Origins of North American arid-land Verbenaceae: More than one way to skin a cat[1]

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PREMISE OF THE STUDY: Verbenaceae originated and initially diversified in South America in wet forest habitats. They have diversified extensively in arid habitats in both South and North America. This study aims to understand the origin of the North American arid-land members of Verbenaceae.

METHODS: A phylogenetic approach is used to examine four genera (*Aloysia*, *Citharexylum*, *Glandularia*, *Verbena*) in three distinct clades with representatives in North American deserts and disjunct South and North American distributions. Phylogenetic analyses were conducted using maximum likelihood and Bayesian approaches. Analyses included both plastid and nuclear DNA regions and include the first study of *Citharexylum* and an expanded sampling of tribe Verbeneae (*Glandularia* and *Verbena*). Ancestral areas were reconstructed for each group.

KEY RESULTS: North American desert species of *Aloysia* and *Glandularia* were likely derived from ancestors in arid temperate South America, perhaps by long-distance dispersal. The pattern for *Verbena* was less clear, with evidence from plastid DNA implicating an Andean dispersal route to the North American clade, whereas nuclear data suggest that the Andean and North American species resulted from independent dispersals from southern South America. A previously unrecognized clade of Andean Verbeneae was discovered, raising the possibility of an Andean origin of *Verbena* or *Verbena* and *Glandularia*. North American desert species of *Citharexylum* represent multiple, independent origins from mesic habitat ancestors in Mesoamerica.

CONCLUSIONS: North American arid-zone Verbenaceae are derived from South and Central American ancestors via multiple avenues, including long-distance, amphitropical dispersal, Andean migration corridors, and in situ evolution of desert-adapted species.

KEY WORDS   *Aloysia*; Andean corridor; biogeography; *Citharexylum*; *Glandularia*; long-distance dispersal; *Verbena*

Arid regions of North and South America lie between ca. 25° and 40° N and S, respectively (with extensions outside of that range in both continents due to local conditions), and are separated by over 5000 km of predominantly mesic ecosystems. During the last 120 million years (Myr) of Earth's history, the two continents were widely separated until a terrestrial corridor connecting North and South America at ca. 5–15° N first appeared no more than ca. 20 Myr ago (Ma) and became continuous possibly no more than ca. 3.5 Ma (Cody et al., 2010; Montes et al., 2012; Bacon et al., 2015). Despite this long history of separation, many plant groups exhibit disjunct distributions between the arid lands in North America and

South America (Raven, 1963; Solbrig, 1972; Wen and Ickert-Bond, 2009).

Verbenaceae originated in South America ca. 50–40 Ma (Marx et al., 2010; D. Tank, University of Idaho, and R. Olmstead, University of Washington, unpublished data), most likely in the wet tropical forests that predominated at that time (Olmstead, 2013; Greenwood and Wing, 1995). With subsequent cooling and drying ca. 15–10 Ma, arid regions expanded from small topographically controlled sites to the extensive regions present in both continents today (Graham, 2011). Verbenaceae diversified significantly in the arid zones of what is now Argentina and adjacent regions (Marx et al., 2010; Lu-Irving and Olmstead, 2013; P. Lu-Irving et al., unpublished manuscript). Verbenaceae also are represented in North America with seven of nine major lineages occurring north of the Isthmus of Panama (Olmstead, 2013) and several lineages represented in the arid zones of Mexico and the southern United States. The disjunctions between North and South American arid regions have been described for *Glandularia* and *Verbena* (Lewis and Oliver, 1961; Umber, 1979) and *Aloysia* (Lu-Irving et al., 2014). *Citharexylum* also includes several species distributed in the desert

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regions of North America (Rzedowski, 1983; Villaseñor, 2016), but is absent from the comparable regions in South America. No prior phylogenetic study of *Citharexylum* has been published that might shed light on the origin of the North American desert species.

In this study, we explore the origin of four lineages of Verbenaceae (*Aloysia*, *Citharexylum*, *Glandularia*, and *Verbena*) found in North American arid habitats. Because Verbenaceae arose and diversified first in South America, North American lineages are all inferred to have South American ancestry (Yuan and Olmstead, 2008a; Marx et al., 2010; Lu-Irving and Olmstead, 2013; Olmstead, 2013; Lu-Irving et al., 2014). However, several biogeographic patterns are possible for the North American lineages, including (1) long-distance dispersal from South American arid zones, (2) a migration route connecting South American and North American arid zones, or (3) de novo evolution of plants in North American arid zones from wet tropical forest ancestors. We review previous evidence from Lantaneae and Verbeneae (Yuan and Olmstead, 2008a, b; O'Leary et al., 2009; Lu-Irving et al., 2014) and present new evidence from *Verbena* and *Citharexylum* to improve our understanding of the origin of North American arid-zone Verbenaceae.

MATERIALS AND METHODS

Because this study integrates previously published results (*Aloysia*: Lu-Irving et al., 2014), a previously studied group for which additional sampling was obtained (Verbeneae: Yuan and Olmstead, 2008a; O'Leary et al., 2009), and a group (*Citharexylum*) not previously subject to phylogenetic analysis, sampling and DNA regions vary among the different case studies. For a table of vouchers, herbaria housing voucher specimens, and GenBank accession numbers accompanying sequence data used in this study, see Appendix S1 in the Supplemental Data with this article. Herbaria abbreviations follow Index Herbariorum (Thiers, 2017: http://sweetgum.nybg.org/science/ih/). 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).

