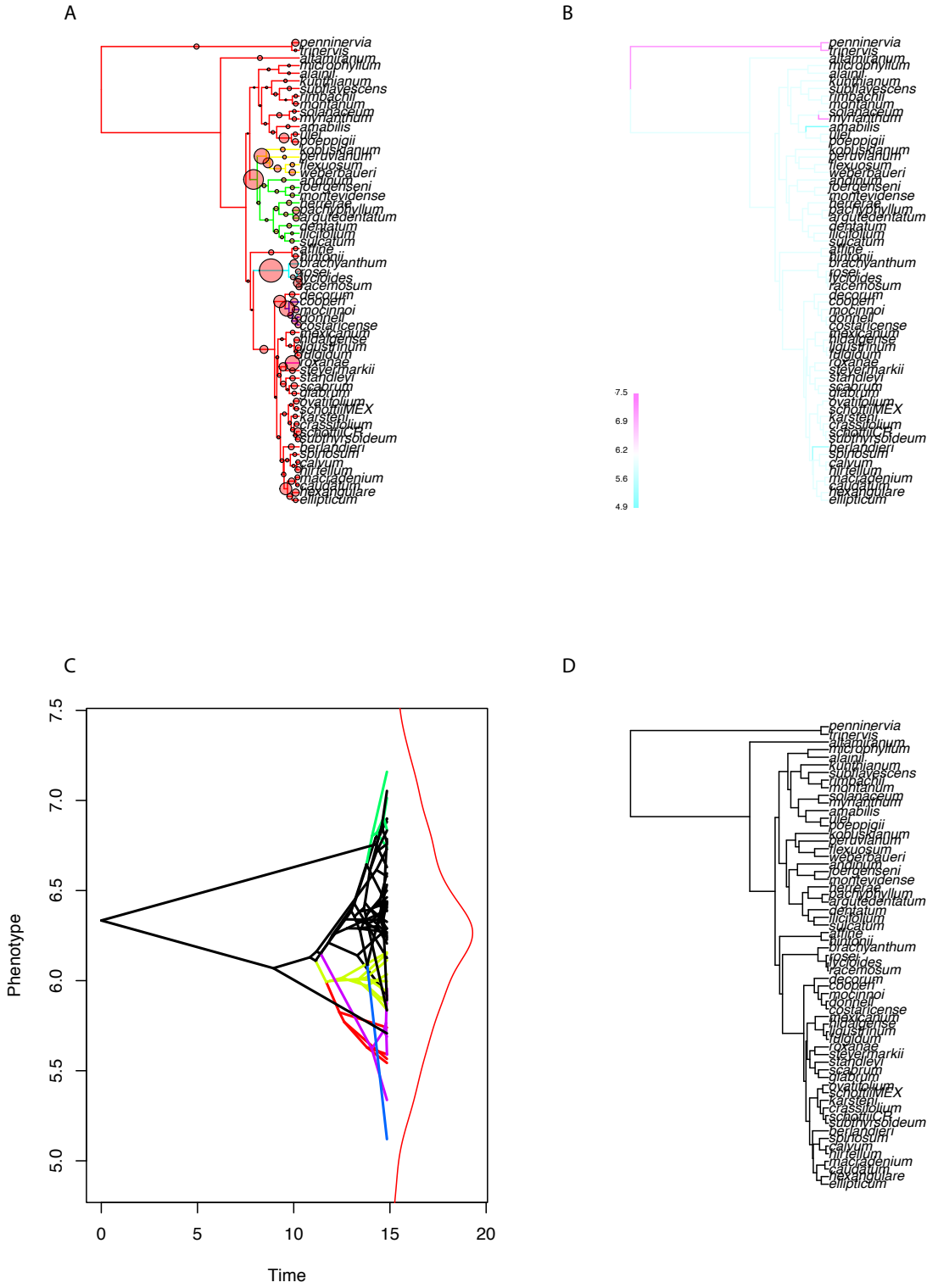


Fig. S19. Bio 17: precipitation of driest quarter

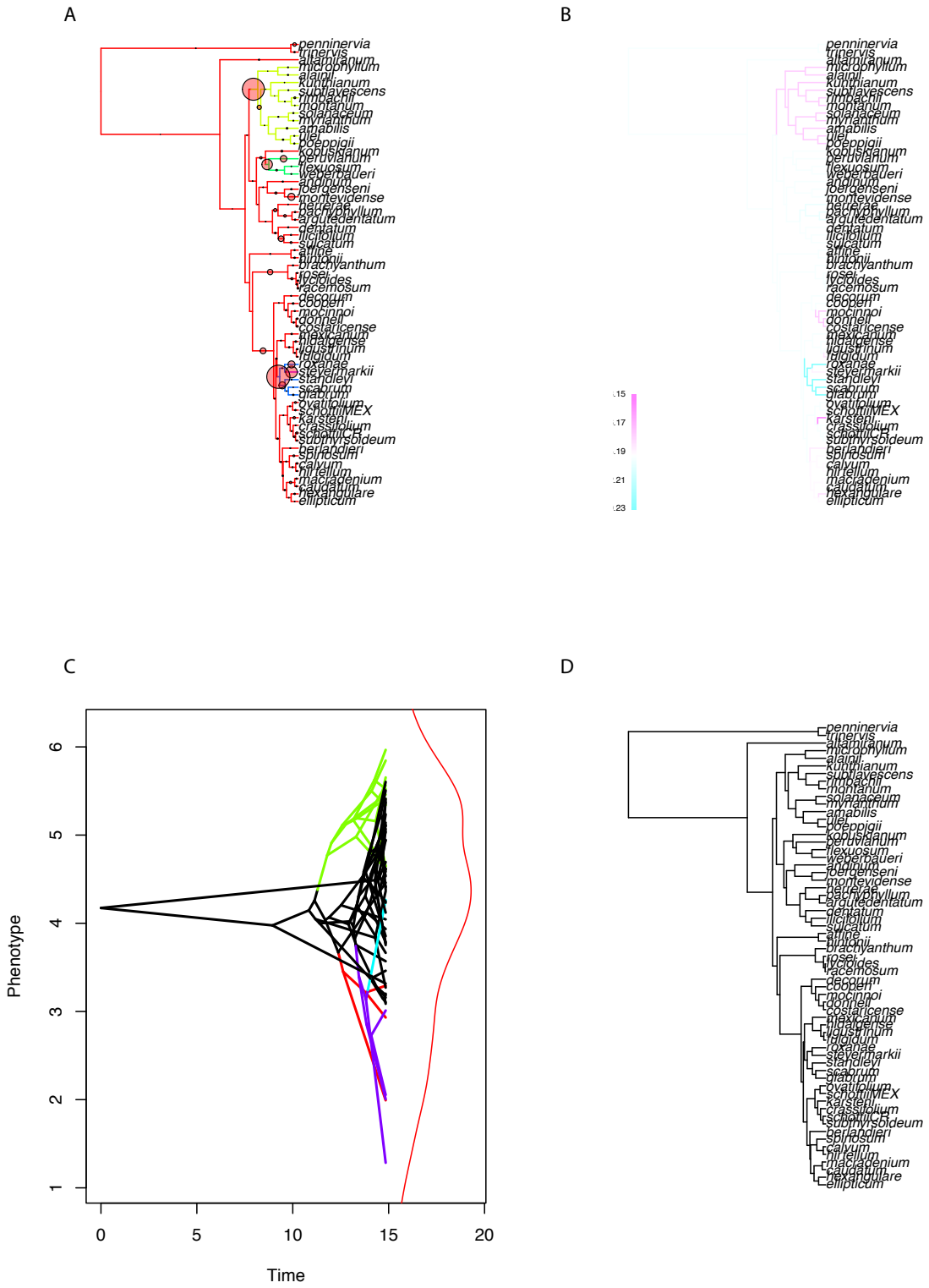


Fig. S20. Bio 18: precipitation of warmest quarter

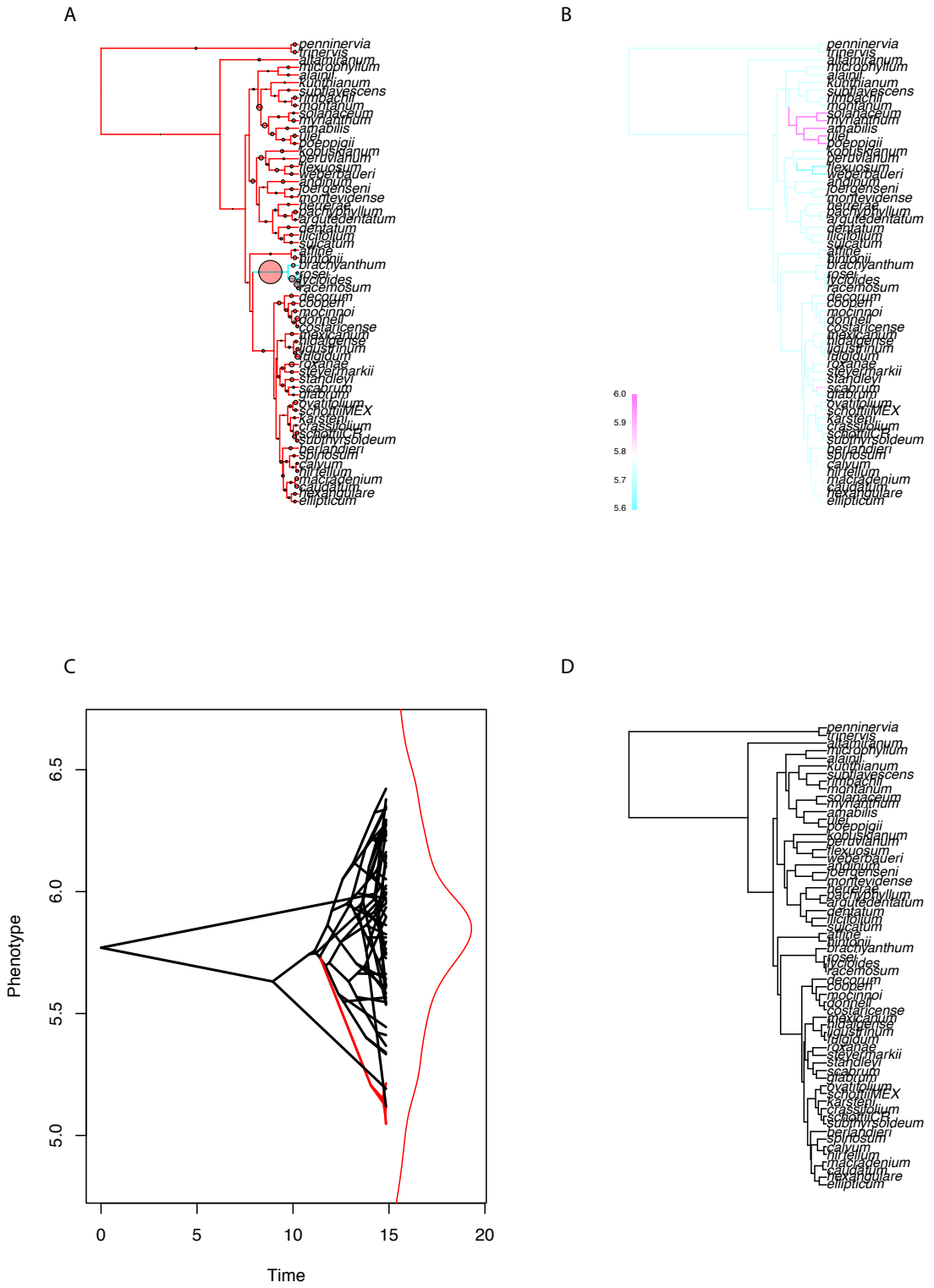


Fig. S21. Bio 19: precipitation of coldest quarter

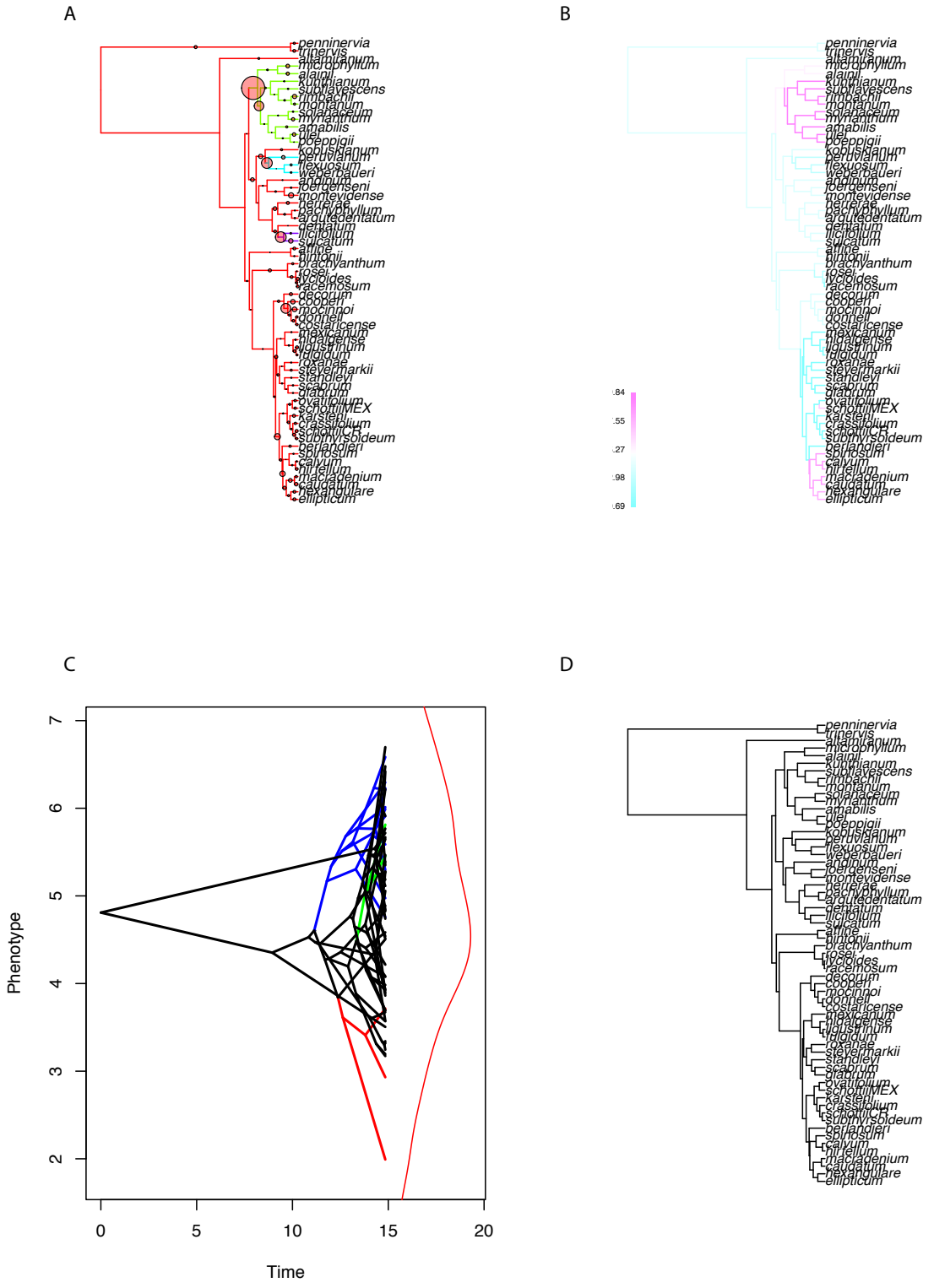


Fig. S22. 95% confidence set of diversification rate shift configurations inferred in BAMM using the default model.