No new data were generated for *Aloysia* in this study. The previously published tree of Lu-Irving et al. (2014) based on the chloroplast and nuclear loci *rpl32-trnL*, *trnT-L*, *trnQ-rps16* and ETS, PPR11 (AtG09680), PPR81 (AtG25970), PPR123 (AtG39980), respectively, is used here (PPR loci numbered sequentially as done by Yuan et al. [2009] and as formally designated in GenBank). The species tree from the *BEAST coalescence-based Bayesian analysis in Lu-Irving et al. (2014) is used in this study over the tree based on maximum likelihood (ML)/Bayesian analyses of the concatenated sequence data, despite conflict in the placement of the North American desert species, for the reasons described by Lu-Irving et al. (2014). All voucher data and GenBank accession numbers are found in Lu-Irving et al. (2014).

For this study, we included 47 new accessions of Verbeneae representing 17 new taxa, with an emphasis on Andean and North American species. New accessions included two species of *Glandularia*, two accessions of *Hierobotana inflata*, 14 accessions of *Junellia*, including two species from Peru, and 24 accessions of *Verbena*, including 13 from the Andes of Peru, Ecuador (including *V. sedula* from the Galapagos), and Colombia, and eight from North America. Many of these species have been infrequently collected and treatments have varied with respect to species circumscription and generic assignment, with several species having been reassigned between *Junellia* and *Verbena*. In Verbeneae, target loci include three chloroplast regions (*trnT-L-F*, *ndhF*, *trnS-G*) and three nuclear regions (ETS, ITS, PPR11). Polymerase chain reaction protocols for the two chloroplast regions, *ndhF* and the *trnT-F* fragment, the latter of which includes the *trnT-trnL* spacer, *trnL* intron, and *trnL-trnF* spacer, followed those of Marx et al. (2010) and Yuan and Olmstead (2008a) for *ndhF* and *trnT-F*, respectively. Internal (ITS) and external transcribed spacer (ETS) regions of nuclear 18S/26S rDNA were amplified as done by O'Leary et al. (2009). The PPR11 gene was amplified as done by Yuan et al. (2010).

Single accessions representing 28 taxa of *Citharexylum* were included in this study; two accessions of the sister genus *Rehdera* were included as the outgroup. Taxa were chosen on the basis of preliminary results from a broader phylogenetic study of the genus. Clades including arid-adapted taxa and those representing the geographic distribution of the genus were selected. A more comprehensive study of biogeography in *Citharexylum* is underway (L. Frost and R. G. Olmstead, unpublished manuscript).

Target loci included *ndhF*, the *trnL-trnF* spacer, and ETS. Universal primers were used to amplify the *trnL-trnF* (Taberlet et al., 1991) spacer. Sequences for *ndhF* were obtained with primers designed for Verbenaceae (Marx et al., 2010). ETS was amplified with the 18S-IGS primer (Baldwin and Markos, 1998) and a custom primer optimized for use in Lamiales (ETS-B: 5′-ATAGAGCGCGTGAGTGGTG-3′).

Polymerase chain reactions were performed in a Perkin-Elmer (Waltham, Massachusetts, USA) thermocycler with the following general reaction conditions: 94°C for 2 min; 35 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1.5–2.5 min; 72°C for 10 min. PCR products were purified by polyethylene glycol precipitation. Cycle sequencing reactions were carried out in a Perkin-Elmer thermocycler using BigDye ver. 3.1 (Applied Biosystems, Foster City, California, USA) following a standard Applied Biosystems sequencing protocol. Raw sequence data were generated using either an Applied Biosystems 3130XL or 3730 genetic analyzer; reads were edited and assembled with Geneious R7 7.0.6 (Biomatters, Auckland, New Zealand). Sequences were aligned in Geneious R7 7.0.6 (Biomatters) with the MUSCLE Alignment function with a maximum of eight iterations. Alignments were inspected and manually adjusted as 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 under the Akaike information criterion.

Phylogenetic reconstructions for data sets were carried out using ML and Bayesian inference (BI) methods. The ML analyses were performed using the program GARLI 2.0 (Zwickl, 2006). Default parameters were used for two replicate runs with the generation threshold for termination at 20,000 generations and termination score threshold at 0.05. Bootstrap values for nodes were estimated by running 200 replicates with the default settings. The BI analyses were executed with MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Two runs of four chains of Markov chain Monte Carlo were performed, sampling one tree every 100 generations, for 1 million generations. Runs were assessed for convergence and stationarity in the program Tracer v1.6.0 (Rambaut et al., 2014); runs were considered to have converged when effective sample size (ESS) values were above 200. The first 2000 trees were discarded as burn-in; remaining trees were summarized as a majority rule consensus tree.

Ancestral areas were reconstructed using the program RASP v3.2 (Yu et al., 2015). Biogeographic regions were defined for *Aloysia*