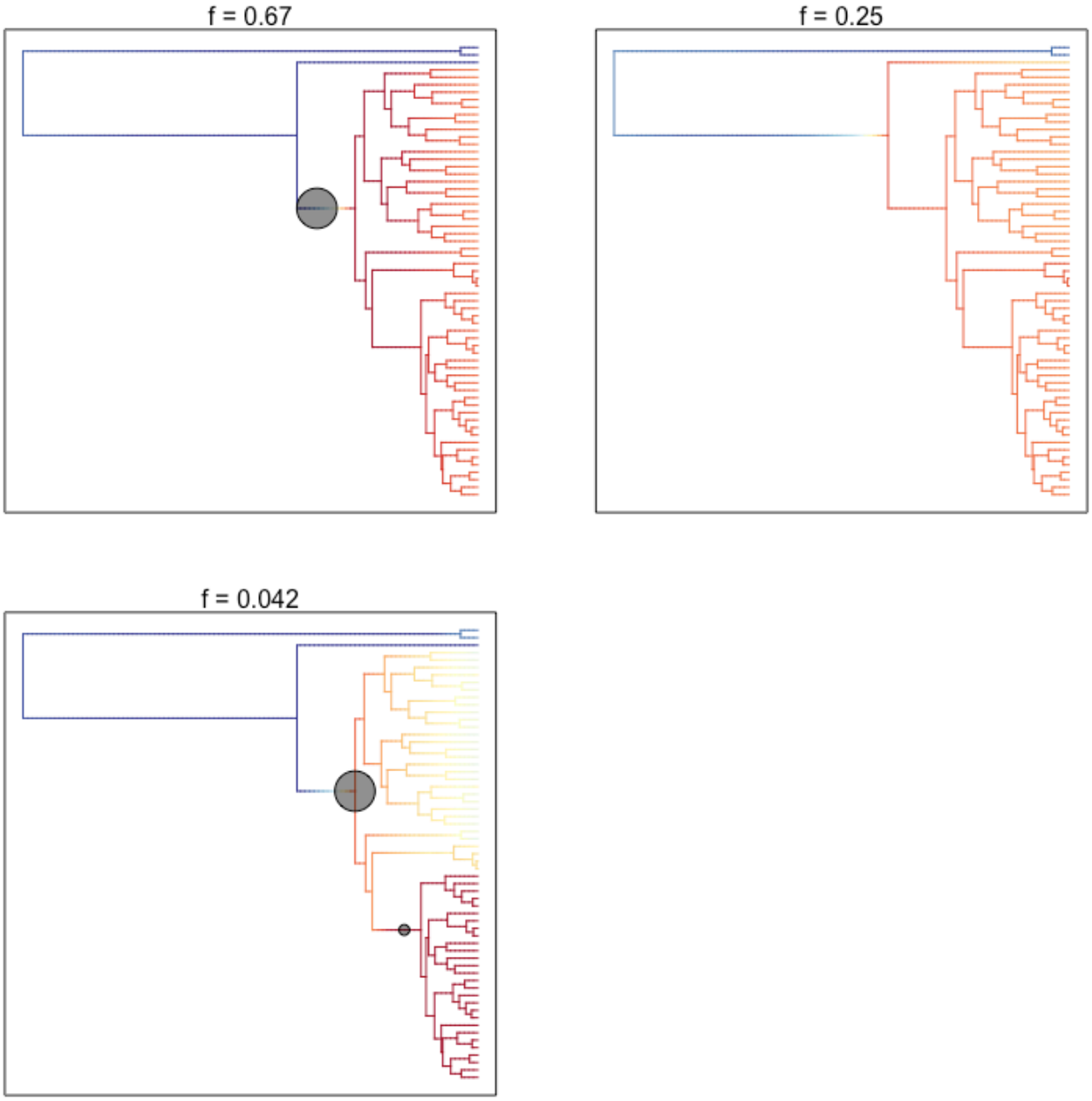
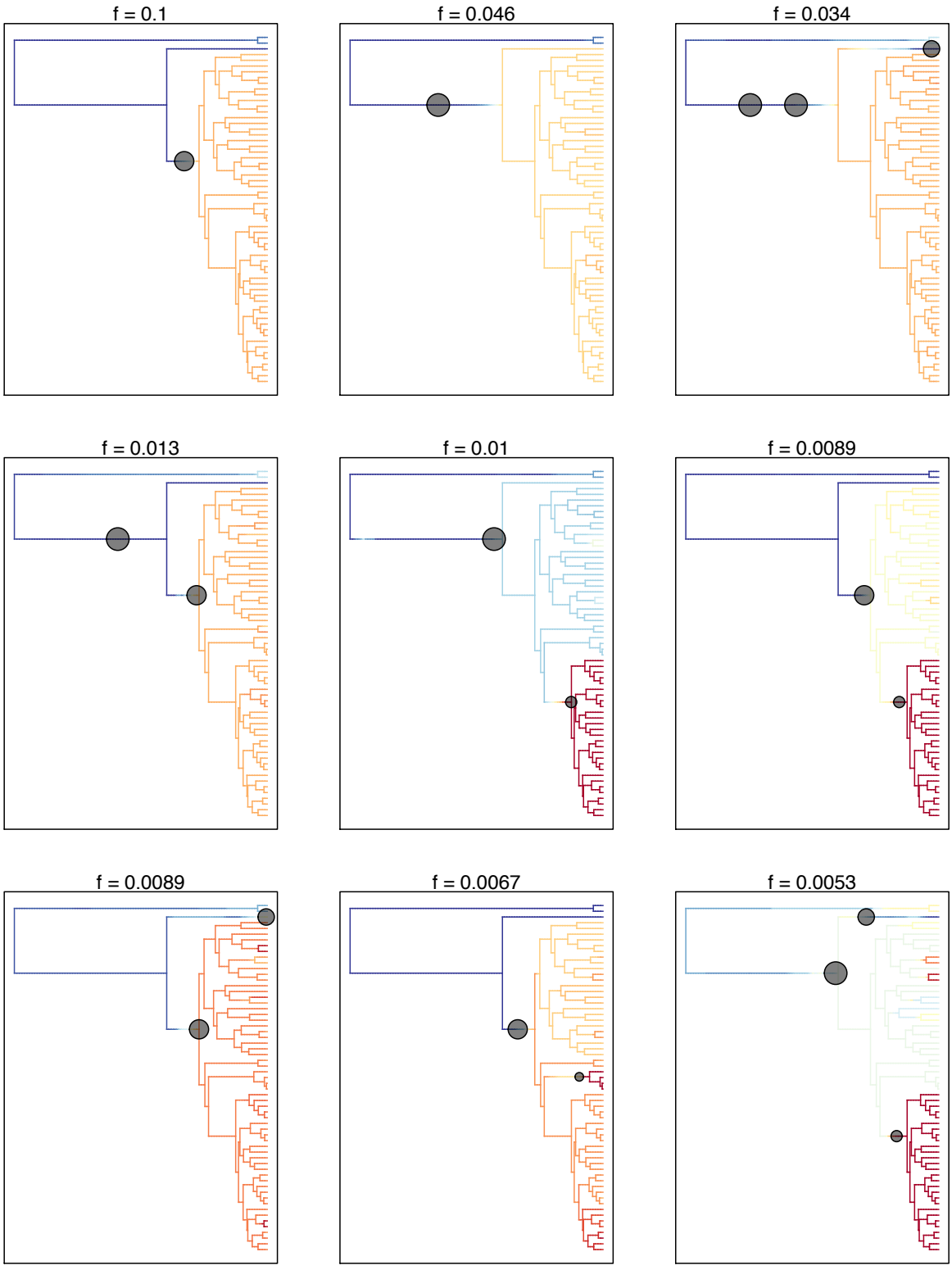


Fig. S23. 95% confidence set of diversification rate shift configurations inferred in BAMM using the MEDUSA-like model.



CHAPTER 3:

An updated phylogeny of Verbenaceae

ABSTRACT

- Premise of the study: Verbenaceae is family of ca. 770 species broadly distributed in the New World and with limited representation in Africa and Europe. The family is an important element in floras of the New World, but lacked extensive phylogenetic work until recently. In the last ten years, both family-wide phylogenetic studies and in-depth studies of major clades have been published. The available data from previous studies and new data were combined to produce an updated phylogeny of Verbenaceae including over 360 species, over three times as many species as included in previous family-wide studies.
- Methods: Twelve chloroplast regions, two nuclear ribosomal spacers, and eight low-copy nuclear loci were analyzed for 366 species of Verbenaceae. Phylogenetic analyses were conducted using maximum likelihood, Bayesian inference, and Bayesian multi-species coalescent approaches.
- Key results: Classification of genera into higher order groups was confirmed and relationships of unassigned genera were resolved. Discovery of a new genus in Verbeneae was also confirmed. The genus Scleröon Benth in Lindl. was resurrected to classify Citharexylum oleinum and C. tetramerum as a new genus in Duranteae.
- Conclusions: Previous inferences of major clades were robust; continued study within clades is needed. The updated phylogeny of Verbenaceae provides a framework for further study of evolutionary patterns in the family.

Key words: classification, microfluidic PCR, molecular phylogenetics, systematics, Scleröon, Verbenaceae

INTRODUCTION

Verbenaceae s.s. comprises 28 genera and *ca*. 780 species of trees, shrubs, lianas, and herbs distributed primarily in the New World (Atkins, 2004; O'Leary et al., 2009; Marx et al. 2010, Thode et al., 2013; Lu-Irving et al., 2014; Frost et al., in prep.). Once considered to be much larger, over 50 genera classified in Verbenacea s.l. were transferred to Lamiaceae after cladistic analyses of morphological characters (Cantino et al., 1992). Marx et al. (2010) produced a phylogeny of Verbenaceae s.s. including complete generic sampling and data from seven chloroplast regions for 109 species. The Marx et al. (2010) study provided a framework from which to describe major clades in the family and produced an updated classification of 8 tribes. Current generic and species diversity for tribes are as follows: Lantaneae (4 genera, ca. 275 spp.; Lu-Irving and Olmstead, 2012; Lu-Irving et al., 2014; O'Leary et al., 2016), Verbeneae (5 genera, 177 spp.; O'Leary et al., 2007; Peralta et al., 2008; O'Leary et al., 2009; O'Leary et al., 2010; O'Leary, 2014; O'Leary and Mulgura, 2014), Neospartoneae (3 genera, 7 spp.), Priveae (2 genera, 21 spp.), Citharexyleae (2 genera, 72 spp.; Frost et al., 2017), Casselieae (3 genera, 14 spp.), Duranteae (5 genera, ca. 190 spp.; Thode et al., 2013), and Petreae (1 genus, 12 spp.). In addition to the eight tribes were two unassigned genera, *Dipyrena* (1 spp.) and *Rhaphithamnus* (2 spp.), which exhibited conflict in their placement between chloroplast (Marx et al., 2010) and nuclear (Yuan et al., 2010) datasets. *Coelocarpum*, a genus that was unassigned by Atkins (2004), nested within Lantaneae in Marx et al. (2010) with weak support and was tentatively described with Lantaneae. Later work (Lu-Irving and Olmstead, 2012; Lu-Irving et al., 2014) confirmed it's placement outside Lantaneae.

Alongside or since the study by Marx et al. (2010), multi-locus phylogenetic studies of tribes within Verbenaceae have been conducted, namely on Verbeneae (Yuan and Olmstead, 2008a; Yuan and Olmstead, 2008b; O'Leary et al., 2009; Yuan et al., 2010; Frost et al., 2017), Lantaneae (Lu-Irving and Olmstead, 2012; Lu-Irving et al. 2014), Citharexyleae (Frost et al., 2017; Frost et al., in prep.), and Neospartoneae (Lu et al., in prep.). These studies provide a large pool of existing data from which to generate an updated phylogeny of Verbenaceae with increased taxonomic and molecular sampling. Additionally, novel results from these studies required further testing, which may be accomplished through an expanded family-wide study. Frost et al. (2017) discovered a potential new clade in Verbeneae, dubbed the Andean clade. The Andean clade of Verbeneae comprised three species of *Junellia* and *Verbena* (*Junellia fasciculata*, *Junellia occulta*, and *Verbena vilifolia*) for both chloroplast and nuclear datasets, but datasets varied on the relationship of these species to other clades of Verbeneae and on the inclusion or exclusion of *Hierobotana* (O'Leary, 2014), a monotypic genus endemic to Ecuador, in the Andean clade. Frost et al. (in prep.) discovered that two species of *Citharexylum*, *C. oleinum* and *C. tetramerum*, did not nest within Citharexyleae, but could not confidently place the species in Verbenaceae based only on outgroup sampling.

Through pooling of the available data from phylogenetic studies of Verbenaceae and generation of new data for this study, we generated an updated phylogeny of Verbenaceae including over 360 species. This phylogeny was used to confirm major clades of Verbenaceae, test conflicted relationships for unassigned genera and the novel Andean clade of Verbeneae, and place *C. oleinum* and *C. tetramerum* within Verbenaceae.

MATERIALS AND METHODS

***Sampling*—**In total, 645 accessions representing 368 species of Verbenaceae were sampled. This study incorporates published data (Yuan and Olmstead, 2008a; Yuan and Olmstead, 2008b; O'Leary et al., 2009; Marx et al., 2010; Yuan et al., 2010; Lu-Irving and Olmstead, 2012; Thode et al., 2013; Lu-Irving et al., 2014; Frost et al., 2017) and newly generated data for 275 accessions. Because this study incorporates data from multiple sources using different molecular loci, new data was generated for 179 accessions included in those studies in order to create more overlapping data between datasets. Additionally, we included 96 new accessions representing 81 taxa. New accessions included two accessions (covering two spp.) of *Chascanum*, 11 (10 spp.) of *Duranta*, 31 (24 spp.) of *Glandularia*, 19 (15 spp.) of *Junellia*, four (three spp.) of *Lantana*, ten (ten spp.) of *Lippia*, one (one spp.) of *Mulgurea*, one (one spp.) of *Petrea*, one (one spp.) of *Stachytarpheta*, and 16 (14 spp.) of *Verbena*. Twenty-one molecular loci from the combined studies were utilized, including 12 chloroplast loci (*ccs*A, *mat*K, *ndh*F, *psb*Z, *rbc*L, *rpl*32, *rpo*C2, *rps*16, *trn*G-S, *trn*T, *trn*L-F, and *trn*Y), two nuclear ribosomal spacer regions (ETS and ITS), and eight low-copy nuclear genes (PPR11, PPR62, PPR70, PPR81, PPR90, PPR91, PPR97, and PPR123; PPR loci numbered sequentially as in Yuan et al., 2009). Generation of new sequence data was carried out using a subset of loci: *mat*K, *ndh*F, *rbc*L, *rpl*32, *rpo*C2, *trn*T, *trn*L-F, ETS, and ITS. These loci were chosen for their broad use in studies and universality of primer sequences for family-wide use. For a table of vouchers, herbaria housing voucher specimens, and GenBank accession numbers accompanying sequence data used in this study, see

Appendix S1 (see the Supplementary Data with this article). Herbaria acronyms follow Index Herbariorum (Thiers, constantly updated: http://sweetgum.nybg.org/science/ih/).

DNA extraction, Microfluidic PCR, and sequencing—Genomic DNA was extracted from either field-collected, silica-gel-dried tissues or herbarium specimens using a modified CTAB method (Doyle and Doyle, 1987). Microfluidic PCR was performed on an Access Array System (Fluidigm) using two Juno 192.24 IFCs. For detailed primer information about targeted loci (*mat*K, *ndh*F, *rbc*L, *rpl*32, *rpo*C2, *trn*T, *trn*L-F, ETS, and ITS), see Frost et al. (in prep.). Amplicons were harvested and pooled as described in Uribe-Convers et al. (2016). For each IFC, The resulting pools were multiplexed in an Illumina Miseq using the Reagent Kit v3 600 cycles. Microfluidic PCR in the Access Array System, downstream quality control, and Illumina sequencing were performed in the University of Idaho (Moscow, ID) Intstitute for Bioinformatics and Evolutionary Studies (ibest) Genomic Resources Core facility. Data processing follows Frost et al. (in prep.). Briefly, reads from the Illumina Miseq runs were processed using the Fluidigm2PURC pipeline (Blischak et al., in review). Reads were processed with clustering thresholds of 0.975, 0.99, 0.995, 0.997 and a size threshold of 5.

Phylogenetic analyses—Sequences were aligned using AliView ver. 1.18.1 (Larsson, 2014); alignments were inspected and manually adjusted where necessary. Individual alignments were analyzed with jModelTest 2.1.7 (Darriba et al., 2012; Guindon and Gascuel, 2003) to select the best-fit model of nucleotide substitution. The GTR + G

model was selected and applied to all analyses. For regions with shared evolutionary histories, alignments were concatenated using Geneious R7 7.0.6 (Biomatters, Auckland, New Zealand) and treated as a single dataset. Regions *ccs*A, *mat*K, *ndh*F, *psb*Z, *rbc*L, *rpl*32, *rpo*C2, *rps*16, *trn*G-S, *trn*T, *trn*L-F, and *trn*Y were combined as the chloroplast (cpDNA) dataset, and ETS and ITS were combined as the nuclear ribsomal (nrDNA) dataset. Ten independent datasets were analyzed: cpDNA, nrDNA, PPR11, PPR62, PPR70, PPR81, PPR90, PPR91, PPR97, and PPR123. The eight PPR genes were combined and analyzed as a low-copy nuclear dataset, and all of the data were concatenated for an "all combined" dataset.

Another dataset was constructed using one representative for each species and a subset of the data in order to reduce the amount of noise from missing data in the all combined dataset. For species with multiple individuals with overlapping data, the individual with the most data was retained as the representative for that species. For species with individuals with non-overlapping data, the non-overlapping sequences were combined to yield greater coverage; the combined sequence served as the representative for that species. The data set was reduced to the nine targeted loci in this study and PPR11, PPR70, and PPR123, which had the best taxonomic sampling of the PPR regions (>200 accessions).

Phylogenetic reconstructions were conducted for individual datasets, low-copy nuclear genes combined, and all data combined, both with all representatives and one representative, using maximum likelihood and Bayesian approaches, as implemented in RAxML and MrBayes ver. 3.2.6 (Ronquist & Huelsenbeck, 2003), respectively.

Maximum likelihood analyses consisted of bootstrap analyses with 500 replicates for each dataset. Datasets were partitioned into individual gene regions with individual parameters unlinked in Bayesian analyses. Analyses consisted of two replicate runs, each with four chains, which were run for at least one million generations, sampled every 100 generations. Runs were assessed for convergence and stationarity in Tracer v1.6.0 (Rambaut et al., 2014); runs were considered to have converged when effective sample sizes (ESS) for parameter values were above 200 for combined files. The first 20% of sampled trees were discarded as burn-in from each run; the remaining trees were summarized into a majority rule consensus tree with all compatible groups.

RESULTS

Dataset characteristics—Taxonomic sampling included 366 species of Verbenaceae, ca. half (47.5%) of the estimated species diversity (Table1) The total concatenated dataset consisted of 27,794 aligned bp and 645 accessions (Table 2). Among the characters, 11,139 were variable, of which 7,270 were parsimony-informative. The reduced dataset with one representative per species was 15,630 aligned bp and included 368 accessions/ species. The selected subset included less missing data than the all combined dataset, 57.56% and 74.00% missing data, respectively.

Phylogenetic analyses—The tribes described by Marx et al. (2010) were recovered for all datasets, except Casselieae with nrDNA (Fig. 1; supplementary). All analyses reconstructed a grade of Petreeae, Duranteae, Citharexyleae and Casselieae at the base.

The rest of the tree was composed of two large clades, Lantaneae and Verbeneae, and several small clades including tribes Priveae and Neospartoneae and *Coelocarpum*, *Dipyrena*, and *Rhaphithamnus*, genera that are unassigned to tribes. Most datasets (cpDNA, low copy combined, and all combined) inferred *Coelocarpum* as sister to Lantaneae. Coelocarpum was reconstructed as sister to Verbeneae by nrDNA with low support. Verbeneae was reconstructed as sister to Lantaneae and *Coelocarpum* with high support for low-copy and all combined datasets. *Dipyrena* was inferred to be sister to a clade of Lantaneae, *Coelocarpum*, and Verbeneae by nrDNA, low-copy, and all combined datasets. For cpDNA, *Dipyrena* was reconstructed as sister to Verbeneae, as in Marx et al. (2010). For relationships within clades, see Fig. 2.

Rhaphithamnus, Priveae, Neospartoneae, and *Dipyrena* form a grade leading to the large clade including Lantaneae and Verbeaneae. All datasets reconstructed Neospartoneae as sister to the clade of Lantaneae, *Coelocarpum*, Verbeneae, and *Dipyrena*. Relationships of Priveae and *Rhaphithamnus* relative to the aforementioned groups varied and/or were not well supported across analyses. Priveae, as sister to the clade including Neospartoneae and Lantaneae, and *Rhaphithamnus,* as sister to that, was reconstructed with high support by low-copy nuclear data and with low support by nrDNA and all combined datasets. The reversed relationship, *Rhaphithamnus* sister to the clade including Neospartoneae and Lantaneae and Priveae sister to that, was reconstructed with low support by cpDNA.

The clade of *Junellia fasciculata, Junellia occulta,* and *Verbena vilifolia,* was reconstructed by all datasets (Fig. 3); the relationship of this clade relative to other clades

Verbeneae and its relationship to *Hierobotana* varied across loci. These species nested with *Hierobotana* in nrDNA and all combined trees and separate from *Hierobotana* in cpDNA and low-copy nuclear combined trees. Chloroplast (Fig. 3A), low-copy nuclear (Fig. 3C), and all combined data (one representative dataset; Fig. 3D) reconstructed the clade as sister to *Verbena*; nrDNA (Fig. 3B) inferred it to be sister to *Verbena* and *Glandularia*.

Citharexylum oleinum and *C. tetramerum*, the two species that fell outside of *Citharexylum* in previous studies, nested in tribe *Duranteae* (Fig. 4). In all analyses, the two species were found to be sister to the clade of *Bouchea*, *Chascanum*, and *Stachytarpheta*. This relationship was highly supported by nrDNA and the all combined (one representative) dataset.

DISCUSSION

Relationships reconstructed by different sources of data (cpDNA, nrDNA, and low-copy nuclear genes) were, for the most part, concordant with each other and previously published studies of Verbenaceae. The eight tribes classified by Marx et al. (2010) were recovered by analyses, except for Casselieae for nrDNA, which may be an artifact of low sampling for that group in that dataset. Relationships between tribes were also mostly consistent with those reconstructed by Marx et al. (2010). Unassigned genera in Verbenaceae, *Coelocarpum, Dipyrena,* and *Rhapthithamnus,* are supported as independent lineages in this study and remain unassigned to tribes.