and Verbeneae as (A) Southern South America, (B) Andean South America, and (C) North America (including *V. scabra*, which is predominantly Caribbean in distribution, but including some populations in the United States and Mexico), for which the geographical origin is of interest. In *Citharexylum*, for which the ancestral biome is of interest, areas were defined as (A) mesic Neotropical, (B) Andean dry valleys, and (C) arid North America. See Fig. 1 for maps of localities and biogeographical assignments. For *Aloysia*, geographic distributions were assigned and mapped onto the *BEAST tree in Lu-Irving et al. (2014), but ancestral areas were not reconstructed. The biogeographic coding of that study is used here as the basis for the biogeographic analysis. Analyses were performed only on the Aloysia clade. Taxa outside of the Aloysia clade included by Lu-Irving et al. (2014) are, for the most part, not distributed in regions defined in this study. However, we are confident that the early-diverging lineages of *Aloysia* reflect the geographical origin of the clade. For Verbeneae (*Glandularia* and *Verbena*), outputs from Bayesian analyses of both nuclear and chloroplast data sets for Verbeneae were used. All taxa included in the phylogenetic analyses were assigned areas and analyzed in RASP to reconstruct the ancestral area of the *Verbena*/*Glandularia* clade (Appendix S2). The output from the Bayesian analysis of *Citharexylum* was used (Appendix S3). Areas were assigned based on consultations of databases (GBIF, 2016), personal observations of herbarium specimens, and field collections. Ancestral ranges were reconstructed with Bayesian binary Markov chain Monte Carlo (BBM) and dispersal–extinction cladogenesis (DEC) analyses. The maximum number of areas was set to three, and default settings were used for all analyses. All possible areas were included in DEC analyses. BBM and DEC analyses were largely in agreement. Results

from the BBM were chosen to present because ancestral reconstructions at major nodes were thought to be more congruent with the previously inferred evolutionary history of these groups.

RESULTS

Aloysia—The ancestral area reconstruction for *Aloysia* (Fig. 2) inferred with high probability an arid southern South American origin with two dispersals directly to the North American deserts; North American representatives of the *A. gratissima* complex form one, and *A. macrostachya* and *A. wrightii* form the other. Three separate shifts are inferred into central/northern Andean South America from southern South America, one involving the *A. gratissima* complex (also including *A. virgata*), one comprising a mostly Andean endemic clade (including *A. peruviana*), and the third leading to *A. herrerae*.

Verbeneae—The data for the Verbeneae analysis expand upon previous studies (Yuan and Olmstead, 2008a; O'Leary et al., 2009; Marx et al., 2010; Yuan et al., 2010) by acquiring new sequences from herbarium and field-collected specimens. All included taxa have at least two of the three chloroplast regions, except *Junellia succulentifolia*, which only had data for *trnT-L-F*, and likewise all taxa except *Verbena valerianoides* had at least two of the three regions of nuclear DNA. Seven species were included in the plastid data set that were not included in the nuclear data set, and 11 species were included in the nuclear data set that were not included in the plastid data set. Multiple accessions were included for several species, especially ones with Andean distributions.
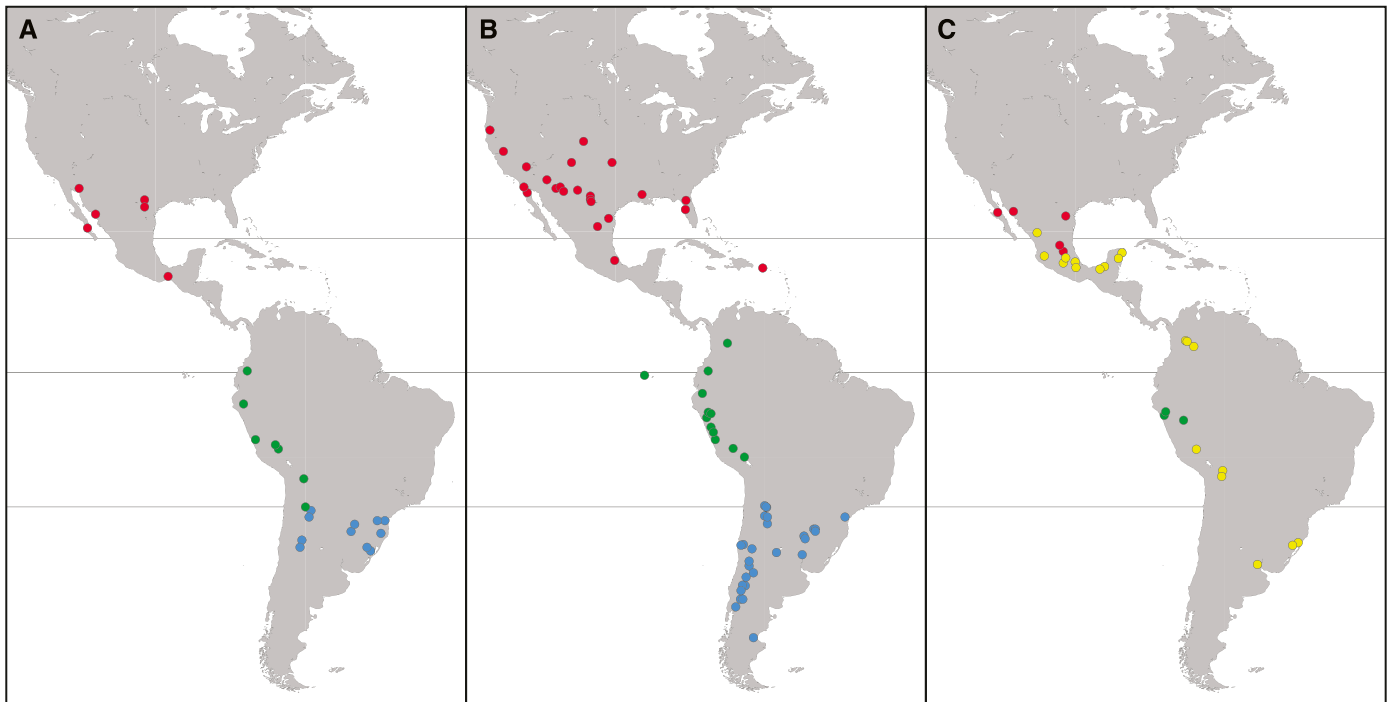