Unassigned genera—Additional nuclear loci support *Dipyrena* as sister to the clade including Lantaneae and Verbeneae, rather than sister to Verbeneae. Prior to this study, Marx et al. (2010) inferred *Dipyrena* to be sister to Verbeneae using chloroplast data. Yuan et al. (2010) using five PPR loci (PPR11, PPR62, PPR70, PPR91, and PPR123) reconstructed *Dipyrena* as sister to the clade including Verbeneae and Lantaneae with PPR11, PPR70, and PPR123. The same relationship to Verbeneae found with chloroplast data was reconstructed with PPR62 by Yuan et al. (2010) with low support. Expanded sampling of Verbenaceae for PPR62 in this study places *Dipyrena* sister to the clade including Verbena and Lantaneae as with other PPR loci. PPR91 reconstructed a different topology altogether. This study also includes a new source of evidence to resolve the conflict between chloroplast regions and PPR genes: nrDNA. Nuclear ribosomal data also infer *Dipyrena* as sister to the clade including Lantaneae and Verbeneae. While the chloroplast reconstructs a slightly different evolutionary history, nuclear data agree that *Dipyrena* is sister to Verbeneae plus Lantaeae.

Nuclear and combined data also support *Rhaphithamnus* as the earliest diverging lineage in the grade of *Rhaphithamnus*, Priveae, Neospartoneae, and *Dipyrena*. The relationship of *Rhaphithamnus* relative to Priveae was uncertain based on results of Marx et al. (2010). Similar to the case with *Dipyrena*, there was conflict between chloroplast and PPR datasets as to whether *Rhaphithamnus* was placed above or below Priveae in the grade. It was placed above Priveae in chloroplast (Marx et al., 2010) and PPR91 (Yuan et al., 2010) datasets and below for PPR11, PPR62, PPR70, and PPR123. Nuclear ribosomal

data in this study also reconstructs *Rhaphithamnus* below, as the earliest diverging lineage in the grade of these four small groups.

New clades

Verbeneae—Frost et al. (2017) discovered a new clade in Verbeaneae composed of three Andean species in *Verbena* and *Junellia*: *Junellia fasciculata*, *Junellia occulta*, and *Verbena vilifolia*. The study, which included data for three chloroplast regions and nuclear loci ETS, ITS, and PPR11, found that this clade, dubbed the Andean clade, along with monotypic *Hierobotana* was sister to Verbena and *Glandularia* in the nuclear tree, but nested within *Verbena* and *Glandularia* in the chloroplast tree. Additionally, in the nuclear tree, *Hierobotana*, which is endemic to the Ecuadorian Andes, was not inferred to be closely related to these other Andean species, but rather to North American Verbena.

Chloroplast results in this study were consistent with relationships found by Frost et al. (2017): *Hierobotana* was separate from the Andean clade and sister to North American *Verbena*. Nuclear ribosomal data (ETS and ITS) reconstructed the Andean clade plus *Hierobotana* as sister to a clade including *Verbena* and *Glandularia*, consistent with the nuclear tree in Frost et al. (2017). Only the results of nuclear loci combined (ETS, ITS, and PPR11; Frost et al., 2017) were presented in that paper. Incorporation of more low copy nuclear loci in this study and separate analyses of low copy nuclear data from nuclear ribosomal data provide new insights into the evolutionary history of these species. Low-copy nuclear genes combined inferred the Andean clade to be sister to all *Verbena* and *Hierobotana* to be nested within the North American *Verbena*. The all

combined (one representative) dataset found the Andean clade plus *Hierobotana* to be sister to all *Verbena*.

Species tree analyses are necessary to resolve discordant relationships among gene trees to determine (1) if *Hierobotana* is related to the Andean clade or distinct and (2) the relationship of the Andean clade relative to other genera in order to resolve classification. If the Andean taxa form a monophyletic group with *Verbena*, they could be included in *Verbena*. However, if they are reconstructed as paraphyletic to other genera, as in the nuclear ribosomal dataset where the Andean clade comes out sister to both *Verbena* and Glandularia, the Andean clade would need to be classified as a distinct genus.

Duranteae—*Citharexylum oleinum* and *C. tetramerum*, two species of shrubs with reduced inflorescences distributed in Mexico, were placed outside of Citharexyleae by Frost et al. (in prep.). All phylogenetic reconstructions inferred that these species form a small clade sister to *Bouchea*, *Chascanum*, and *Stachytarpheta* in tribe Duranteae. Placement outside of and paraphyletic to other genera in the tribe necessitates classification of new genus in Verbenaceae. We propose to resurrect the genus *Scleröon* Benth in Lindl. for which *Scleröon oleinum* was designate the type (Edwards Botanical Register, 1843). Citharexylum tetramerum also belongs in *Scleröon*.

Scleröon Benth in Lindl., Edwards's Bot. Reg. 29 (Misc.): 65. 1843. Type: *Scleröon oleinum* Benth in Lindl.

i. ***Scleröon tetramerum*** (T.S. Brandeg.) L.A. Frost, **comb. nov**

Basionym: *Citharexylum tetramerum* T.S. Brandeg., Univ. Calif. Publ. Bot. 3: 390.1909.

CONCLUSIONS

Our work finds that higher level classification within Verbenaceae is robust, but there is still much to learn within major groups. Focused research on clades will continue to refine our understanding of relationships and revise classification. This updated family-wide phylogeny provides a framework from which to test broad patterns of evolution in a family that is an important element of the New World floras. Future work will explore the biogeographical history, via reconstructions of dispersal between continents and transitions from the tropics into temperate zone, and how patterns of diversification relate to biogeographic events, geologic events, and character evolution.

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REFERENCES

Blischak, P. D., M. Latvis, D. F. Morales-Briones, J. C. Johnson, V. S. Di Stilio, A. D. Wolfe, and D. C. Tank. In Review. Fluidigm2PURC: automated processing and haplotype inference for double-barcoded PCR amplicons. bioRxiv. doi: 10.1101/242677.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and high-performance computing. Nature Methods 9(8): 772.

Doyle, J. J., and J. L. Doyle. 1987. A rapid isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Guindon, S., and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52(5): 696-704.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17(8): 754-755.

Frost, L. A., Tyson, S. M., Lu-Irving, P., O'Leary, N., & Olmstead, R. G. (2017). Origins of North American arid-land Verbenaceae: More than one way to skin a cat. American journal of botany, 104(11), 1708-1716.

Lu-Irving, P., & Olmstead, R. G. (2012). Investigating the evolution of Lantaneae (Verbenaceae) using multiple loci. Botanical Journal of the Linnean Society, 171(1), 103-119.

Lu-Irving, P., O'Leary, N., O'Brien, A., & Olmstead, R. G. (2014). Resolving the genera Aloysia and Acantholippia within tribe Lantaneae (Verbenaceae), using chloroplast and nuclear sequences. Systematic Botany, 39(2), 644-655.

Marx, H. E., O'Leary, N., Yuan, Y. W., Lu-Irving, P., Tank, D. C., Múlgura, M. E., & Olmstead, R. G. (2010). A molecular phylogeny and classification of Verbenaceae. American journal of Botany, 97(10), 1647-1663.

O'Leary, N. 2014. Hierobotana Briq., an intriguing monotypic genus of tribe Verbeneae (Verbenaceae). Phytotaxa 164: 286-290.

O'Leary, N., M. E. Múlgura, and O. Morrone. 2007. Revisión taxonómica de las especies del género Verbena (Verbenaceae): serie Pachystachyae. Annals of the Missouri Botanical Garden 94: 571-621.

O'Leary, N., Y.-W. Yuan, A. Chemisquy, and R. G. Olmstead. 2009. Reassignment of species of paraphyletic Junellia s.l. to the new genus Mulguraea (Verbenaceae) and new circumscription of genus Junellia: molecular and morphological congruence. Systematic Botany 34: 777-786.

O'Leary, N., M. E Múlgura, and O. Morrone. 2010. Revisión taxonómica de las especies del género Verbena L. (Verbenaceae) II: serie Verbena. Annals of the Missouri Botanical Garden 97: 369-428.

O'Leary, N., and M. E. Múlgura. 2014. Synopsis of tribe Verbeneae Dumortier (Verbenaceae) in Peru. Phytotaxa 163: 121-148.

O'Leary, N., P. Lu-Irving, P. Moroni, and S. Siedo. 2016. Recircumscription and taxonomic revision of Aloysia (Lantaneae: Verbenaceae) in South America. Annals of the Missouri Botanical Garden 101: 568-609.

Olmstead, R. G. (2013). Phylogeny and biogeography in Solanaceae, Verbenaceae and Bignoniaceae: a comparison of continental and intercontinental diversification patterns. Botanical Journal of the Linnean Society, 171(1), 80-102.

Peralta, P., M. E. Múlgura, S. S. Denham, and S. M. Botta. 2008. Revisión del género Junellia (Verbenaceae). Annals of the Missouri Botanical Garden 95: 338-390.

Rambaut, A., M. A. Suchard, D. Xie, and A. J. Drummond. 2014. Tracer v1.6. Available from http://beast.bio.ed.ac.uk/Tracer.

Thiers, B. [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/.

Thode, V. A., O'Leary, N., Olmstead, R. G., & Freitas, L. B. (2013). Phylogenetic position of the monotypic genus Verbenoxylum (Verbenaceae) and new combination under Recordia. Systematic Botany, 38(3), 805-817.

Uribe-Convers, S., M. L. Settles, and D. C. Tank. 2016. A Phylogenomic Approach Based on PCR Target Enrichment and High Throughput Sequencing: Resolving the Diversity within the South American Species of Bartsia L. (Orobanchaceae). PLOS ONE 11(2): e0148203. doi: 10.1371/journal.pone.0148203

Yuan, Y. W., & Olmstead, R. G. (2008a). A species-level phylogenetic study of the Verbena complex (Verbenaceae) indicates two independent intergeneric chloroplast transfers. Molecular Phylogenetics and Evolution, 48(1), 23-33.

Yuan, Y. W., & Olmstead, R. G. (2008b). Evolution and phylogenetic utility of the PHOT gene duplicates in the Verbena complex (Verbenaceae): dramatic intron size variation and footprint of ancestral recombination. American Journal of Botany, 95(9), 1166-1176.

Yuan, Y.-W., C. Liu, H. E. Marx, and R. G. Olmstead. 2009. The PPR (pentatricopeptide repeat) gene family, a tremendous resource for plant phylogenetic studies. New Phytologist 182: 272-283.

Yuan, Y. W., Liu, C., Marx, H. E., & Olmstead, R. G. (2010). An empirical demonstration of using pentatricopeptide repeat (PPR) genes as plant phylogenetic tools: Phylogeny of Verbenaceae and the Verbena complex. Molecular Phylogenetics and Evolution, 54(1), 23-35.

TABLES

Table 1. Taxonomic sampling

Taxon	Sampling: spp. sampled (estimated species diversity)	Taxon	Sampling: spp. sampled (estimated species diversity)
Petreeae	6 (12)	**Neospartoneae**	5 (7)
Petrea	6 (12)	*Diostea*	1 (1)
Duranteae	33 (191)	*Lampayo*	2 (2)
Bouchea	5 (10)	*Neosparton*	2 (4)
Chascanum	6 (27)	**Verbeneae**	124 (177)
Duranta	13 (20)	*Glandularia*	52 (84)
Recordia	2 (2)	*Hierobotana*	1 (1)
Stachytarpheta	7 (130)	*Junellia*	27 (37)
Casselieae	6 (14)	*Mulguraea*	5 (11)
Casselia	3 (6)	*Verbena*	39 (44)
Parodianthus	1 (2)	**Lantaneae**	121 (270)
Tamonea	2 (6)	*Aloysia*	28 (36)
Citharexyleae	61 (72)	*Acantholippia*	1 (1)
Citharexylum	59 (70)	*Lantana*	34 (100)
Rehdera	2 (2)	*Lippia*	58 (125)
Priveae	3 (21)	**Unassigned genera**	
Pitrea	1 (1)	*Coelocarpum*	2 (5)
Priva	2 (20)	*Dipyrena*	1 (1)
		Rhaphithamnus	2 (2)

Table 2. Characteristics of individual datasets

Alignment	No. of taxa	Proportion of taxa	Alignment length (bp)	% missing data	No. of variable sites	Proportion of variable sites	No. of parsimony informative sites	Proportion of parsimony informative sites	GC content
all comb.	645	1.000	27794	74.00	11139	0.401	7270	0.262	0.392
cpDNA	627	0.972	16961	69.45	5176	0.305	3175	0.187	0.358
nrDNA	580	0.899	1401	43.94	906	0.647	764	0.545	0.592
lowcopy	400	0.620	9948	74.957	5142	0.517	3417	0.343	0.398
PPR11	204	0.316	1461	29.76	866	0.593	621	0.425	0.401
PPR62	151	0.234	1452	44.65	717	0.494	457	0.315	0.411
PPR70	202	0.313	1128	52.35	697	0.618	464	0.411	0.382
PPR81	123	0.191	1180	7.96	652	0.553	433	0.367	0.395
PPR90	72	0.112	986	3.29	314	0.318	182	0.185	0.405
PPR91	45	0.070	1306	8.74	524	0.401	335	0.257	0.394
PPR97	62	0.096	747	5.47	266	0.356	158	0.212	0.41
PPR123	241	0.374	1394	31.18	739	0.53	533	0.382	0.405

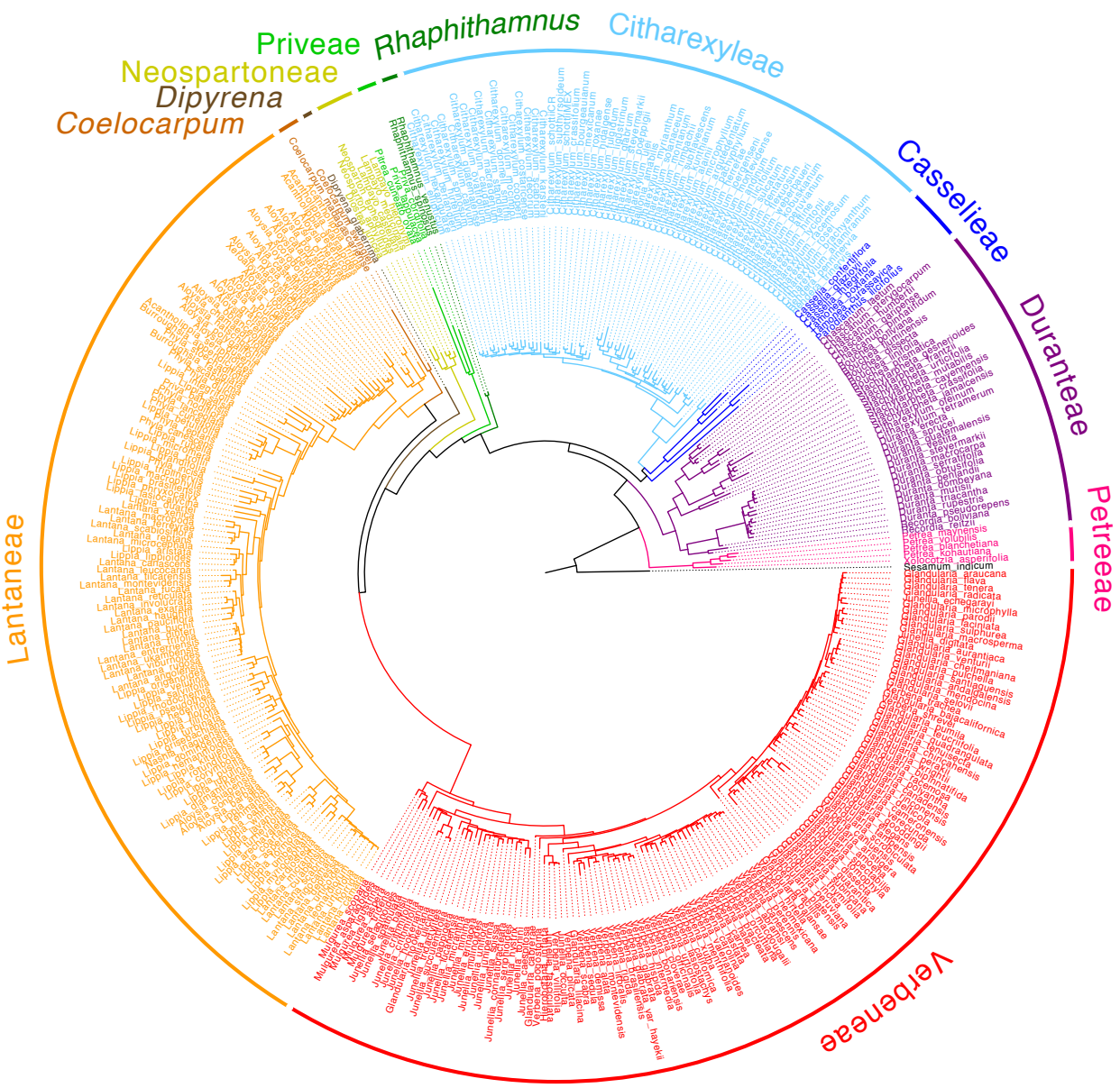
FIGURE CAPTIONS

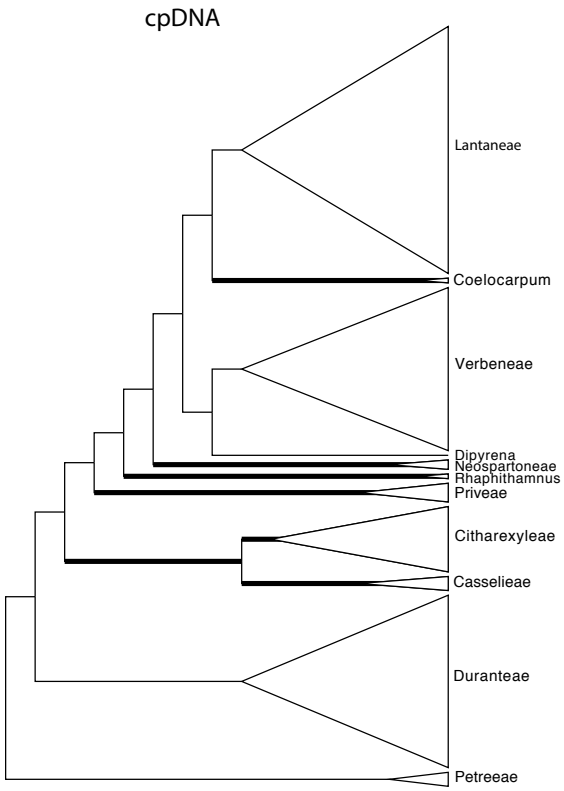
Fig. 1. Cartoon trees for cp, nr, lowcopy nuclear combined, and all combined (one representative) datasets showing topological differences among trees and support for relationships. Thickened branches indicate high support (≥ 70 bootstrap support from maximum likelihood (ML) analyses or ≥ 95 % posterior probability from Bayesian inference (BI)).

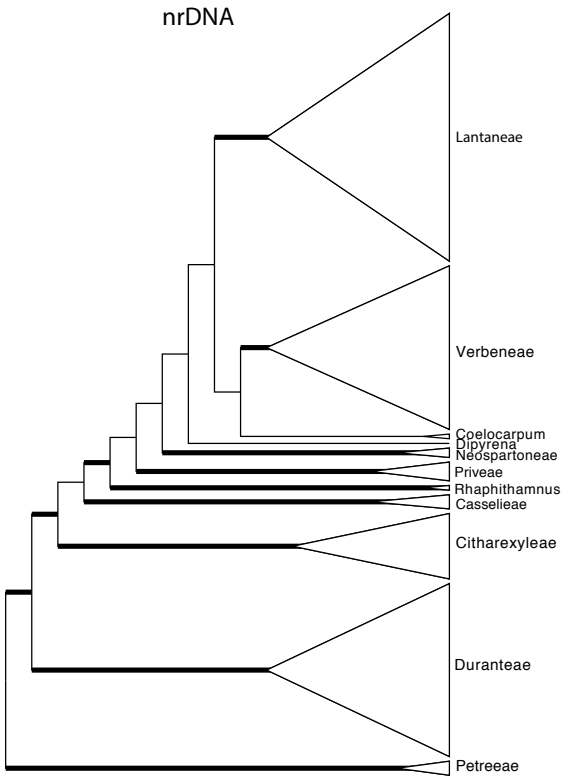
Fig. 2. Bayesian phylogenetic reconstructions for the all combined (one representative) dataset with tribes/major clades labelled and color coded.

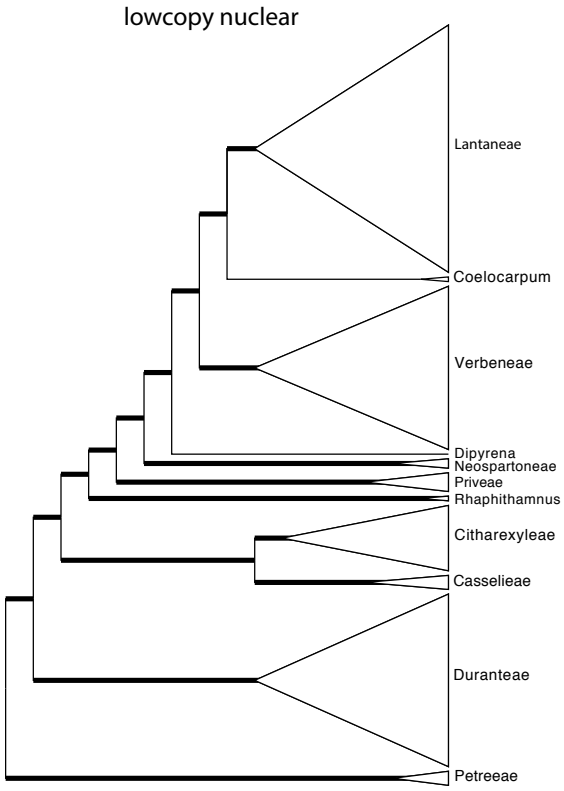
Fig. 3. Bayesian phylogenetic reconstructions for tribe Verbeneae showing relationships of the Andean clade (*Junellia fasiculata*, *Junellia occulta*, and *Verbena villifolia*) and *Hierobotana inflata* (highlighted blue) from (A) cpDNA, (B) nrDNA, (C) low-copy nuclear combined, and (D) all combined (one representative dataset).

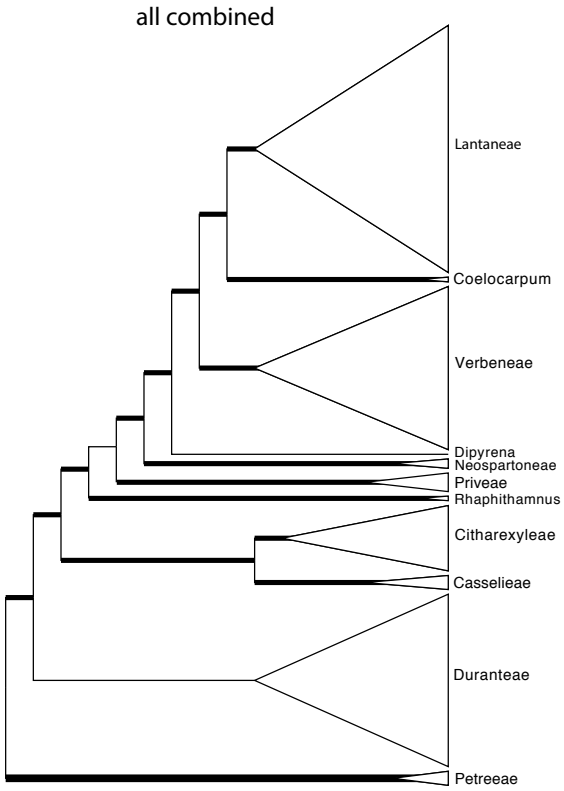
Fig. 4. Bayesian phylogenetic reconstructions for tribe Duranteae showing relationship of C. oleinum and C. tetramerum (highlighted blue) from the all combined (one representative) dataset. The same relationships were recovered by other datasets. Numbers indicate support values for high supportably nodes (≥ 95 % posterior probability).


Priveae
Rhaphithamnus
Citharexyleae
Neospartoneae
Dipyrena
Coelocarpum
Casselieae
Duranteae
Petreeae
Lantaneae
Verbeneae
Sesamum_indicum

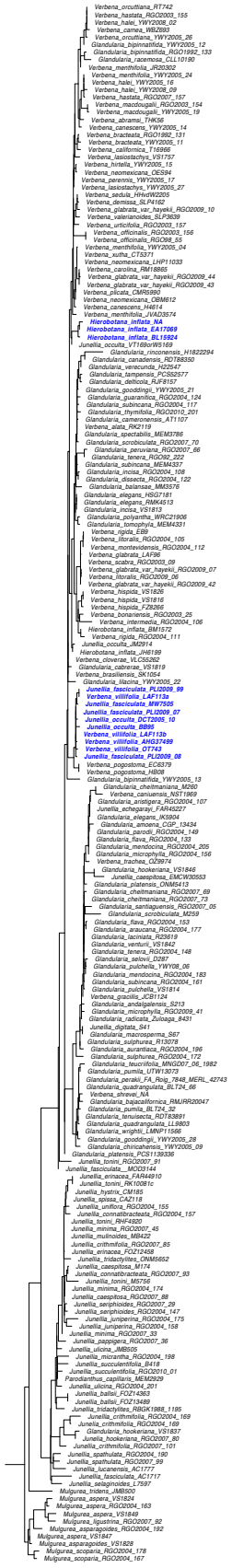

cpDNA
Lantaneae
Coelocarpum
Verbeneae
Dipyrena
Neospartoneae
Rhaphithamnus
Priveae
Citharexyleae
Casselieae
Duranteae
Petreeae


nrDNA
Lantaneae
Verbeneae
Coelocarpum
Dipyrena
Neospartoneae
Priveae
Rhaphithamnus
Casselieae
Citharexyleae
Duranteae
Petreeae


lowcopy nuclear
Lantaneae
Coelocarpum
Verbeneae
Dipyrena
Neospartoneae
Priveae
Rhaphithamnus
Citharexyleae
Casselieae
Duranteae
Petreeae


all combined
Lantaneae
Coelocarpum
Verbeneae
Dipyrena
Neospartoneae
Priveae
Rhaphithamnus
Citharexyleae
Casselieae
Duranteae
Petreeae

A



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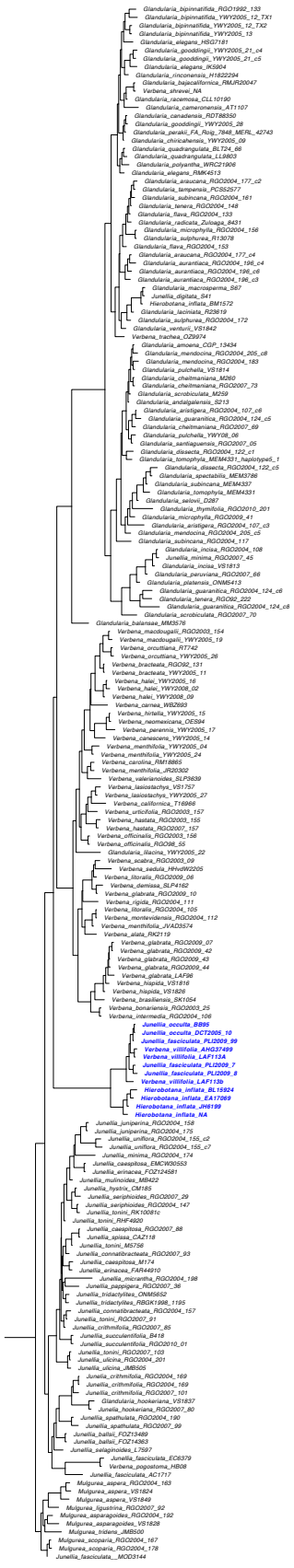

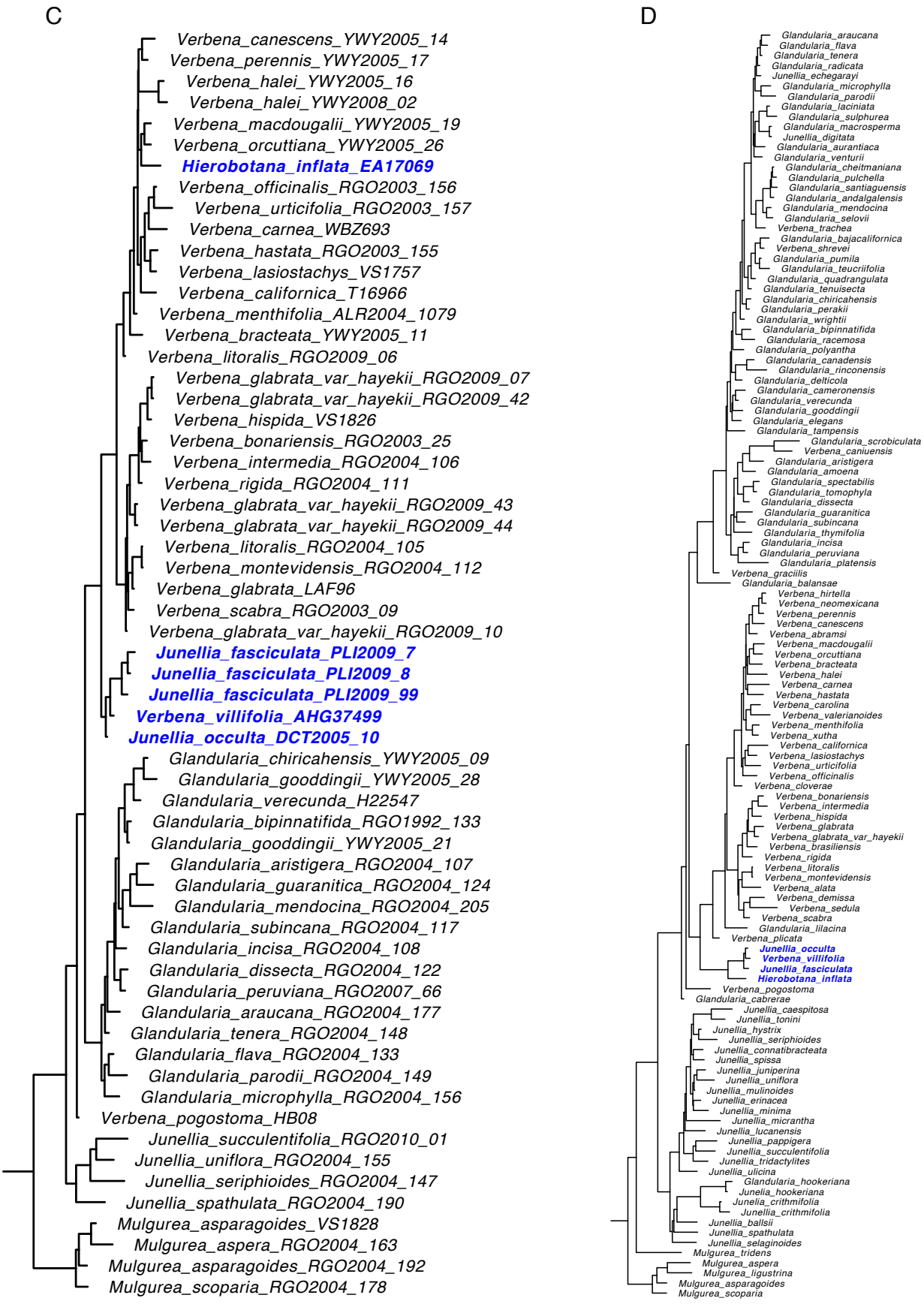
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Verbena_macdougalii_YWY2005_19
Verbena_orcuttiana_YWY2005_26
Hierobotana_inflata_EA17069
Verbena_officinalis_RGO2003_156
Verbena_urticifolia_RGO2003_157
Verbena_carnea_WBZ693
Verbena_hastata_RGO2003_155
Verbena_lasiostachys_VS1757
Verbena_californica_T16966
Verbena_menthifolia_ALR2004_1079
Verbena_bracteata_YWY2005_11
Verbena_litoralis_RGO2009_06
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Verbena_hispida_VS1826
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Verbena_rigida_RGO2004_111
Verbena_glabrata_var_hayekii_RGO2009_43
Verbena_glabrata_var_hayekii_RGO2009_44
Verbena_litoralis_RGO2004_105
Verbena_montevidensis_RGO2004_112
Verbena_glabrata_LAF96
Verbena_scabra_RGO2003_09
Verbena_glabrata_var_hayekii_RGO2009_10
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Junellia_fasciculata_PLI2009_8
Junellia_fasciculata_PLI2009_99
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Glandularia_gooddingii_YWY2005_21
Glandularia_aristigera_RGO2004_107
Glandularia_guaranitica_RGO2004_124
Glandularia_mendocina_RGO2004_205
Glandularia_subincana_RGO2004_117
Glandularia_incisa_RGO2004_108
Glandularia_dissecta_RGO2004_122
Glandularia_peruviana_RGO2007_66
Glandularia_araucana_RGO2004_177
Glandularia_tenera_RGO2004_148
Glandularia_flava_RGO2004_133
Glandularia_parodii_RGO2004_149
Glandularia_microphylla_RGO2004_156
Verbena_pogostoma_HB08
Junellia_succulentifolia_RGO2010_01
Junellia_uniflora_RGO2004_155
Junellia_seriphioides_RGO2004_147
Junellia_spathulata_RGO2004_190
Mulgurea_asparagoides_VS1828
Mulgurea_aspera_RGO2004_163
Mulgurea_asparagoides_RGO2004_192
Mulgurea_scoparia_RGO2004_178
D
Glandularia_araucana
Glandularia_flava
Glandularia_tenera
Glandularia_radicata
Junellia_echegarayi
Glandularia_microphylla
Glandularia_parodii
Glandularia_laciniata
Glandularia_sulphurea
Glandularia_macrosperma
Junellia_digitata
Glandularia_aurantiaca
Glandularia_venturii
Glandularia_cheitmaniana
Glandularia_pulchella
Glandularia_santiaguensis
Glandularia_andalgalensis
Glandularia_mendocina
Glandularia_selovii
Verbena_trachea
Glandularia_bajacalifornica
Verbena_shrevei
Glandularia_pumila
Glandularia_teucriifolia
Glandularia_quadrangulata
Glandularia_tenuisecta
Glandularia_chiricahensis
Glandularia_perakii
Glandularia_wrightii
Glandularia_bipinnatifida
Glandularia_racemosa
Glandularia_polyantha
Glandularia_canadensis
Glandularia_rinconensis
Glandularia_delticola
Glandularia_cameronensis
Glandularia_verecunda
Glandularia_gooddingii
Glandularia_elegans
Glandularia_tampensis
Glandularia_scrobiculata
Verbena_caniuensis
Glandularia_aristigera
Glandularia_amoena
Glandularia_spectabilis
Glandularia_tomophyla
Glandularia_dissecta
Glandularia_guaranitica
Glandularia_subincana
Glandularia_thymifolia
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Glandularia_peruviana
Glandularia_platensis
Verbena_gracilis
Glandularia_balansae
Verbena_hirtella
Verbena_neomexicana
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Verbena_canescens
Verbena_abramsi
Verbena_macdougalii
Verbena_orcuttiana
Verbena_bracteata
Verbena_halei
Verbena_carnea
Verbena_hastata
Verbena_carolina
Verbena_valerianoides
Verbena_menthifolia
Verbena_xutha
Verbena_californica
Verbena_lasiostachys
Verbena_urticifolia
Verbena_officinalis
Verbena_cloverae
Verbena_bonariensis
Verbena_intermedia
Verbena_hispida
Verbena_glabrata
Verbena_glabrata_var_hayekii
Verbena_brasiliensis
Verbena_rigida
Verbena_litoralis
Verbena_montevidensis
Verbena_alata
Verbena_demissa
Verbena_sedula
Verbena_scabra
Glandularia_lilacina
Verbena_plicata
Junellia_occulta
Verbena_villifolia
Junellia_fasciculata
Hierobotana_inflata
Verbena_pogostoma
Glandularia_cabrerae
Junellia_caespitosa
Junellia_tonini
Junellia_hystrix
Junellia_seriphioides
Junellia_connatibracteata
Junellia_spissa
Junellia_juniperina
Junellia_uniflora
Junellia_mulinoides
Junellia_erinacea
Junellia_minima
Junellia_micrantha
Junellia_lucanensis
Junellia_pappigera
Junellia_succulentifolia
Junellia_tridactylites
Junellia_ulicina
Glandularia_hookeriana
Junellia_hookeriana
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Junellia_crithmifolia
Junellia_ballsii
Junellia_spathulata
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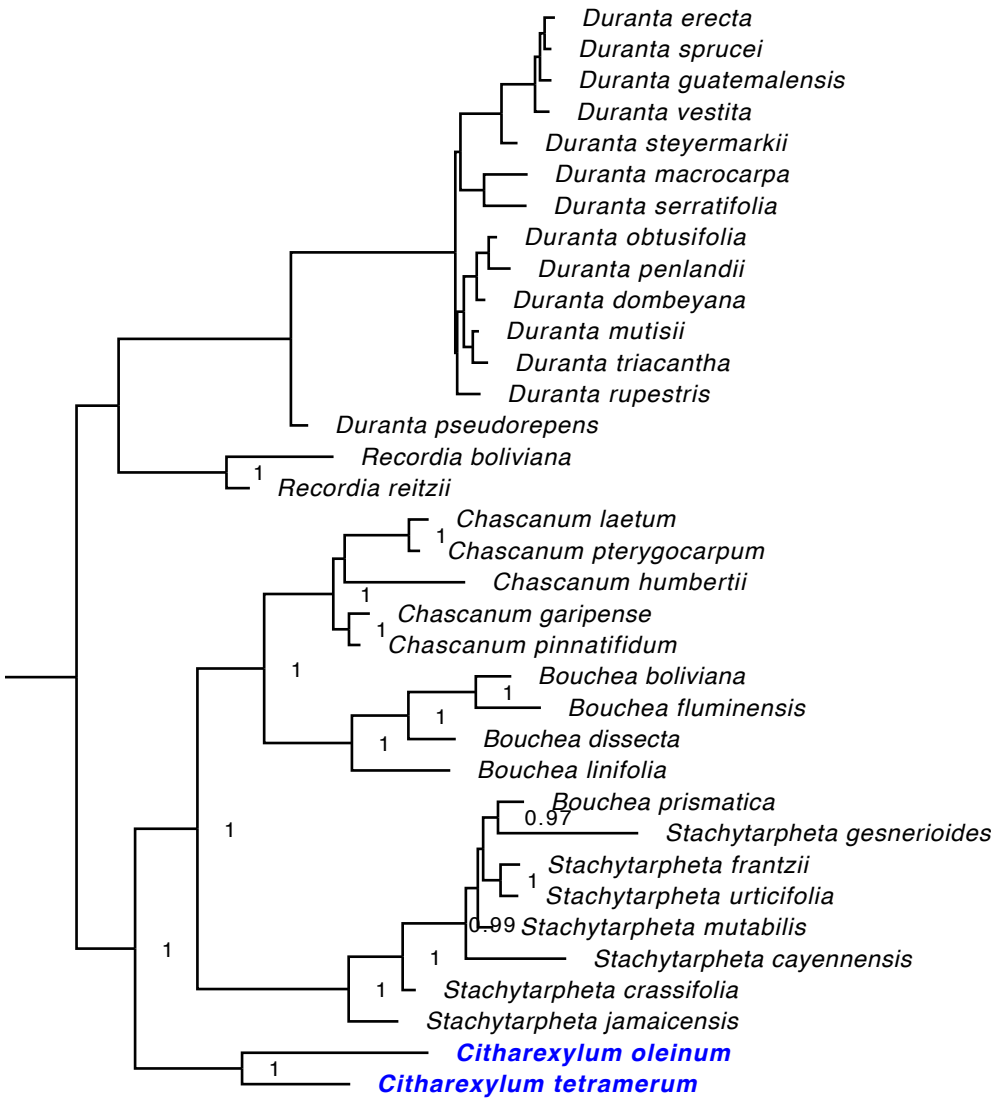
Mulgurea_scoparia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Duranta vestita
Duranta steyermarkii
Duranta macrocarpa
Duranta serratifolia
Duranta obtusifolia
Duranta penlandii
Duranta dombeyana
Duranta mutisii
Duranta triacantha
Duranta rupestris
Duranta pseudorepens
Recordia boliviana
Recordia reitzii
Chascanum laetum
Chascanum pterygocarpum
Chascanum humbertii
Chascanum garipense
Chascanum pinnatifidum
Bouchea boliviana
Bouchea fluminensis
Bouchea dissecta
Bouchea linifolia
Bouchea prismatica
Stachytarpheta gesnerioides
Stachytarpheta frantzii
Stachytarpheta urticifolia
Stachytarpheta mutabilis
Stachytarpheta cayennensis
Stachytarpheta crassifolia
Stachytarpheta jamaicensis
Citharexylum oleinum
Citharexylum tetramerum
1
0.97
0.99