FIGURE 1 Map of North and South America showing collection localities for individuals included in this study, except those collected in cultivation. Colors correspond to the assigned region or biome in biogeographical analysis. Blue = southern South America, green = Andean South America, red = North America for (A) *Aloysia* and (B) Verbeneae. Yellow = mesic neotropical, green = Andean dry valley, red = arid North American biomes for (C) *Citharexylum*.
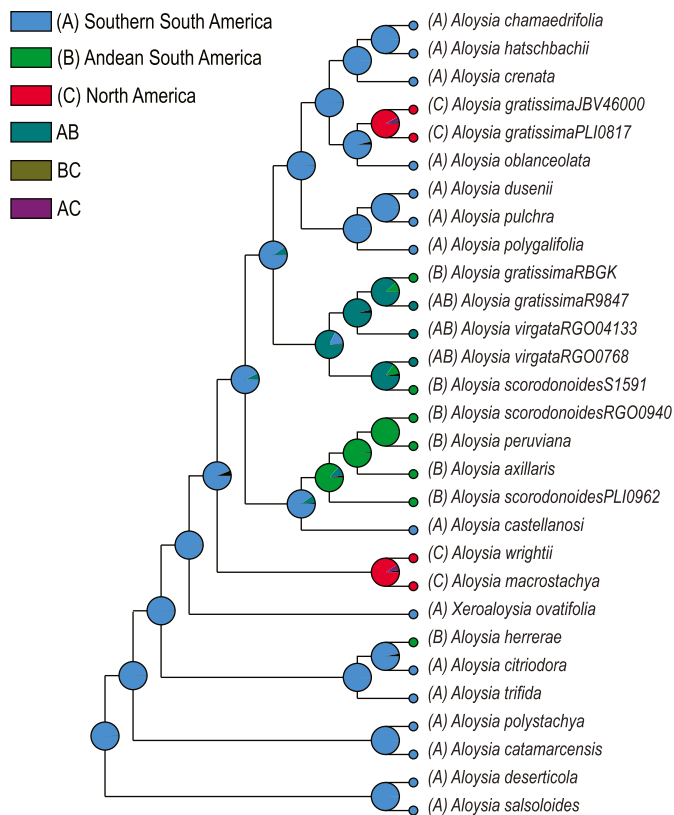
FIGURE 2 Graphical output from Bayesian binary MCMC (BBM) analysis in RASP for *Aloysia*. Areas are defined as (A) southern South America (B) Andean South America and (C) North America. Outgroups used by Lu-Irving et al. (2014) were removed for clarity. Pie charts at nodes indicate the probability of an area as the ancestral area; black portions of pie charts are reconstructions with less than 5% probability.

The plastid and nuclear trees (Fig. 3; Appendix S2) each recovered the same major clades, including clades representing *Verbena* (within which occurs a clade of *Glandularia* bearing a putative *Verbena* plastid derived from an ancient event of plastid transfer; Yuan and Olmstead (2008a), *Glandularia*, and a newly discovered Andean clade (hereafter referred to as the Andean clade) comprising three Andean species in the genera *Junellia* and *Verbena*. In the following discussion, reference to a clade composed of *Verbena* will be understood to include a subset of *Glandularia* taxa in the plastid tree. Within *Verbena*, the nuclear DNA tree found clades representing the South American and North American species sister to each other, whereas in the plastid tree some of the Andean accessions of *Verbena* were included in the North American clade. The trees varied in the placement of the Andean clade and the monotypic *Hierobotana*. In the nuclear tree, these groups form a clade sister to *Glandularia* plus *Verbena*. However, in the plastid tree, *Hierobotana* is sister to North American *Verbena*, the Andean clade is sister to *Verbena* (which includes those *Glandularia* species with *Verbena*-derived plastids), and *Glandularia* is sister to *Verbena*, *Hierobotana,* and the Andean clade.

The BBM analysis for Verbeneae (Fig. 3) indicates a southern South American origin for the clade comprising *Glandularia*, *Verbena*, *Hierobotana*, and the novel Andean clade. *Glandularia* also is inferred to have a southern South American ancestral area, with the

stem node for the North American *Glandularia* also in southern South America. However, the nuclear and plastid trees imply different things about the origin of North American *Verbena*. The nuclear tree (Fig. 3A) includes all South American species of *Verbena* in one clade, except for *V. valerianoides* (Colombia), which occurs in the otherwise entirely North American *Verbena* clade. The ancestral area for the stem node of *Verbena* is southern South America. The clade of South American *Verbena* is inferred to have an ancestral area in southern South America with two or more dispersals into the Andes, whereas the stem node for the North American *Verbena* clade is southern South America with a single dispersal event to North America. The BBM analysis of the plastid tree (Fig. 3B) infers a southern South American ancestral area for the South American clade of *Verbena*, but yields an Andean distribution as the highest probability ancestral area for the stem node of the North American *Verbena* clade. The stem node for all of *Verbena* is unresolved with both southern South America and the Andes having similar probability.

Citharexylum—All included taxa have at least partial sequences for the three loci sampled in this study (*trnL-F*, *ndhF*, and ETS) with the exception of *C. berlandieri*, which is missing a sequence for ETS. Two major clades of *Citharexylum* are reconstructed (Fig. 4; Appendix S3): a North American and a South American clade. *Citharexylum altamiranum*, a mid-elevation forest tree endemic to Mexico, is inferred to be sister to the rest of the genus. This result is consistent with analyses using expanded taxonomic sampling in the genus (L. Frost and R. G. Olmstead, unpublished manuscript). The South American clade is divided into a lower-elevation clade (Northern Andes, Brazil, and Argentina) and a high-elevation clade (Peru).