APPENDICES

Appendix S1. Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Acantholippia deserticola	Acantholippia_deserticola_FB4963	F. Biurrun 4963 (SI)
Acantholippia deserticola	Acantholippia_deserticola_RK8847	R. Keisling 8847 (SI)
Acantholippia salsaloides	Acantholippia_salsaloides_RGO2007_28	R.G. Olmstead 2007-28 (WTU)
Acantholippia salsoloides	Acantholippia_salsoloides_RGO2007_23	R.G. Olmstead 2007-23 (WTU)
Acantholippia salsoloides	Acantholippia_salsoloides_RGO2007_52	R.G. Olmstead 2007-52 (WTU)
Acantholippia seriphioides	Acantholippia_seriphioides_C10152	Correa et al. 10152 (SI)
Acantholippia seriphioides	Acantholippia_seriphioides_RGO2004_146	R.G. Olmstead 2004-146 (WTU)
Acantholippia seriphioides	Acantholippia_seriphioides_RGO2004_152	R.G. Olmstead 2004-152 (WTU)
Acantholippia trifida	Acantholippia_trifida_B7706	Biurrun 7706 (SI)
Aloysia axillaris	Aloysia_axillaris_WA21575	Wood & Atahuachi 21575 (KEW)
Aloysia barbata	Aloysia_barbata_CF3902	Carter & Ferris 3902 (US)
Aloysia castellanosii	Aloysia_castellanosii_F41191	Ferriencia 41191 (MERL)
Aloysia catamarcensis	Aloysia_catamarcensis_RGO2007_82	R.G. Olmstead 2007-82 (WTU)
Aloysia chamaedryfolia	Aloysia_chamaedryfolia_HR1131	H. Rimpler 1131 (FB)
Aloysia chamaedryfolia	Aloysia_chamaedryfolia_VT102	V. Thode 102 (ICN)
Aloysia chiapensis	Aloysia_chiapensis_M932	Martinez 932 (TEX)
Aloysia citriodora	Aloysia_citriodora_RGO2003_24	R.G. Olmstead 2003-24 (WTU)
Aloysia citriodora	Aloysia_citriodora_RGO2007_13	R.G. Olmstead 2007-13 (WTU)
Aloysia crenata	Aloysia_crenata_ALC29106	A. L. Cabrera 29106 (SI)
Aloysia dusenii	Aloysia_dusenii_KS38344	Krapocikas & Schinini 38344 (TEX)
Aloysia dusenii	Aloysia_dusenii_RGO2010_217	R.G. Olmstead 2010-217
Aloysia gratissima	Aloysia_gratissima_BLT26_28	B.L. Turner 26-28 (TEX)
Aloysia gratissima	Aloysia_gratissima_KJK12803	K.-J. Kim 12803 (TEX)
Aloysia gratissima	Aloysia_gratissima_PLI2008_17	P. Lu-Irving 08-17 (WTU)
Aloysia gratissima	Aloysia_gratissima_RGO2004_103	R.G. Olmstead 2004-103 (WTU)
Aloysia gratissima	Aloysia_gratissima_RGO2004_109	R.G. Olmstead 2004-109 (WTU)
Aloysia hatschbachii	Aloysia_hatschbachii_GH51897	G. Hatschbach 51897 (US)
Aloysia herrerae	Aloysia_herrerae_RGO2009_30	R.G. Olmstead 2009-30 (WTU)
Aloysia herrerae	Aloysia_herrerae_WS14658	Wood & Serrano 14658 (KEW)
Aloysia looseri	Aloysia_looseri_FAR9847	F.A. Roig 9847 (MERL)
Aloysia lycioides	Aloysia_lycioides_46000	#460-00 (VAL)
Aloysia lycioides	Aloysia_lycioides_RBGK2517602169	251-76-02169 (KEW)
Aloysia macrostachya	Aloysia_macrostachya_PLI2008_14	P. Lu-Irving 2008-14 (WTU)
Aloysia macrostachya	Aloysia_macrostachya_PLI2008_19	P. Lu-Irving 08-19 (WTU)
Aloysia oblanceolata	Aloysia_oblanceolata_VT96	Thode 96 (ICN)
Aloysia peruviana	Aloysia_peruviana_RGO2009_45	R.G. Olmstead 2009-45 (WTU)
Aloysia polygalifolia	Aloysia_polygalifolia_VT398	Thode 398 (ICN)
Aloysia polystachya	Aloysia_polystachya_K817	Kranz 817 (CESJ)
Aloysia pulchra	Aloysia_pulchra_RGO2004_129	R.G. Olmstead 2004-129 (WTU)
Aloysia pulchra	Aloysia_pulchra_VT157	Thode 157 (ICN)
Aloysia scorodonioides	Aloysia_scorodonioides_PLI2009_62	P. Lu-Irving 09-62 (WTU)
Aloysia scorodonioides	Aloysia_scorodonioides_RGO2009_40	R.G. Olmstead 09-40 (WTU)
Aloysia scorodonioides	Aloysia_scorodonioides_S1591	Saravia 1591 (SI)
Aloysia sonorensis	Aloysia_sonorensis_R85_1108	Reichenbacher 85-1108 (TEX)
Aloysia virgata	Aloysia_virgata_RGO2004_133	R.G. Olmstead 2004-133 (WTU)
Aloysia virgata	Aloysia_virgata_RGO2007_68	R.G. Olmstead 07-68 (WTU)
Aloysia virgata	Aloysia_virgata_VJB232_97	Valencia J. B. #232-97

Appendix S1. (cont.) Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Aloysia wrightii	Aloysia_wrightii_O1765	Ocampo 1765 (WTU)
Aloysia wrightii	Aloysia_wrightii_RGO1991_04	R.G. Olmstead 1991-004 (WTU)
Baillonia amabilis	Baillonia_amabilis_GM2188	Guaglianon & Mulgura 2188 (SI)
Baillonia amabilis	Baillonia_amabilis_MC4522	M. Cardenas 4522 (US)
Bouchea boliviana	Bouchea_boliviana_AAM2573	A. Arauja-M et.al. 2573 (MO)
Bouchea dissecta	Bouchea_dissecta_ALRG2004_951	A.L. Reina G. et al., 2004-951 (TEX)
Bouchea fluminensis	Bouchea_fluminensis_HR1141	H. Rimpler 1141 (FB)
Bouchea linifolia	Bouchea_linifolia_BLT20_423	B.L. Turner 20-423 (TEX)
Bouchea prismatica	Bouchea_prismatica_JRA10965	J.R. Abbott 10965 (MO)
Burroughsia appendiculata	Burroughsia_appendiculata_JH14254	James Henrickson 14254 (TEX)
Burroughsia appendiculata	Burroughsia_appendiculata_JH14273	James Henrickson 14273 (LL)
Burroughsia fastigiata	Burroughsia_fastigiata_LLW7847	LL Wiggins 7847 (US)
Burroughsia fastigiata	Burroughsia_fastigiata_SB294	Sikes & Babcock 294 (TEX)
Casselia confertiflora	Casselia_confertiflora_RCM2859	R.C. Mendonca et al. 2859 (US)
Casselia glaziovii	Casselia_glaziovii_MAdS3630	M.A de Silva et al. 3630 (US)
Casselia integrifolia	Casselia_integrifolia_JRP3449	J.R. Pirani et.al. 3449 (US)
Chascanum garipense	Chascanum_garipense_LS11230	L. Smook 11230 (MO)
Chascanum humbertii	Chascanum_humbertii_BL505	B. Lewis et al. 505 (MO)
Chascanum humbertii	Chascanum_humbertii_MR6127	Miller & Randrianasola 6127 (MO)
Chascanum laetum	Chascanum_laetum_JD6923	J. DeWilde 6923 (MO)
Chascanum marrubifolium	Chascanum_marrubifolium_MGMT1099	M. Gilbert & M. Thulin 1099 (MO)
Chascanum marrubiifolium	Chascanum_marrubiifolium_JJL15802	J.J. Lavranos 15802 (MO)
Chascanum pinnatifidum	Chascanum_pinnatifidum_JLBP12207	J. Lanvranos & B. Pehlemann 12207 (MO)
Chascanum pterygocarpum	Chascanum_pterygocarpum_BV759S	B. Verdcourt 759S (MO)
Citharexylum affine	Citharexylum_affine_TW3014	T. Wendt et al. 3014 (F)
Citharexylum affine	Citharexylum_affine_V6743	Villanueva 6743 (MEXU)
Citharexylum affine	Citharexylum_affine_VS	Velasco-Sinaca (MEXU)
Citharexylum alainii	Citharexylum_alainii_VSR2959A	Veloz, Salazar, Reyes 2959-A (FTG)
Citharexylum altamiranum	Citharexylum_altamiranum_EC2609	E. Carranza 2609 (MEXU)
Citharexylum altamiranum	Citharexylum_altamiranum_GJD9971	G. and J. Davidse 9971 (MO)
Citharexylum altamiranum	Citharexylum_altamiranum_M12032	Magaña 12032
Citharexylum altamiranum	Citharexylum_altamiranum_OH9864	Orozoco H. 9864 (MEXU)
Citharexylum altamiranum	Citharexylum_altamiranum_RH4704	R. Hernandez 4704 (MO)
Citharexylum andinum	Citharexylum_andinum_BB981	B. Becker 981 (TEX)
Citharexylum andinum	Citharexylum_andinum_F2628	E. Fernandez et al 2628 (MO)
Citharexylum argutedentatum	Citharexylum_argutedentatum_RGO2009_32	R.G. Olmstead 2009-32 (WTU)
Citharexylum argutedentatum	Citharexylum_argutedentatum_RGO2009_36	R.G. Olmstead 2009-36 (WTU)
Citharexylum berlandieri	Citharexylum_berlandieri_JT674	J. Trusty 674 (FTG)
Citharexylum berlandieri	Citharexylum_berlandieri_LAF65	L.A. Frost 65 (WTU)
Citharexylum berlandieri	Citharexylum_berlandieri_LW803	L. Woodbury 803 (FTG)
Citharexylum berlandieri	Citharexylum_berlandieri_RHM11926	R.H. Magaña 11926 (MEXU)
Citharexylum bourgeauianum	Citharexylum_bourgeauianum_W20066	Webster et al 20066 (HUH)
Citharexylum brachyanthum	Citharexylum_brachyanthum_FC9112	F. Chiang 9112 (F)
Citharexylum brachyanthum	Citharexylum_brachyanthum_JAE2609	J.A. Encina 2609 (MEXU)
Citharexylum brachyanthum	Citharexylum_brachyanthum_JWC11623	Johnston, Wendt, Chiang 11623 (F)
Citharexylum brachyanthum	Citharexylum_brachyanthum_RTC17479	R.T. Colín 17479 (MEXU)
Citharexylum calvum	Citharexylum_calvum_LAF79	L.A. Frost 79 (WTU)

Appendix S1. (cont.) Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Citharexylum calvum	Citharexylum_calvum_LAF80	L.A. Frost 80 (WTU)
Citharexylum caudatum	Citharexylum_caudatum_CSM2352	Clase, Santana Montilla 2352 (FTG)
Citharexylum caudatum	Citharexylum_caudatum_RGO96_73	R.G. Olmstead 1996-73A (WTU)
Citharexylum cooperi	Citharexylum_cooperi_BH25538	B. Hammel 25538 (INB)
Citharexylum costaricense	Citharexylum_costaricense_E626	Espinoza 626
Citharexylum costaricense	Citharexylum_costaricense_LAF2014_01	L.A. Frost 14-01 (WTU)
Citharexylum costaricense	Citharexylum_costaricense_RL1136	R. Lawton 1136 (F)
Citharexylum crassifolium	Citharexylum_crassifolium_RW733	R. Wanderlin et al. 733 (MO)
Citharexylum decorum	Citharexylum_decorum_IA1252	I. Aldoz 1252 (MO)
Citharexylum decorum	Citharexylum_decorum_LAF142	L.A. Frost 142 (WTU)
Citharexylum decorum	Citharexylum_decorum_LAF143	L.A. Frost 143 (WTU)
Citharexylum decorum	Citharexylum_decorum_LAF144	L.A. Frost 144 (WTU)
Citharexylum decorum	Citharexylum_decorum_LAF145	L.A. Frost 145 (WTU)
Citharexylum decorum	Citharexylum_decorum_LAF146	L.A. Frost 146 (WTU)
Citharexylum decorum	Citharexylum_decorum_LAF147	L.A. Frost 147 (WTU)
Citharexylum dentatum	Citharexylum_dentatum_BS2014	B. Stein et al. 2014 (F)
Citharexylum dentatum	Citharexylum_dentatum_D3130	Dillon et al. 3130 (F)
Citharexylum dentatum	Citharexylum_dentatum_LAF90	L.A. Frost 090 (WTU)
Citharexylum dentatum	Citharexylum_dentatum_PLI09_09	P. Lu-Irving 2009-09 (WTU)
Citharexylum donnell-smithii	Citharexylum_donnel_smithii_BH26607	B. Hammel 26607 (INB)
Citharexylum donnell-smithii	Citharexylum_donnel_smithii_LAF2014_10	L.A. Frost 14-10 (WTU)
Citharexylum donnell-smithii	Citharexylum_donnel_smithii_LAF2014_11	L.A. Frost 14-11 (WTU)
Citharexylum donnell-smithii	Citharexylum_donnell_smithii_UWBG	cultivated UWBG, seed from CR
Citharexylum ellipticum	Citharexylum_ellipticum_EC14571	E. Cabrera 14571 (MO)
Citharexylum ellipticum	Citharexylum_ellipticum_LAF74	L.A. Frost 74 (WTU)
Citharexylum ellipticum	Citharexylum_ellipticum_LAF75	L.A. Frost 75 (WTU)
Citharexylum ellipticum	Citharexylum_ellipticum_LAF77	L.A. Frost 77 (WTU)
Citharexylum ellipticum	Citharexylum_ellipticum_LAF78	L.A. Frost 78 (WTU)
Citharexylum flabellifolium	Citharexylum_flabellifolium_F85_903B	Felger 85-903B (TEX)
Citharexylum flabellifolium	Citharexylum_flabellifolium_FS6115	F. Shreve 6115 (F)
Citharexylum flabellifolium	Citharexylum_flabellifolium_TRVD93_818	VanDevender et al. 93-818 (MO)
Citharexylum flexuosum	Citharexylum_flexuosum_LAF109	L.A. Frost 109 (WTU)
Citharexylum flexuosum	Citharexylum_flexuosum_LAF110	L.A. Frost 110 (WTU)
Citharexylum flexuosum	Citharexylum_flexuosum_LAF111	L.A. Frost 111 (WTU)
Citharexylum flexuosum	Citharexylum_flexuosum_LAF117	L.A. Frost 117 (WTU)
Citharexylum flexuosum	Citharexylum_flexuosum_LAF118	L.A. Frost 118 (WTU)
Citharexylum flexuosum	Citharexylum_flexuosum_LAF89	L.A. Frost 089 (WTU)
Citharexylum flexuosum	Citharexylum_flexuosum_PLI2009_13	P. Lu-Irving 2009-13 (WTU)
Citharexylum flexuosum	Citharexylum_flexuosum_PLI2009_19	P. Lu-Irving 2009-19 (WTU)
Citharexylum fulgidum	Citharexylum_fulgidum_MRR544	M. Rosas R. 544 (HUH-Arnold Arboretum)
Citharexylum glabrum	Citharexylum_glabrum_GM10169	Garcia-Mendoza 10169 (MEXU)
Citharexylum glabrum	Citharexylum_glabrum_JAM5312	J.A. Machuca 5312 (MO)
Citharexylum glabrum	Citharexylum_glabrum_JCSN17646	J.C. Soto N. 17646 (MEXU)
Citharexylum glabrum	Citharexylum_glabrum_RCD3121	R.C. Durán 3121 (MEXU)
Citharexylum guatemalense	Citharexylum_guatemalense_IC1827	I. Coronado et al. 1827 (MO)
Citharexylum herrerae	Citharexylum_herrerae_RGO09_11	R.G. Olmstead 2009-11 (WTU)
Citharexylum herrerae	Citharexylum_herrerae_RGO09_21	R.G. Olmstead 2009-21 WTU)

Appendix S1. (cont.) Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Citharexylum hexangulare	Citharexylum_hexangulare_C22275	Calzada 22275 (MEXU)
Citharexylum hexangulare	Citharexylum_hexangulare_C27709	Chagata 27709 (MEXU)
Citharexylum hexangulare	Citharexylum_hexangulare_LAF51	L.A. Frost 51 (WTU)
Citharexylum hexangulare	Citharexylum_hexangulare_LAF73	L.A. Frost 73 (WTU)
Citharexylum hexangulare	Citharexylum_hexangulare_LAF86	L.A. Frost 86 (WTU)
Citharexylum hexangulare	Citharexylum_hexangulare_LAF87	L.A. Frost 87 (WTU)
Citharexylum hexangulare	Citharexylum_hexangulare_M20	Martinez 20
Citharexylum hidalgense	Citharexylum_hidalgense_A1971	Ayala 1971
Citharexylum hidalgense	Citharexylum_hidalgense_BBG740291	Berkley Botanical Garden 740291
Citharexylum hidalgense	Citharexylum_hidalgense_JR22356	J. Rzedowski 22356 (F)
Citharexylum hidalgense	Citharexylum_hidalgense_PT530	P. Tenorio 530 (MO)
Citharexylum hintonii	Citharexylum_hintonii_GH7534	G. Hinton 7531 (MO)
Citharexylum hirtellum	Citharexylum_hirtellum_EMMS	E.M. Martínez Salas (MO)
Citharexylum hirtellum	Citharexylum_hirtellum_LAF82	L.A. Frost 82 (WTU)
Citharexylum hirtellum	Citharexylum_hirtellum_LAF84	L.A. Frost 84 (WTU)
Citharexylum ilicifolium	Citharexylum_ilicifolium_KEW	cultivated Royal Botanic Gardens, Kew
Citharexylum ilicifolium	Citharexylum_ilicifolium_LAF135	L.A. Frost 135 (WTU)
Citharexylum ilicifolium	Citharexylum_ilicifolium_LAF137	L.A. Frost 137 (WTU)
Citharexylum ilicifolium	Citharexylum_ilicifolium_LAF152	L.A. Frost 152 (WTU)
Citharexylum joergenseni	Citharexylum_joergenseni_EF1711	E. Fernandez et al. 1711 (MO)
Citharexylum karsteni	Citharexylum_karsteni_LAF2013_03	L.A. Frost 13-03 (WTU)
Citharexylum karsteni	Citharexylum_karsteni_LAF2013_15	L.A. Frost 13-15 (WTU)
Citharexylum karsteni	Citharexylum_karsteni_LAF2013_16	L.A. Frost 13-16 (WTU)
Citharexylum kobuskianum	Citharexylum_kobuskianum_LAF114	L.A. Frost 114 (WTU)
Citharexylum kobuskianum	Citharexylum_kobuskianum_LAF115	L.A. Frost 115 (WTU)
Citharexylum kobuskianum	Citharexylum_kobuskianum_LAF116	L.A. Frost 116 (WTU)
Citharexylum kunthianum	Citharexylum_kunthianum_LAF140	L.A. Frost 13-19 B (WTU)
Citharexylum kunthianum	Citharexylum_kunthianum_LAF141	L.A. Frost 13-23 A (WTU)
Citharexylum kunthianum	Citharexylum_kunthianum_LAF2013_19B	L.A. Frost 13-26 (WTU)
Citharexylum kunthianum	Citharexylum_kunthianum_LAF2013_23A	L.A. Frost 140 (WTU)
Citharexylum kunthianum	Citharexylum_kunthianum_LAF2013_26	L.A. Frost 141 (WTU)
Citharexylum ligustrinum	Citharexylum_ligustrinum_RGBK0006951235	cultivated Royal Botanic Gardens, Kew
Citharexylum ligustrinum	Citharexylum_ligustrinum_V18711	Ventura 18711 (MO)
Citharexylum lycioides	Citharexylum_lycioides_C154	Chávez et al. rjchm 154
Citharexylum lycioides	Citharexylum_lycioides_S955	Spellman et al. 955 (F)
Citharexylum macradenium	Citharexylum_macradenium_BH26602	B. Hammel 26602 (INB)
Citharexylum mexicanum	Citharexylum_mexicanum_FC_F243	F. Chiang et al. F-243 (MO, MEXU)
Citharexylum mexicanum	Citharexylum_mexicanum_GP2165	Gomez Pompa 2165 (MEXU)
Citharexylum mexicanum	Citharexylum_mexicanum_S625	Salinas 625
Citharexylum mexicanum	Citharexylum_mexicanum_TN318	Taylor and Nee 318 (MO)
Citharexylum microphyllum	Citharexylum_microphyllum_ARSG8502	Acasado-Rdgz., Siaca, Garcia 8502 (FTG)
Citharexylum microphyllum	Citharexylum_microphyllum_RGNR4084	R. Garcia, N. Ramirez 4084 (F)
Citharexylum mocinnoi	Citharexylum_mocinnoi_LAF54	L.A. Frost 54 (WTU)
Citharexylum mocinnoi	Citharexylum_mocinnoi_LAF59	L.A. Frost 59 (WTU)
Citharexylum mocinnoi	Citharexylum_mocinnoi_LAF60	L.A. Frost 60 (WTU)
Citharexylum mocinnoi var. longibracteolata	Citharexylum_mocinnoi_longibracteolata_LAF71	L.A. Frost 71 (WTU)
Citharexylum montanum	Citharexylum_montanum_LAF129	L.A. Frost 129 (WTU)

Appendix S1. (cont.) Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Citharexylum montanum	Citharexylum_montanum_LAF131	L.A. Frost 131 (WTU)
Citharexylum montanum	Citharexylum_montanum_LAF132	L.A. Frost 132 (WTU)
Citharexylum montanum	Citharexylum_montanum_LAF133	L.A. Frost 133 (WTU)
Citharexylum montanum	Citharexylum_montanum_LAF2013_10	L.A. Frost 13-10 (WTU)
Citharexylum montanum	Citharexylum_montanum_LAF2013_25	L.A. Frost 13-25 (WTU)
Citharexylum montevidensis	Citharexylum_montevidensis_RGO2004_101	R.G. Olmstead 2004-101 (WTU)
Citharexylum montevidensis	Citharexylum_montevidensis_RGO2004_102	R.G. Olmstead 2004-102 (WTU)
Citharexylum myrianthum	Citharexylum_myrianthum_AK578	A. Kegler 578 (MO)
Citharexylum oleinum	Citharexylum_oleinum_B3587	Bartholomew et al. 3587 (HUH-Gray)
Citharexylum oleinum	Citharexylum_oleinum_DN2473	Diggs & Nee 2473 (F)
Citharexylum ovatifolium	Citharexylum_ovatifolium_GW11813	G. Webster et al. 11813 (F)
Citharexylum ovatifolium	Citharexylum_ovatifolium_MY00781	M. Yánez 00781 (MO)
Citharexylum pachyphyllum	Citharexylum_pachyphyllum_BV471	B. Vuilleumier 471 (HUH)
Citharexylum pachyphyllum	Citharexylum_pachyphyllum_D1655	E.W. Davis et al. 1655 (F)
Citharexylum pachyphyllum	Citharexylum_pachyphyllum_H1066	Hudson 1066 (MO)
Citharexylum peruvianum	Citharexylum_peruvianum_LAF100	L.A. Frost 100 (WTU)
Citharexylum peruvianum	Citharexylum_peruvianum_LAF121	L.A. Frost 121 (WTU)
Citharexylum peruvianum	Citharexylum_peruvianum_LAF123	L.A. Frost 123 (WTU)
Citharexylum peruvianum	Citharexylum_peruvianum_LAF99	L.A. Frost 99 (WTU)
Citharexylum peruvianum	Citharexylum_peruvianum_S17122	Segasteguii 17122 (USM)
Citharexylum poeppigii	Citharexylum_poeppigii_LAF2013_11	L.A. Frost 13-11 (WTU)
Citharexylum poeppigii	Citharexylum_poeppigii_LAF2013_12	L.A. Frost 13-12 (WTU)
Citharexylum racemosum	Citharexylum_racemosum_EC5797	E. Carrauza 5797 (TEX)
Citharexylum racemosum	Citharexylum_racemosum_M5997	Martinez 5997
Citharexylum reticulatum	Citharexylum_reticulatum_AA581	A. Araijo et al. 581 (MO)
Citharexylum rimbachii	Citharexylum_rimbachii_LAF138	L.A. Frost 138 (WTU)
Citharexylum rimbachii	Citharexylum_rimbachii_LAF139	L.A. Frost 139 (WTU)
Citharexylum rosei	Citharexylum_rosei_CWJ8180	Chiang, Wendt, Johnston 8180 (F)
Citharexylum roxanae	Citharexylum_roxanae_AC5083	A. Carter 5083 (F)
Citharexylum roxanae	Citharexylum_roxanae_RM23834	R. Moran 23834 (MO)
Citharexylum scabrum	Citharexylum_scabrum_SLF359_94	Friedman 359-94
Citharexylum schottiiCR	Citharexylum_schottiiCR_LAF2014_04	L.A. Frost 14-04 (WTU)
Citharexylum schottiiCR	Citharexylum_schottiiCR_LAF2014_07	L.A. Frost 14-07 (WTU)
Citharexylum schottiiCR	Citharexylum_schottiiCR_LAF2014_09	L.A. Frost 14-09 (WTU)
Citharexylum schottiiMEX	Citharexylum_schottiiMEX_LAF62	L.A. Frost 62 (WTU)
Citharexylum schottiiMEX	Citharexylum_schottiiMEX_LAF63	L.A. Frost 63 (WTU)
Citharexylum solanaceum	Citharexylum_solanaceum_RW341	R. Wasum 341 (MO)
Citharexylum spinosum	Citharexylum_spinosum_JCS5727	J.C. Solomon 5727 (TEX)
Citharexylum spinosum	Citharexylum_spinosum_RGO14_04	R.G. Olmstead 2014-04 (WTU)
Citharexylum spinosum	Citharexylum_spinosum_RGO96_113	R.G. Olmstead 1996-113 (WTU)
Citharexylum standleyi	Citharexylum_standleyi_AM3011	A. Magallanes 3011 (F)
Citharexylum standleyi	Citharexylum_standleyi_L1797	Lott 1796
Citharexylum standleyi	Citharexylum_standleyi_LAPJ2182b	LAPJ 2182b
Citharexylum steyermarkii	Citharexylum_steyermarkii_DEB40360	D.E. Breedlove 40360 (MO)
Citharexylum subflavescens	Citharexylum_subflavescens_LAF2013_01	L.A. Frost 13-01 (WTU)
Citharexylum subflavescens	Citharexylum_subflavescens_LAF2013_06	L.A. Frost 13-06 (WTU)
Citharexylum subflavescens	Citharexylum_subflavescens_LAF2013_27	L.A. Frost 13-27 (WTU)

Appendix S1. (cont.) Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Citharexylum subthyrsoideum	Citharexylum_subthyrsoideum_MGG30	M. Gabriela Gomez 30 (MO)
Citharexylum sulcatum	Citharexylum_sulcatum_LAF2013_07B	L.A. Frost 13-07 B (WTU)
Citharexylum sulcatum	Citharexylum_sulcatum_LAF2013_08	L.A. Frost 13-08 (WTU)
Citharexylum tetramerum	Citharexylum_tetramerum_M8638	Mendoza 8638
Citharexylum tetramerum	Citharexylum_tetramerum_PT4717	P. Teuoria 4717 (F)
Citharexylum tristachyum	Citharexylum_tristachyum_G11484	Gillis 11484 (FTG)
Citharexylum ulei	Citharexylum_ulei_LAF128	L.A. Frost 128 (WTU)
Citharexylum ulei	Citharexylum_ulei_LAF130	L.A. Frost 130 (WTU)
Citharexylum weberbaueri	Citharexylum_weberbaueri_LAF103	L.A. Frost 103 (WTU)
Citharexylum weberbaueri	Citharexylum_weberbaueri_LAF105	L.A. Frost 105 (WTU)
Citharexylum weberbaueri	Citharexylum_weberbaueri_LAF106	L.A. Frost 106 (WTU)
Citharexylum weberbaueri	Citharexylum_weberbaueri_LAF120	L.A. Frost 120 (WTU)
Citharexylum weberbaueri	Citharexylum_weberbaueri_LAF124	L.A. Frost 124 (WTU)
Citharexylum weberbaueri	Citharexylum_weberbaueri_LAF125	L.A. Frost 125 (WTU)
Citharexylum weberbaueri	Citharexylum_weberbaueri_LAF126	L.A. Frost 126 (WTU)
Citharexylum weberbaueri	Citharexylum_weberbaueri_PLI09_04	P. Lu-Irving 2009-04 (WTU)
Coelocarpum madagascariense	Coelocarpum_madagascariense_GES2977	G.E. Schatz 2977 (MO)
Coelocarpum madagascariense	Coelocarpum_madagascariense_PM3569	Phillipson and Milijaona 3569 (MO)
Coelocarpum swinglei	Coelocarpum_swinglei_PBP3443	Phillopson et al. 3443 (MO)
Diostea juncea	Diostea_juncea_RBGK1969_35347	RBG Kew 1969-35347
Diostea juncea	Diostea_juncea_RGBE19300262	RBG Edinburgh 19300262
Diostea juncea	Diostea_juncea_RGO2010_3	R.G. Olmstead 2010-03 (WTU)
Dipryena glaberrima	Dipryena_glaberrima_RGO2004_179	R.G. Olmstead 2004-179 (WTU)
Duranta costaricana	Duranta_costaricana_EA2025	E. Alfaro 2025 (MO)
Duranta dombeyana	Duranta_dombeyana_AS9723	A. Sagastegui 9723 (TEX)
Duranta erecta	Duranta_erecta_RGO1992_211	R.G. Olmstead 1992-211 (WTU)
Duranta erecta	Duranta_erecta_RGO1996_100	R.G. Olmstead 1996-100 (WTU)
Duranta erecta	Duranta_erecta_RGO2009_39	R.G. Olmstead 2009-39 (WTU)
Duranta erecta variegata	Duranta_erecta_variegata_RGO92_206	R.G. Olmstead 92-206 (WTU)
Duranta fletcheriana	Duranta_fletcheriana_1996_71	R.G. Olmstead 1996-71 (WTU)
Duranta guatemalensis	Duranta_guatemalensis_SOG4741	S. Ochoa Gaona 4741 (TEX)
Duranta macrocarpa	Duranta_macrocarpa_ML88672	M. Lewis 88672 (MO)
Duranta mutisii	Duranta_mutisii_LAF2013_09	L.A. Frost 13-09 (WTU)
Duranta mutisii	Duranta_mutisii_PJ1666	P. Jorgensen et al. 1666 (MO)
Duranta obtusifolia	Duranta_obtusifolia_AM1048	A. Macurdy 1048 (MO)
Duranta obtusifolia	Duranta_obtusifolia_LAF2013_28	L.A. Frost 13-28 (WTU)
Duranta penlandii	Duranta_penlandii_BS10673	B. Sparre 10673 (US) (Maybe 18673)
Duranta pseudorepens	Duranta_pseudorepens_C12474	Can et al. 12474 (USOR)
Duranta rupestris	Duranta_rupestris_RGO2009_12	R.G. Olmstead 2009-12 (WTU)
Duranta rupestris	Duranta_rupestris_RGO2009_33	R.G. Olmstead 2009-33 (WTU)
Duranta serratifolia	Duranta_serratifolia_RGO2007_09	R.G. Olmstead 2007-09 (WTU)
Duranta sprucei	Duranta_sprucei_RGO1992_221	R.G. Olmstead 1992-221 (WTU)
Duranta steyermarkii	Duranta_steyermarkii_SLP2767	S. Lopez-Palacios 2767 (TEX)
Duranta triacantha	Duranta_triacantha_CA432	C. Antezama 432 (MO)
Duranta triacantha	Duranta_triacantha_IP2046	I. Padilla 2046 (MO)
Duranta triacantha	Duranta_triacantha_RGO2009_20	R.G. Olmstead 2009-20 (WTU)
Duranta vestita	Duranta_vestita_MRRW50	M. Rossato & R. Wasum 50 (MO)