A mesic-adapted ancestor was reconstructed for all major clades in *Citharexylum*, the genus as a whole, the North American clade, and the South American clade. There have been at least three independent transitions to arid regions in Mexico. At least two transitions from more mesic Andean habitats to inter-Andean dry valleys are inferred.

DISCUSSION

Verbenaceae have been inferred to have originated in mesic tropical ecosystems before adapting to xeric conditions and diversifying extensively in arid and semiarid regions of southern South America (Marx et al., 2010; Lu-Irving and Olmstead, 2013; Olmstead, 2013). Expansion of the distribution of Verbenaceae into North America occurred in seven of nine main lineages, with only two species-poor clades restricted to South America (Olmstead, 2013). Six of the clades occurring in North America include representatives in arid regions of Mexico and the southwestern United States. In *Glandularia*, disjunctions exist between the arid regions of North and southern South America (Yuan and Olmstead, 2008a). In *Aloysia* and *Verbena*, species are found in both of these regions, but also in scattered pockets of arid habitat in the Andes (Lu-Irving et al., 2014; O'Leary and Múlgura, 2014). In *Citharexylum*, arid-adapted species are found in dry habitats in the Andes and in North American deserts (L. Frost and R. Olmstead, unpublished data), but not in southern South America.

Aloysia—Lantaneae are the largest tribe of Verbenaceae and are widely distributed throughout tropical regions of the New World,
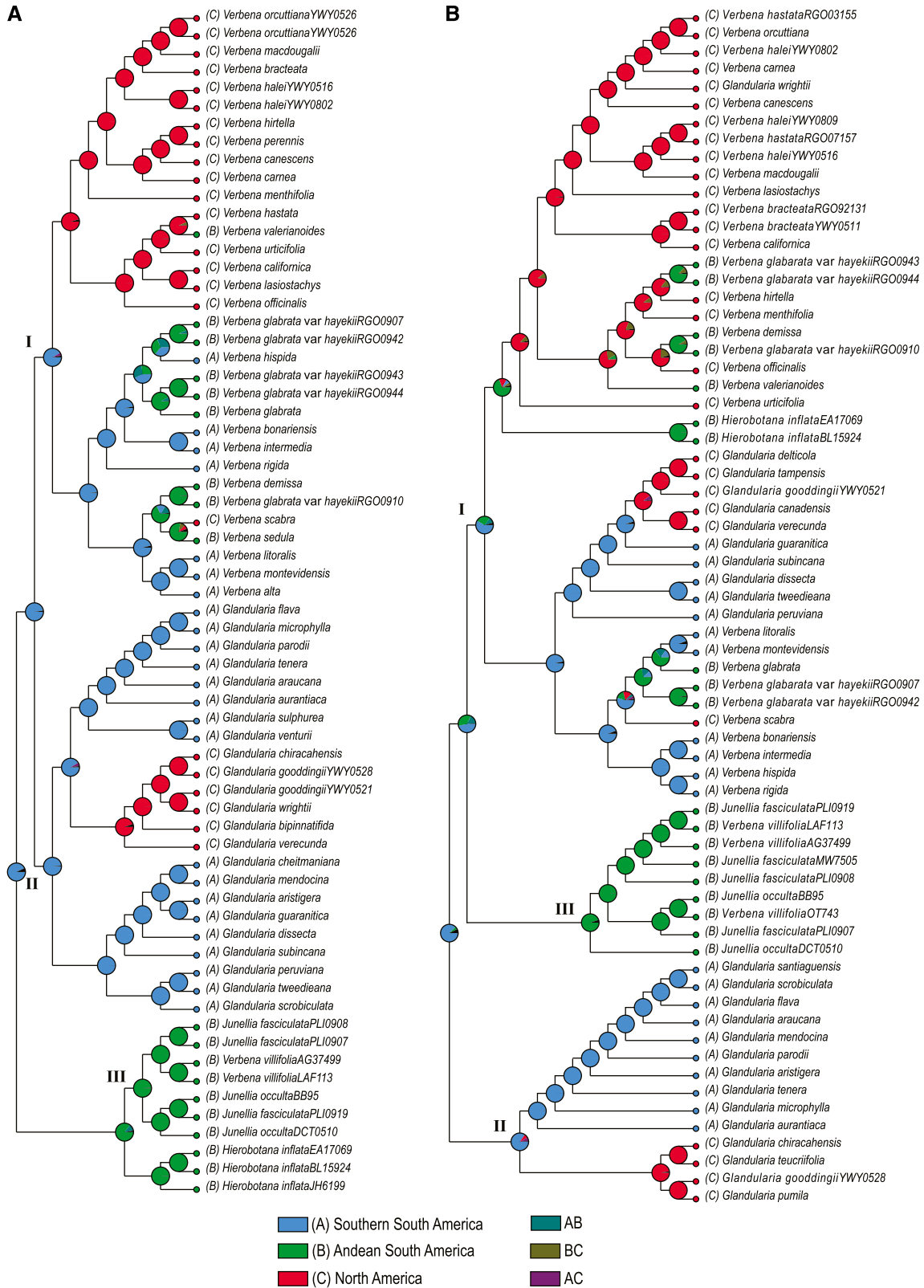
FIGURE 3 Graphical output from Bayesian binary MCMC (BBM) analysis in RASP for (A) nuclear and (B) chloroplast data sets for Verbeneae. Areas are defined as (A) southern South America (B) Andean South America and (C) North America. Biogeographical analyses were performed with all taxa included in the phylogenetic study, but basal clades of Verbeneae comprised of *Mulguraea* and *Junellia* were removed from the figure clade for clarity. Major clades are numbered as (I) Verbena clade, (II) Glandularia clade, and (III) Andean clade.
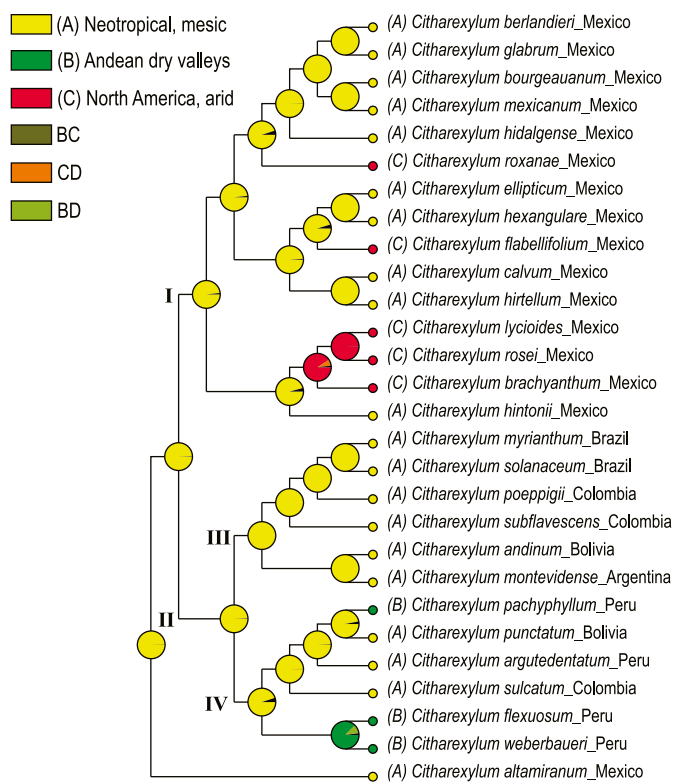
FIGURE 4 Graphical output from Bayesian binary MCMC (BBM) analysis in RASP for *Citharexylum*. Areas are defined as (A) neotropical, mesic, (B) Andean dry valleys, (C) North America, arid. Biogeographical analyses were performed with all taxa included in the phylogenetic study, but the outgroup was removed for clarity. Major clades are numbered as (I) North American clade, (II) South American clade, (III) low elevation clade, and (IV) high elevation clade.