Appendix S1. (cont.) Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Duranta vestita	Duranta_vestita_RW1624	R. Wasum 1624 (US)
Glandularia amoena CGP	Glandularia_amoena_CGP_13434	C.G. Pringle 13434
Glandularia andalgalensis	Glandularia_andalgalensis_S213	Solomon 213
Glandularia araucana	Glandularia_araucana_RGO2004_177	R.G. Olmstead 2004-177 (WTU)
Glandularia aristigera	Glandularia_aristigera_RGO2004_107	R.G. Olmstead 2004-107 (WTU)
Glandularia aurantiaca	Glandularia_aurantiaca_RGO2004_196	R.G. Olmstead 2004-196 (WTU)
Glandularia bajacalifornica	Glandularia_bajacalifornica_RMJRR20047	R. Moran & J. Reveal 20047 (US)
Glandularia balansae	Glandularia_balansae_MM3576	M.E. Mulgara 3576 (CTES,FCQ, SI)
Glandularia bipinnatifida	Glandularia_bipinnatifida_RGO1992_133	R.G. Olmstead 1992-133 (WTU)
Glandularia bipinnatifida	Glandularia_bipinnatifida_YWY2005_12	Y.-W. Yuan 2005-12 (WTU)
Glandularia bipinnatifida	Glandularia_bipinnatifida_YWY2005_13	Y.-W. Yuan 2005-13 (WTU)
Glandularia cabrerae	Glandularia_cabrerae_VS1819	V. Soza 1819 (WTU)
Glandularia cameronensis	Glandularia_cameronensis_AT1107	A. Traverse 1107
Glandularia canadensis	Glandularia_canadensis_RDT88350	R. Dale Thomas 88350 (US)
Glandularia cheitmaniana	Glandularia_cheitmaniana_M260	Marazzi 260 (SI)
Glandularia cheitmaniana	Glandularia_cheitmaniana_RGO2007_69	R.G. Olmstead 2007-69 (WTU)
Glandularia cheitmaniana	Glandularia_cheitmaniana_RGO2007_73	R.G. Olmstead 2007-73 (WTU)
Glandularia chiricahensis	Glandularia_chiricahensis_YWY2005_09	Y.-W. Yuan 2005-09 (WTU)
Glandularia delticola	Glandularia_delticola_RJF8157	Raymond J. Fleetwood 8157 (US)
Glandularia dissecta	Glandularia_dissecta_RGO2004_122	R.G. Olmstead 2004-122 (WTU)
Glandularia elegans	Glandularia_elegans_HSG7181	H. S. Gentry 7181
Glandularia elegans	Glandularia_elegans_IK5904	I. Knobloch 5904
Glandularia elegans	Glandularia_elegans_RMK4513	R. M. King 4513
Glandularia flava	Glandularia_flava_RGO2004_133	R.G. Olmstead 2004-133 (WTU)
Glandularia flava	Glandularia_flava_RGO2004_153	R.G. Olmstead 2004-153 (WTU)
Glandularia gooddingii	Glandularia_gooddingii_YWY2005_21	Y.-W. Yuan 2005-21 (WTU)
Glandularia gooddingii	Glandularia_gooddingii_YWY2005_28	Y.-W. Yuan 2005-28 (WTU)
Glandularia guaranitica	Glandularia_guaranitica_RGO2004_124	R.G. Olmstead 2004-124 (WTU)
Glandularia hookeriana	Glandularia_hookeriana_VS1837	V. Soza 1837 (WTU)
Glandularia hookeriana	Glandularia_hookeriana_VS1846	V. Soza 1846 (WTU)
Glandularia incisa	Glandularia_incisa_RGO2004_108	R.G. Olmstead 2004-108 (WTU)
Glandularia incisa	Glandularia_incisa_VS1813	V. Soza 1813
Glandularia laciniata	Glandularia_laciniata_R23619	Reughoal 23619 (MERL)
Glandularia lilacina	Glandularia_lilacina_YWY2005_22	Y.-W. Yuan 2005-22 (WTU)
Glandularia macrosperma	Glandularia_macrosperma_S67	Solomon 67
Glandularia mendocina	Glandularia_mendocina_RGO2004_183	R.G. Olmstead 2004-183 (WTU)
Glandularia mendocina	Glandularia_mendocina_RGO2004_205	R.G. Olmstead 2004-205 (WTU)
Glandularia microphylla	Glandularia_microphylla_RGO2004_156	R.G. Olmstead 2004-156 (WTU)
Glandularia microphylla	Glandularia_microphylla_RGO2009_41	R.G. Olmstead 2009-41 (WTU)
Glandularia parodii	Glandularia_parodii_RGO2004_149	R.G. Olmstead 2004-149 (WTU)
Glandularia perakii	Glandularia_perakii_FA_Roig_7848_MERL_42743	F.A. Roig 7848 (MERL 42743)
Glandularia peruviana	Glandularia_peruviana_RGO2007_66	R.G. Olmstead 2007-66 (WTU)
Glandularia platensis	Glandularia_platensis_ONM5413	O.N. Morrone 5413
Glandularia platensis	Glandularia_platensis_PCS1139336	P. C. Standley 1139336 (US)
Glandularia polyantha	Glandularia_polyantha_WRC21906	W.R. Carr 21906
Glandularia pulchella	Glandularia_pulchella_VS1814	V. Soza 1814
Glandularia pulchella	Glandularia_pulchella_YWY08_06	Y.-W. Yuan 08-06

Appendix S1. (cont.) Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Glandularia pumila	Glandularia_pumila_BLT24_32	B.L. Turner 24-32
Glandularia pumila	Glandularia_pumila_UTW13073	U. T. Waterfall 13073 (US)
Glandularia quadrangulata	Glandularia_quadrangulata_BLT24_66	Lundell & Lundell 9803
Glandularia quadrangulata	Glandularia_quadrangulata_LL9803	B.L. Turner 24-66
Glandularia racemosa	Glandularia_racemosa_CLL10190	C.L. Lundell 10190 (US)
Glandularia radicata Zuloaga	Glandularia_radicata_Zuloaga_8431	F.O. Zuloaga 8431
Glandularia rinconensis	Glandularia_rinconensis_H1822294	H. et al. (US 1822294)
Glandularia santiaguensis	Glandularia_santiaguensis_RGO2007_05	R.G. Olmstead 2007-05 (WtU)
Glandularia scrobiculata	Glandularia_scrobiculata_M259	Marazzi 259 (SI)
Glandularia scrobiculata	Glandularia_scrobiculata_RGO2007_70	R.G. Olmstead 2007-70 (WTU)
Glandularia selovii	Glandularia_selovii_D287	Denham s.s. 287
Glandularia spectabilis	Glandularia_spectabilis_MEM3786	M.E. Mulgura et al 3786
Glandularia subincana	Glandularia_subincana_MEM4337	M.E. Mulgara et al 4337
Glandularia subincana	Glandularia_subincana_RGO2004_117	R.G. Olmstead 2004-117 (WTU)
Glandularia subincana	Glandularia_subincana_RGO2004_161	R.G. Olmstead 2004-161 (WTU)
Glandularia sulphurea	Glandularia_sulphurea_R13078	Reughoal 13078 (MERL)
Glandularia sulphurea	Glandularia_sulphurea_RGO2004_172	R.G. Olmstead 2004-172 (WTU)
Glandularia tampensis	Glandularia_tampensis_PCS52577	P. C. Standley 52577 (US)
Glandularia tenera	Glandularia_tenera_RGO1992_222	R.G. Olmstead 92-222 (WTU)
Glandularia tenera	Glandularia_tenera_RGO2004_148	R.G. Olmstead 2004-148 (WTU)
Glandularia tenuisecta	Glandularia_tenuisecta_RDT83891	R. Dale Thomas 83891
Glandularia teucriifolia	Glandularia_teucriifolia_MNGD07_06_1982	M. Nee & G. Diggs 07/06/1982 (US)
Glandularia thymifolia	Glandularia_thymifolia_RGO2010_201	R.G. Olmstead 2010-201 (WTU)
Glandularia tomophyla	Glandularia_tomophyla_MEM4331	M.E. Mulgara et al 4331
Glandularia venturii	Glandularia_venturii_VS1842	V. Soza 1842 (WTU)
Glandularia verecunda	Glandularia_verecunda_H22547	Henrickson 22547 (US)
Glandularia wrightii	Glandularia_wrightii_LMNP11566	Lehto, McGill, Nash & Pinkava 11566 (US)
Hierobotana inflata	Hierobotana_inflata_BL15924	B. Lojtant 15924 (MO)
Hierobotana inflata	Hierobotana_inflata_BM1572	B. MacBryde 1572 (MO)
Hierobotana inflata	Hierobotana_inflata_EA17069	E. Asplund 17069 US
Hierobotana inflata	Hierobotana_inflata_JH6199	Jack Humbles 6199 (TEX)
Hierobotana inflata	Hierobotana_inflata_NA	No Voucher
Junelia crithmifolia	Junellia_crithmifolia_RGO2004_169	R.G. Olmstead 2004-169 (WTU)
Junelia hookeriana	Junellia_hookeriana_RGO2007_80	R.G. Olmstead 2007-80 (WTU)
Junellia ballsii	Junellia_ballsii_FOZ13489	F.O. Zuloaga 14363
Junellia ballsii	Junellia_ballsii_FOZ14363	F.O. Zuloaga 13489
Junellia caespitosa	Junellia_caespitosa_EMCW30553	E. Mendez & C. Waillond 30553 (MERL)
Junellia caespitosa	Junellia_caespitosa_M174	Meglioli 174 (SI)
Junellia caespitosa	Junellia_caespitosa_RGO2007_88	R.G. Olmstead 2007-88 (WTU)
Junellia connatibracteata	Junellia_connatibracteata_RGO2004_157	R.G. Olmstead 2004-157 (WtU)
Junellia connatibracteata	Junellia_connatibracteata_RGO2007_93	R.G. Olmstead 2007-93 (WTU)
Junellia crithmifolia	Junellia_crithmifolia_RGO2004_169	R.G. Olmstead 2004-169 (WTU)
Junellia crithmifolia	Junellia_crithmifolia_RGO2007_101	R.G. Olmstead 2007-101 (WTU)
Junellia crithmifolia	Junellia_crithmifolia_RGO2007_85	R.G. Olmstead 2007-85 (WTU)
Junellia digitata	Junellia_digitata_S41	Solomon 41
Junellia echegarayi	Junellia_echegarayi_FAR45227	F.A. Roig et al 45227 (MERL)
Junellia erinacea	Junellia_erinacea_FAR44910	F.A. Roig et al 44910 (MERL)

Appendix S1. (cont.) Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Junellia erinacea	Junellia_erinacea_FOZ12458	F.O. Zuloaga 12458
Junellia fasciculata	Junellia_fasciculata_AC1717	A. Cano 1717 (MO)
Junellia fasciculata	Junellia_fasciculata_EC6379	E. Cerrate 6379 (USM)
Junellia fasciculata	Junellia_fasciculata_MOD3144	M.O. Dillon et al. 3144 (USM)
Junellia fasciculata	Junellia_fasciculata_MW7505	M. Weigand et al. 7505 (USM)
Junellia fasciculata	Junellia_fasciculata_PLI2009_07	P. Lu-Irving 2009-07 (WTU)
Junellia fasciculata	Junellia_fasciculata_PLI2009_08	P. Lu-Irving 2009-08 (WTU)
Junellia fasciculata	Junellia_fasciculata_PLI2009_99	P. Lu-Irving 2009-99 (WTU)
Junellia hystrix	Junellia_hystrix_CM185	C. Meglioli et al 185 (WTU)
Junellia juniperina	Junellia_juniperina_RGO2004_158	R.G. Olmstead 2004-158 (WTU)
Junellia juniperina	Junellia_juniperina_RGO2004_175	R.G. Olmstead 2004-175 (WTU)
Junellia lucanensis	Junellia_lucanensis_AC1777	A. Cano 1777 (MO)
Junellia micrantha	Junellia_micrantha_RGO2004_198	R.G. Olmstead 2004-198 (WTU)
Junellia minima	Junellia_minima_RGO2004_174	R.G. Olmstead 2004-174 (WTU)
Junellia minima	Junellia_minima_RGO2007_33	R.G. Olmstead 2007-33 (WTU)
Junellia minima	Junellia_minima_RGO2007_45	R.G. Olmstead 2007-45 (WTU)
Junellia mulinoides	Junellia_mulinoides_MB422	M. Bonifacino 422
Junellia occulta	Junellia_occulta_BB95	B. Becker et al. 95 (TEX)
Junellia occulta	Junellia_occulta_DCT2005_10	D.C. Tank 2005-10 (WTU)
Junellia occulta	Junellia_occulta_JM2914	J. Mostacero et al. 2914 (MO)
Junellia occulta	Junellia_occulta_VT169orW5169	VT 169 or Weigand 5169
Junellia pappigera	Junellia_pappigera_RGO2007_36	R.G. Olmstead 2007-36 (WTU)
Junellia selaginoides	Junellia_selaginoides_L7597	Lammers et al., 7597 (US)
Junellia seriphioides	Junellia_seriphioides_RGO2004_147	R.G. Olmstead 2004-147 (WTU)
Junellia seriphioides	Junellia_seriphioides_RGO2007_29	R.G. Olmstead 2007-29 (WTU)
Junellia spathulata	Junellia_spathulata_RGO2004_190	R.G. Olmstead 2004-190 (WTU)
Junellia spathulata	Junellia_spathulata_RGO2007_99	R.G. Olmstead 2007-99 (WTU)
Junellia spissa	Junellia_spissa_CAZ118a	C.A. Zanotti 118
Junellia succulentifolia	Junellia_succulentifolia_B418	Bonifacino 418 (US)
Junellia succulentifolia	Junellia_succulentifolia_RGO2010_01	R.G. Olmstead 2010-01 (WtU)
Junellia tonini	Junellia_tonini_M5756	Morrone 5756 (SI)
Junellia tonini	Junellia_tonini_RGO2007_103	R.G. Olmstead 2007-103 (WTU)
Junellia tonini	Junellia_tonini_RGO2007_91	R.G. Olmstead 2007-91 (WTU)
Junellia tonini	Junellia_tonini_RHF4920	R.H. Fortunato 4920
Junellia tonini	Junellia_tonini_RK10081c	R. Kiesling 10081c (SI)
Junellia tridactylites	Junellia_tridactylites_ONM5652	O.N. Morrone et al. 5652
Junellia tridactylites	Junellia_tridactylites_RBGK1988_1195	RBG Kew #1988-1195; Voucher unknown
Junellia ulicina	Junellia_ulicina_JMB505	J.M. Bonifacino 505
Junellia ulicina	Junellia_ulicina_RGO2004_201	R.G. Olmstead 2004-201 (WTU)
Junellia uniflora	Junellia_uniflora_RGO2004_155	R.G. Olmstead 2004-155 (WTU)
Lampayo castellani	Lampayo_castellani_BV386	B.Vuilleumier 386 (TEX)
Lampayo castellani	Lampayo_castellani_RGO2007_63	R.G. Olmstead 2007-063 (WTU)
Lampayo hieronymi	Lampayo_hieronymi_EMC2092	E. Marilienez Carretero 2092 (MERL)
Lampayo hieronymi	Lampayo_hieronymi_FB4960	F. Biurran et al. 4960 (SI)
Lampayo medicinalis	Lampayo_medicinalis_BGHNR311	BGHNR 311 (RGBE)
Lantana angolensis	Lantana_angolensis_H915	Hines 915 (PRE)
Lantana angolensis	Lantana_angolensis_LR572	Long & Rae 572 (MO)

Appendix S1. (cont.) Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Lantana buchii	Lantana_buchii_PLI2012_107	P. Lu-Irving 12-107 (WTU)
Lantana camara	Lantana_camara_PLI2012_37	P. Lu-Irving 12-37 (WTU)
Lantana camara	Lantana_camara_PLI2012_3A	P. Lu-Irving 2012-3A (WTU)
Lantana camara	Lantana_camara_RGO1992_140	R.G. Olmstead 92-140 (WTU)
Lantana canascens	Lantana_canascens_RGO2007_06	R.G. Olmstead 2007-06 (WTU)
Lantana cujabensis	Lantana_cujabensis_PLI2010_19	P. Lu-Irving 10-19 (WTU)
Lantana depressa	Lantana_depressa_PLI2012_1	P. Lu-Irving 12-1 (WTU)
Lantana dinteri	Lantana_dinteri_MG30347	Merxmuller & Gress 30347 (PRE)
Lantana entrerriensis	Lantana_entrerriensis_RGO2004_126	R.G. Olmstead 2004-126 (WTU)
Lantana exarata	Lantana_exarata_PLI2012_49	P. Lu-Irving 12-49 (WTU)
Lantana ferreyrae	Lantana_ferreyrae_PLI05_06_2010	P. Lu-Irving s.n. May 6, 2010 (WTU)
Lantana fucata	Lantana_fucata_FRGS2952	F.R.G. Salimena 2952 (CESJ)
Lantana haughtii	Lantana_haughtii_PLI2009_34	P. Lu-Irving 09-34 (WTU)
Lantana horrida	Lantana_horrida_KJK12808	K.-J. Kim 12808 (TEX)
Lantana horrida	Lantana_horrida_PLI2012_61	P. Lu-Irving 12-61 (WTU)
Lantana involucrata	Lantana_involucrata_PLI2012_13	P. Lu-Irving 12-13
Lantana involucrata	Lantana_involucrata_RGO1996_124	R.G. Olmstead 1996-124 (WTU)
Lantana leonardiorum	Lantana_leonardiorum_PLI2012_102	P. Lu-Irving 12-102 (WTU)
Lantana leucocarpa	Lantana_leucocarpa_PLI2012_70	P. Lu-Irving 12-70 (WTU)
Lantana macropoda	Lantana_macropoda_NM7355	Nesom & Mayfield 7355 (TEX)
Lantana micrantha	Lantana_micrantha_RGO2007_08	R.G. Olmstead 2007-08 (WTU)
Lantana microcephala	Lantana_microcephala_PLI2008_07	P. Lu-Irving 2008-07 (WTU)
Lantana montevidensis	Lantana_montevidensis_PLI2008_15	P. Lu-Irving 2008-15 (WTU)
Lantana montevidensis	Lantana_montevidensis_RGO2010_203	R.G. Olmstead 2010-203 (WTU)
Lantana pauciflora	Lantana_pauciflora_PLI2012_106	P. Lu-Irving 2012-106
Lantana reptans	Lantana_reptans_PLI2009_14	P. Lu-Irving 2009-14 (WTU)
Lantana reticulata	Lantana_reticulata_PLI2012_66	P. Lu-Irving 2012-66 (WTU)
Lantana rugosa	Lantana_rugosa_PLI2008_25	P. Lu-Irving 2008-25 (WTU)
Lantana rugosa	Lantana_rugosa_VWV389	VW. Vos 389 (NU)
Lantana scabiosiflora	Lantana_scabiosiflora_PLI2009_01	P. Lu-Irving 2009-1 (WTU)
Lantana scabrida	Lantana_scabrida_PLI2012_89	P. Lu-Irving 2012-89 (WTU)
Lantana strigocamara	Lantana_strigocamara_PLI2012_22	P. Lu-Irving 2012-22 (WTU)
Lantana tilcarensis	Lantana_tilcarensis_RGO2007_18	R.G. Olmstead 2007-18 (WTU)
Lantana trifolia	Lantana_trifolia_PLI2009_59	P. Lu-Irving 2009-59 (WTU)
Lantana trifolia	Lantana_trifolia_PLI2012_90	P. Lu-Irving 2012-90 (WTU)
Lantana trifolia	Lantana_trifolia_RGO1996_98	R.G. Olmstead 1996-98 (WTU)
Lantana ukambensis	Lantana_ukambensis_FM80	Mawi 80 (MO)
Lantana urticifolia	Lantana_urticifolia_RGO1996_75	R.G. Olmstead 1996-75 (WTU)
Lantana urticoides	Lantana_urticoides_PLI2008_02	P. Lu-Irving 2008-2 (WTU)
Lantana viburnoides	Lantana_viburnoides_M991013R29	Miyazaki 991013R29 (TEX)
Lantana viburnoides	Lantana_viburnoides_PK23167	P. Kuchar 23167 (MO)
Lantana xenica	Lantana_xenica_VS1838	V. Soza 1838 (WTU)
Lippia alba	Lippia_alba_FTG71293A	FTG 71293A (FTG)
Lippia alba	Lippia_alba_RGO2004_110	RGO 04-110 (WTU)
Lippia arechavaletae	Lippia_arechavaletae_NA	No Voucher
Lippia aristata	Lippia_aristata_PLI2010_05	PLI 2010-5 (WTU)
Lippia asperrima	Lippia_asperrima_ERG3282	E.R. Guaglianon et al. 3282