with limited representation in temperate zones of both North and South America (Marx et al., 2010; Lu-Irving and Olmstead, 2013). Early diversification of Lantaneae, including the clade comprising *Aloysia*, and the included genera *Acantholippia* and *Xeroaloysia*, occurred in the xeric regions of southern South America, and most of the diversity of *Aloysia* is found there today (Lu-Irving et al., 2014; O'Leary et al., 2016). Early diversification of *Aloysia* occurred in these arid zones, before diversifying into northeastern Argentina and southern Brazil (where they occur in more mesic habitats), the Andes, and North America (Lu-Irving et al., 2014). The distribution of *Aloysia* includes several species in the Andes, two species endemic to North America (where they occur in deserts of Mexico and the southwestern United States), and one widespread species, *A. gratissima*, which is found in both North and South America (Lu-Irving et al., 2014; O'Leary et al., 2016). Another group of five species traditionally treated as *Aloysia*, but lacking the defining four-lobed calyx, also occur in Mexico. These are nested within a large clade comprising *Lantana* and *Lippia* (Lu-Irving et al., 2014) and are not included in this study.

The North American species of *Aloysia* represent two distinct lineages, each with ancestry in southern South America. One of these lineages comprises the North American representatives of the widespread and variable *A. gratissima* and is nested within a clade of mostly Argentinian and Brazilian species, but not with the South American representatives of *A. gratissima*, which occur in a clade

with other species from Argentina. Additional sampling of South American *A. gratissima* (P. Lu-Irving, unpublished data) suggests extensive polyphyly of this species, with additional accessions forming a complex with several other species from southern South America unrelated to the Andean endemic and North American endemic species. The other North American lineage diverges relatively early in the radiation of *Aloysia* in temperate South America and includes the two endemic North American species (*A. macrostachya*, *A. wrightii*). Our results, which are based on the coalescent tree obtained by Lu-Irving et al. (2014), infer that the ancestral area for the stem node of each North American lineage is southern South America. These results are consistent with an origin of each of the North American lineages via a long-distance dispersal event from southern South America (although this conclusion is tempered by the fact that the optimal tree from the analysis of concatenated sequences in that paper found the North American and Andean endemic species (i.e., the clade including *A. peruviana*) to form sister clades). Although relationships within the large *Lantana/Lippia* clade are insufficiently resolved (Lu-Irving and Olmstead, 2013; Lu-Irving et al., 2014) to identify the precise origin of the clade of arid-land Mexican species excluded from *Aloysia* (noted above), they likely represent another long-distance dispersal from their nearest relatives in South America. The Andean species represent three distinct dispersals from southern South America to the Andes, leading to a clade of Andean endemic species of *Aloysia* (*A. scorodonoides*, *A. peruviana*, *A. axillaris*), some representatives of the A. gratissima complex, and the distinctive Andean species, *A. herrerae*. Thus, the present distribution of North American desert species of *Aloysia* appears to have arisen from two (perhaps three counting the clade within *Lantana/Lippia*) long distance-dispersal events from arid regions of South America, without leaving evidence of an Andean migration corridor.