Appendix S1. (cont.) Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Lippia asperrima	Lippia_asperrima_RGO2004_140	RGO 04-140
Lippia betulifolia	Lippia_betulifolia_WH4841	W. Hahn 4841 (US)
Lippia brasiliensis	Lippia_brasiliensis_PLI2010_17	PLI 2010-17 (WTU)
Lippia coartacta	Lippia_coartacta_ONM5253	O.N. Morrone 5253
Lippia corymbosa	Lippia_corymbosa_PLI2010_13	PLI 2010-13 (WTU)
Lippia diamantinensis	Lippia_diamantinensis_FRGS2943	F.R.G. Salimena 2943 (CESJ)
Lippia domingensis	Lippia_domingensis_PLI2012_80	PLI 2012-80 (WTU)
Lippia duartei	Lippia_duartei_PLI2010_11	PLI 2010-11 (WTU)
Lippia dulcis	Lippia_dulcis_NA	No Voucher
Lippia filifolia	Lippia_filifolia_VT352	V. Thode 352 (ICN)
Lippia florida	Lippia_florida_FRGS2945	F.R.G. Salimena 2945 (CESJ)
Lippia formosa	Lippia_formosa_O1764	Ocampo 1764
Lippia grandifolia	Lippia_grandifolia_MR5249	M. Reekmans 5249 (PRE)
Lippia grisebachiana	Lippia_grisebachiana_C382	Caldella et al. 382
Lippia hederifolia	Lippia_hederifolia_PLI2010_14	PLI 10-14 (WTU)
Lippia hemannioides	Lippia_hemannioides_VT389	V. Thode 389 (ICN)
Lippia integrifolia	Lippia_integrifolia_RGO2007_78	RGO 2007-78 (WTU)
Lippia javanica	Lippia_javanica_PLI2012_1a	PLI 2012-1a (WTU)
Lippia javanica	Lippia_javanica_WV390	W. Vos 390 (NU)
Lippia kituiensi	Lippia_kituiensi_LF917	L. Festo et al 917 (MO)
Lippia lasiocalycina	Lippia_lasiocalycina_VT363	Thode 363 (WTU)
Lippia lippioides	Lippia_lippioides_MEM4185	M.E. Mulgara et al 4185
Lippia lupulina	Lippia_lupulina_FRGS2941	F.R.G. Salimena 2941 (CESJ)
Lippia macrophylla	Lippia_macrophylla_TS13474	Thomas & Sant'Ana 13474 (CESJ)
Lippia micromera	Lippia_micromera_RGO1992_225	RGO 92-225 (WTU)
Lippia myriocephala	Lippia_myriocephala_SOG144	SOG 144 (HULE)
Lippia oatesii	Lippia_oatesii_B4199	Biegel 4199 (PRE)
Lippia origanoides	Lippia_origanoides_PLI2010_18	PLI 10-18 (WTU)
Lippia origanoides	Lippia_origanoides_RGO1992_210	RGO 92-210 (WTU)
Lippia phryxocalyx	Lippia_phryxocalyx_GE4506	G. Eiten 4506 (US)
Lippia pseudothea	Lippia_pseudothea_FS2940	FS 2940 (CESJ)
Lippia pusilla	Lippia_pusilla_VT337	V. Thode 337 (ICN)
Lippia rehmannii	Lippia_rehmannii_HHWP92	HHW Pearson 2/12/92
Lippia rehmannii	Lippia_rehmannii_PLI2008_20	PLI 2008-20 (WTU)
Lippia rhodocnemis	Lippia_rhodocnemis_PLI2010_06	PLI 2010-6 (WTU)
Lippia rotundifolia	Lippia_rotundifolia_FRGS2958	F.R.G. Salimena 2958 (CESJ)
Lippia rubella	Lippia_rubella_PLI2010_3	PLI 10-3 (WTU)
Lippia salsa	Lippia_salsa_RGO2004_180	RGO 04-180 (WTU)
Lippia salviifolia	Lippia_salviifolia_FRGS2975	F.R.G. Salimena 2975 (CESJ)
Lippia salviifolia	Lippia_salviifolia_RGO2004_131	RGO 2004-131 (WTU)
Lippia triplinervis	Lippia_triplinervis_M2456	Mota 2456 (WTU)
Lippia trollii	Lippia_trollii_FOZ11537	F.O. Zuloaga 11537
Lippia turbinata	Lippia_turbinata_RGO2007_74	RGO 2007-74 (WTU)
Lippia turnerifolia	Lippia_turnerifolia_RGO2004_121	RGO 2004-121 (WTU)
Lippia ukambensis	Lippia_ukambensis_PJG9915	P.J. Greenway 9915 (PRE)
Lippia umbellata	Lippia_umbellata_VD06_194	Van Devender 06-194 (TEX)
Lippia velutina	Lippia_velutina_PLI2010_16	PLI 10-16 (WTU)

Appendix S1. (cont.) Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Lippia wilmsii	Lippia_wilmsii_PLI2012_11	PLI 12-11 (WTU)
Mulgurea asparagoides	Mulgurea_asparagoides_RGO2004_192	R.G. Olmstead 2004-192 (WTU)
Mulgurea asparagoides	Mulgurea_asparagoides_VS1828	V. Soza 1828 (WTU)
Mulgurea aspera	Mulgurea_aspera_RGO2004_163	R.G. Olmstead 2004-163 (WtU)
Mulgurea aspera	Mulgurea_aspera_VS1824	V. Soza 1824 (WTU)
Mulgurea aspera	Mulgurea_aspera_VS1849	V. Soza 1849 (WTU)
Mulgurea ligustrina	Mulgurea_ligustrina_RGO2007_92	R.G. Olmstead 2007-92 (WTU)
Mulgurea scoparia	Mulgurea_scoparia_RGO2004_167	R.G. Olmstead 2004-167 (WTU)
Mulgurea scoparia	Mulgurea_scoparia_RGO2004_178	R.G. Olmstead 2004-178 (WTU)
Mulgurea tridens	Mulgurea_tridens_JMB500	J.M. Bonifacino 500 (US)
Nashia inaguensis	Nashia_inaguensis_FTG8655	Fairchild Tropical Gardens 8655
Nashia inaguensis	Nashia_inaguensis_Hirts	cultivated Hirt's Gardens
Nashia inaguensis	Nashia_inaguensis_WTG13097	William T. Gillis 13097 (TEX)
Neosparton aphyllum	Neosparton_aphyllum_RGO2004_150	RGO 2004-150 (WTU)
Neosparton aphyllum	Neosparton_aphyllum_RGO2004_193	RGO 2004-193 (WTU)
Neosparton ephedroides	Neosparton_ephedroides_RGO2004_194	RGO 2004-194 (WTU)
Neosparton ephedroides	Neosparton_ephedroides_RGO2007_77	RGO 2007-077 (WTU)
Parodianthus capillaris	Parodianthus_capillaris_MEM2929	M.E. Mulgara 2929 (SI)
Parodianthus ilicifolius	Parodianthus_ilicifolius_RGO2004_181	RGO 2004-181 (WTU)
Petrea blanchetiana	Petrea_blanchetiana_GdN5812	G. de Nevers 5812 (MO)
Petrea bracteata	Petrea_bracteata_AHG13325	A.H. Gentry 13325 (MO)
Petrea kohautiana	Petrea_kohautiana_JLC6554	J.L. Clark 6554 (US)
Petrea maynensis	Petrea_maynensis_GV856	G. Villa 856 (MO)
Petrea maynensis	Petrea_maynensis_RB1765	R. Burnham 1765 (MO)
Petrea volubilis	Petrea_volubilis_RBGK3267503134	RBGK #326.75.03134
Petrea volubilis	Petrea_volubilis_RGBK0007317818	W. Ramirez et al. 17 (MO)
Petrea volubilis	Petrea_volubilis_RGO2011_07	RGO 2011-07
Phyla canascens	Phyla_canascens_RGO2004_159	RGO 2004-159 (WTU)
Phyla cuneifolia	Phyla_cuneifolia_RGO1992_134	RGO 1992-134 (WTU)
Phyla dulcis	Phyla_dulcis_RGO1998_56	RGO 1998-56 (WTU)
Phyla incisa	Phyla_incisa_KJK12801	K.-J. Kim 12801 (TEX)
Phyla lanceolata	Phyla_lanceolata_M4090	McCormac 4090 (OS)
Phyla lanceolata	Phyla_lanceolata_PLI2008_16	PLI 2008-16 (WTU)
Phyla nodiflora	Phyla_nodiflora_PLI2008_03	PLI 2008-03 (WTU)
Phyla nodiflora	Phyla_nodiflora_PLI2008_04	PLI 2008-04 (WTU)
Phyla reptans	Phyla_reptans_RGO2007_65	RGO 2007-65 (WTU)
Phyla scaberrima	Phyla_scaberrima_R168	Rothschild 168 (TEX)
Phyla stoechadifolia	Phyla_stoechadifolia_C43312	Correll 43312 (TEX)
Pitrea cuneato-ovata	Pitrea_cuneato_ovata_RGO2004_186	RGO 2004-186 (WTU)
Pitrea cuneato-ovata	Pitrea_cuneato_ovata_RGO2007_81	RGO 2007-81 (WTU)
Priva bahiensis	Priva_bahiensis_MS6025	M. Sobral et al 6025 (SI)
Priva cordifolia	Priva_cordifolia_WV391	W. Vos 391 (NU)
Priva lappulacea	Priva_lappulacea_RGO1996_86	RGO 1996-86 (WTU)
Priva lappulacea	Priva_lappulacea_TSC8651	T.S. Cochrane 8651 (TEX)
Recordia boliviana	Recordia_boliviana_MN42092	M. Nee 42092 (TEX)
Recordia boliviana	Recordia_boliviana_NRJW2932	N. Ritter, J. Wood 2932 (MO)
Recordia reitzii	Recordia_reitzii_166756	166756 (ICN)

Appendix S1. (cont.) Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Rehdera penninervia	Rehdera_penninervia_CLLEC19938	C.L. Lundell, Elias Contreras 19938 (TEX)
Rehdera penninervia	Rehdera_penninervia_MPCNB1378	M. Pena-Chocarro & N. Bonilla 1378 (MO)
Rehdera trinervis	Rehdera_trinervis_DN2386	D. Neil 2386 (MO)
Rehdera trinervis	Rehdera_trinervis_EM31706	E. Martinez et al, 31706 (TEX)
Rehdera trinervis	Rehdera_trinervis_EMS30531	E.Martinez S. 30531 (TEX)
Rhaphithamnus spinosus	Rhaphithamnus_spinosus_RBGK1288301596	Kew 128-83.01596
Rhaphithamnus venustus	Rhaphithamnus_venustus_TFS11855	T.F. Stuessy 11855 (OS)
Stachytarpheta cayannensis	Stachytarpheta_cayannensis_RGO951	RGO 951 (WTU)
Stachytarpheta cayennensis	Stachytarpheta_cayennensis_RGO2004_113	RGO 2004-113 (WTU)
Stachytarpheta crassifolia	Stachytarpheta_crassifolia_PT10	P. Tavares et al 10 (MO)
Stachytarpheta frantzii	Stachytarpheta_frantzii_FTG2001_0533B	Fairchild Tropical Gardens 2001-0533B
Stachytarpheta gesnerioides	Stachytarpheta_gesnerioides_WWT5915	W.W.Thomas et al. 5915 (MO)
Stachytarpheta jamaicensis	Stachytarpheta_jamaicensis_RGO1996_68	RGO 1996-68 (WTU)
Stachytarpheta jamaicensis	Stachytarpheta_jamaicensis_RGO2003_20	RGO 2003-20 (WTU)
Stachytarpheta mutabilis	Stachytarpheta_mutabilis_RGO1992_207	RGO 1992-207 (WTU)
Stachytarpheta urticifolia	Stachytarpheta_urticifolia_JT673	J Trusty 673 (FTG)
Tamonea boxiana	Tamonea_boxiana_RGO2003_12	RGO 2003-12 (WTU)
Tamonea curassavica	Tamonea_curassavica_HR1917	H. Rimpler 1917 (FB)
Tamonea juncea	Tamonea_juncea_EM14514	E. Martinez 14514 (MO)
Verbena abramsi	Verbena_abramsi_THK56	T.H. Kearney 56
Verbena alata	Verbena_alata_RK2119	R. Kurmorow 2119 (TEX)
Verbena bonariensis	Verbena_bonariensis_RGO2003_25	R.G. Olmstead 2003-25 (WTU)
Verbena bracteata	Verbena_bracteata_RGO1992_131	R.G. Olmstead 92-131 (WTU)
Verbena bracteata	Verbena_bracteata_YWY2005_11	Y.-W. Yuan 2005-11 (WTU)
Verbena brasiliensis	Verbena_brasiliensis_SK1054	Strong & Kelloff 1054
Verbena californica	Verbena_californica_T16966	Taylor 16966 (UC/JEPS)
Verbena canescens	Verbena_canescens_H4614	Hinckley 4614 (TEX)
Verbena canescens	Verbena_canescens_YWY2005_14	Y.-W. Yuan 2005-14 (WTU)
Verbena caniuensis	Verbena_caniuensis_NST1969	N.S. Troneso 1969 (TEX)
Verbena carnea	Verbena_carnea_WBZ693	W.B. Zomlefer 693 (WTU)
Verbena carolina	Verbena_carolina_RM18865	R. McVaugh 18865
Verbena cloverae	Verbena_cloverae_VLC55262	V.L. Cory 55262
Verbena demissa	Verbena_demissa_SLP4162	S. Lopez-Palacios 4162 (TEX)
Verbena glabrata	Verbena_glabrata_LAF96	LAF 96 (WTU)
Verbena glabrata var. hayekii	Verbena_glabrata_var_hayekii_RGO2009_07	R.G. Olmstead 2009–07 (WTU)
Verbena glabrata var. hayekii	Verbena_glabrata_var_hayekii_RGO2009_10	R.G. Olmstead 2009-10 (WTU)
Verbena glabrata var. hayekii	Verbena_glabrata_var_hayekii_RGO2009_42	R.G. Olmstead 2009-42 (WTU)
Verbena glabrata var. hayekii	Verbena_glabrata_var_hayekii_RGO2009_43	R.G. Olmstead 2009-43 (WTU)
Verbena glabrata var. hayekii	Verbena_glabrata_var_hayekii_RGO2009_44	R.G. Olmstead 2009-44 (WTU)
Verbena graciilis	Verbena_graciilis_JCB1124	J.C. Blumer 1124
Verbena halei	Verbena_halei_YWY2005_16	Y.-W. Yuan 2005-16 (WTU)
Verbena halei	Verbena_halei_YWY2008_02	Y.-W. Yuan 2008-02 (WTU)
Verbena halei	Verbena_halei_YWY2008_09	Y.-W. Yuan 2008-09 (WTU)
Verbena hastata	Verbena_hastata_RGO2003_155	R.G. Olmstead 2003-155 (WTU)
Verbena hastata	Verbena_hastata_RGO2007_157	R.G. Olmstead 2007-157 (WTU)
Verbena hirtella	Verbena_hirtella_YWY2005_15	Y.-W. Yuan 2005-15 (WTU)
Verbena hispida	Verbena_hispida_FZ8266	F. Zuloaga 8266 (SI)

Appendix S1. (cont.) Specimens included in this study with voucher information

Taxon	Tip label	Voucher (Herbarium)
Verbena hispida	Verbena_hispida_VS1816	V. Soza 1816 (WTU)
Verbena hispida	Verbena_hispida_VS1826	V. Soza 1826 (WTU)
Verbena intermedia	Verbena_intermedia_RGO2004_106	R.G. Olmstead 2004-106 (WTU)
Verbena lasiostachys	Verbena_lasiostachys_VS1757	Soza 1757 (WTU)
Verbena lasiostachys	Verbena_lasiostachys_YWY2005_27	Y.-W. Yuan 2005-27 (WTU)
Verbena litoralis	Verbena_litoralis_RGO2004_105	R.G. Olmstead 2004-105 (WTU)
Verbena litoralis	Verbena_litoralis_RGO2009_06	RGO 2009-06 (WTU)
Verbena macdougalii	Verbena_macdougalii_RGO2003_154	RGO 2003-154 (WTU)
Verbena macdougalii	Verbena_macdougalii_YWY2005_19	Y.-W. Yuan 2005-19 (WTU)
Verbena menthifolia	Verbena_menthifolia_ALR2004_1079	A. L. Reina G. 2004-1079 (TEX)
Verbena menthifolia	Verbena_menthifolia_JR20302	J. Rzedowski 20302 (MICH)
Verbena menthifolia	Verbena_menthifolia_JVAD3574	Jennie V. A. Dieterle 3574 (MICH)
Verbena menthifolia	Verbena_menthifolia_YWY2005_04	Y.-W. Yuan 2005-04 (WTU)
Verbena menthifolia	Verbena_menthifolia_YWY2005_24	Y.-W. Yuan 2005-24 (WTU)
Verbena montevidensis	Verbena_montevidensis_RGO2004_112	R.G. Olmstead 2004-112 (WTU)
Verbena neomexicana	Verbena_neomexicana_LHP11033	Lehto, Hensel & Pinkava 11033
Verbena neomexicana	Verbena_neomexicana_OBM612	O.B. Metcalfe 612
Verbena neomexicana	Verbena_neomexicana_OES94	O.E. Sperry 94
Verbena officinalis	Verbena_officinalis_RGO2003_156	R.G. Olmstead 2003-156 (WTU)
Verbena officinalis	Verbena_officinalis_RGO98_55	RGO 98-55 (WTU)
Verbena orcuttiana	Verbena_orcuttiana_RT742	R. Tallent 742 (MICH)
Verbena orcuttiana	Verbena_orcuttiana_YWY2005_26	Y.-W. Yuan 2005-26 (WTU)
Verbena perennis	Verbena_perennis_YWY2005_17	Y.-W. Yuan 2005-17 (WTU)
Verbena plicata	Verbena_plicata_CMR5990	C.M. Rogers 5990
Verbena pogostoma	Verbena_pogostoma_EC6379	E. Carrate 6379 (USM)
Verbena pogostoma	Verbena_pogostoma_HB08	H. Beltran 08 (USM)
Verbena rigida	Verbena_rigida_EB9	Erixon & Bremer 9 (UPS)
Verbena rigida	Verbena_rigida_RGO2004_111	R.G. Olmstead 2004-111 (WTU)
Verbena scabra	Verbena_scabra_RGO2003_09	R.G. Olmstead 2003-09 (WTU)
Verbena sedula	Verbena_sedula_HHvdW2205	H. H. van der Werff 2205 (TEX)
Verbena shrevei	Verbena_shrevei_NA	No Voucher
Verbena trachea	Verbena_trachea_OZ9974	O. Zollner 9974 (TEX)
Verbena urticifolia	Verbena_urticifolia_RGO2003_157	R.G. Olmstead 2003-157 (WTU)
Verbena valerianoides	Verbena_valerianoides_SLP3639	S. Lopez-Palacios 3639 (TEX)
Verbena villifolia	Verbena_villifolia_AHG37499	A. Gentry et al. 37499 (TEX)
Verbena villifolia	Verbena_villifolia_LAF113A	LAF 113A (WTU)
Verbena villifolia	Verbena_villifolia_LAF113B	LAF 113B (WTU)
Verbena villifolia	Verbena_villifolia_OT743	O. Tovar 743 (TEX)
Verbena xutha	Verbena_xutha_CT5371	Clansen & Trapido 5371
Xeroaloysia ovatifolia	Xeroaloysia_ovatifolia_RGO2004_184	RGO 2004-184 (WTU)
Xolocotzia asperifolia	Xolocotzia_asperifolia_DN5477	David Neill 5477 (MO)
Xolocotzia asperifolia	Xolocotzia_asperifolia_WDS22332	W.D. Stevens 22332 (MO)