Glandularia *and* Verbena—Tribe Verbeneae is sister to Lantaneae (Marx et al., 2010) and also diversified in the arid regions of temperate South America, where *Mulgurea* and *Junellia* comprise the first two diverging lineages. Previously published studies suggested that the rest of Verbeneae comprise a clade in which *Glandularia* is sister to *Verbena* (Yuan and Olmstead, 2008a; O'Leary et al., 2009; Yuan et al., 2010), although a putative ancient plastid transfer event from *Verbena* to *Glandularia* has resulted in a clade of *Glandularia* nested within *Verbena* in the plastid phylogeny (Yuan and Olmstead, 2008b; O'Leary et al., 2009; Marx et al., 2010).

Both *Glandularia* and *Verbena* exhibit amphitropical disjunct distributions with significant species diversity primarily in arid habitats in both North and South America (Lewis and Oliver, 1961; Umber, 1979; Yuan and Olmstead, 2008a) and each genus is inferred to have had a South American origin (Yuan and Olmstead, 2008a). The pattern of South American *Glandularia* being paraphyletic with respect to the North American species is consistent among the several published studies, although the putative plastid transfer event places a clade of *Glandularia* within the *Verbena* clade in the plastid tree (Yuan and Olmstead, 2008a, b; O'Leary et al., 2009). The South American *Verbena* species are paraphyletic in some gene trees and sister to a monophyletic North American clade in others (Yuan and Olmstead, 2008a, b; O'Leary et al., 2009; Yuan et al., 2010).

Most of the studies cited above did not include any of the several Andean species of Verbeneae (O'Leary et al., 2007; Peralta et al., 2008; O'Leary, 2014; O'Leary and Múlgura, 2014), except for the inclusion of the Ecuadorian monotypic *Hierobotana* in the analysis

of Marx et al. (2010). That study, based on plastid DNA, uncovered a tantalizing placement of this northern Andean endemic as sister to the clade of North American *Verbena*, but included fewer species of tribe Verbeneae than the earlier studies. This relationship suggested the possibility that dispersal of *Verbena* from southern South America to North America followed an Andean migration corridor following "stepping stones" of arid habitat in the Andes, following one of the possible scenarios suggested by Solbrig (1972). *Hierobotana* is distinguished from other Verbeneae in having only two stamens, instead of four; it also occurs in a portion of the northern Andes where few other Verbeneae are found (O'Leary, 2014). Its woody, suffruticose habit suggests a relationship with the basal lineages of Verbeneae, *Junellia*, and *Mulguraea*, rather than with the herbaceous *Glandularia* and *Verbena*. For this project, we sampled additional Andean plants in *Hierobotana*, *Junellia*, and *Verbena* from Peru, Ecuador, and Colombia to explore the relationship of the Andean species to the North American and to the southern South American *Verbena* species.

Our results confirm those of prior studies (Yuan and Olmstead, 2008a; O'Leary et al., 2009; Marx et al., 2010) in finding *Mulguraea* and *Junellia* to be the first two diverging lineages in Verbeneae (Appendix S2). As in those studies, the nuclear tree (Fig. 2A; Appendix S2A) identified a *Verbena* clade comprising two sister clades, one representing North American *Verbena* species and the other species from South America, and a clade representing *Glandularia*, which is sister to *Verbena*. Also, as in previous studies, the analysis of plastid genome DNA sequences (Fig. 2B; Appendix S2B) identified the same clades, except that a subset of *Glandularia* species forms a clade nested within *Verbena*, which was interpreted to be evidence of an ancient plastid transfer event (Yuan and Olmstead, 2008a). However, the addition of Andean sampling in this study identified a new clade (Fig. 2), not previously recognized, composed entirely of Andean taxa (*J. fasciculata*, *J. occulta*, and *V. villifolia*). In the analysis of nuclear DNA sequences, *Hierobotana* is strongly supported as part of this clade, sister to the other three species, whereas in the tree based on plastid DNA sequences, *Hierobotana* is sister to the North American *Verbena* clade, as found by Marx et al. (2010). The position of this Andean clade is not consistent between the nuclear and plastid DNA trees, but the conflict is not strong. In the nuclear tree, this clade is sister to both *Glandularia* and *Verbena*, but with weak support in that position. However, in the plastid tree the clade is strongly supported as sister to *Verbena* (with the included clades of *Glandularia* species bearing *Verbena* plastids, and *Hierobotana*) and, although *Hierobotana* is not part of the clade, it is positioned one node away as sister to the North American clade of *Verbena*. Three additional Andean *Verbena* species plus *V. sedula* from the Galapagos (included in the widespread and variable *V. carolina* of O'Leary et al. [2010]) and *V. scabra* from the Greater Antilles and North America (our sample is from Puerto Rico) are dispersed within the clade comprising the rest of *Verbena*. In the nuclear DNA tree, all of these are found in the South American clade, except *V. valerianoides* from Colombia, which belongs with the North American clade. In the plastid DNA tree, the additional Andean accessions are split among the North American and South American clades. These results suggest some other dispersal events leading to some individual species. The North American/Caribbean *V. scabra* has a separate Andean origin, and the Galapagos *V. sedula* dispersed from Andean South America. Depending on whether the North American *Verbena* clade arose via an Andean migration corridor (consistent with the

plastid DNA tree) or long-distance dispersal from southern South America (consistent with the nuclear DNA tree), Colombian *V. valerianoides* could either represent a remnant of the Andean migration, or recolonization of South America from the north.

The distribution of *Glandularia* represents the classic North America/South America interior (i.e., noncoastal) desert disjunction (Lewis and Oliver, 1961; Raven, 1963; Umber, 1979). The northernmost South American distributions of several *Glandularia* species extend into southern Peru, with one species endemic there, but do not have an extensive distribution in the central or northern Andes (O'Leary and Mulgura, 2014). Relative to prior studies (Yuan and Olmstead, 2008a; O'Leary et al., 2009), we added one South American and one North American *Glandularia* species, but the biogeographic conclusions remain the same as reported by Yuan and Olmstead (2008a). The North American clade is nested relatively deeply within the South American species (Fig. 2). A clade of taxa (including *G. araucana*) from the arid western and northwestern Argentina that occur in the Monte and altiplano regions forms the sister group to the North American clade. Sister to this inclusive clade in the nuclear DNA tree is a clade (including *G. dissecta*) that occurs in more mesic habitats of northeastern Argentina, supporting the origin of the North American desert species in the arid regions of Argentina and neighboring countries. In the plastid tree, all of the more mesic-adapted South American species are in the clade with *Verbena* plastids.

In contrast to *Glandularia*, *Junellia*, and *Mulguraea*, *Verbena* is distributed throughout the Andes, albeit with diminishing diversity to the north (O'Leary et al., 2007; Peralta et al., 2008; O'Leary and Múlgura, 2014). Though never very abundant or diverse in any particular region, *Verbena* is distributed mostly in the more xeric habitats in the high puna of the southern Andes, arid Andean foothills, and dry inter-Andean valleys. *Hierobotana* is restricted to the central valley region of Ecuador. Sampling in prior studies of Verbeneae emphasized the centers of diversity in North and South America, with only *H. inflata* representing the central/northern Andean region (O'Leary et al., 2009; Marx et al., 2010). The finding of an Andean clade sister to *Glandularia* plus *Verbena* (nuclear DNA tree) or to *Verbena* (plastid DNA tree) is novel and suggests that the arid regions of the Andes, where these species occur, has played an important role in the biogeographic history of *Verbena* and possibly *Glandularia*. The conflicting placements of the Andean clade and *Hierobotana* complicate the interpretation of the origin of North American *Verbena* species.

Biogeographical analyses suggest that the ancestral area for the crown node of the clade including *Verbena*, *Glandularia*, *Hierobotana*, and the novel Andean clade is southern South America. This result is not surprising, given that southern South America is a nexus for the early diversification of Verbenaceae (Marx et al., 2010; Olmstead, 2013). Also not surprising is that both the crown and stem ancestral areas for *Glandularia* is southern South America. However, the results present uncertainty with respect to the ancestral area of the diversification that gave rise to crown *Verbena*. The nuclear tree suggests a southern South American ancestral area for the stem node for *Verbena*, and the plastid tree suggests nearly equal probability for a southern South American or Andean ancestral area. The difference between these two inferred ancestral areas stems from the placement of the novel Andean clade and *Hierobotana*. The nuclear tree provides relatively weak support for these two groups forming a sister to the clade comprising *Glandularia* plus *Verbena*, whereas the plastid tree provides strong support

for *Hierobotana* as sister to the North American *Verbena* and the Andean clade as the well-supported sister to *Verbena*. This conflict affects the inference of the source of the North American arid-land *Verbena* species. In the nuclear tree, the stem node ancestral area is southern South America, implying a much longer disjunction between the South American and North American arid-land species and possible long-distance dispersal between them, whereas in the plastid DNA tree, the ancestral area of the stem node of the North American clade is Andean South America, with *Hierobotana*, distributed in the northern Andes, as sister to North American *Verbena*, suggesting an Andean stepping-stone dispersal route for the North American clade.

Citharexylum—*Citharexylum* is widely distributed in the neotropics—from northern Mexico to southern Brazil and Argentina—and inhabits biomes ranging from mesic to xeric and from low to high elevations. The genus originated in North America and diversified in humid forest biomes in both North and South America. Since this period of early diversification, *Citharexylum* has adapted to multiple, different biomes including arid regions and high alpine zones. Most of the taxonomic diversity exists in humid/seasonally humid forests, but eight to ten arid-adapted species are distributed in the deserts of Mexico and four or five in the inter-Andean dry valleys in Peru.

Although *Citharexylum* is found in arid regions of both North and South America, it does not exhibit the distribution in arid lands that is common among disjunct lineages. That is, *Citharexylum* is not found in any of the major deserts of South America, the Monte, Patagonian, or Atacama deserts, all of which would be comparable to deserts of North America. Thus, North American, arid-adapted *Citharexylum* species are not closely related to South American, arid-adapted species. Instead, members of the genus acquired adaptations to arid biomes de novo on each continent.

In North America, there have been at least three independent shifts from mesic to arid biomes of the Mexican xerophytic region (as defined by Rzedowski, 1983). Two species are distributed in the Baja California floristic province, *Citharexylum roxanae*, which is endemic to Baja California Sur, and *C. flabellifolium,* which is also more broadly distributed in the Northwest Coastal Plain province in Sonora (Rzedowski, 1983). Each represents a separate colonization of the region. Morphology suggests that each has adopted a different strategy for life in the desert. *Citharexylum roxanae* has an ephedroid habit; it is a many-branched shrub with green stems and small, ephemeral leaves. *Citharexylum flabellifolium* is a woody shrub with large spines and thick, hirsute leaves. The clade of *C. lycoides*, *C. rosei*, and *C. brachyanthum* represents one colonization event followed by diversification in the central Plateau floristic province, which contains the Chihuahuan desert (Rzedowski, 1983). *Citharexylum brachyanthum* is distributed in the Chihuahuan desert, whereas *C. lycoides* and *C. rosei* are distributed further south. All of these North American arid-adapted clades are inferred to have stem ancestral areas in mesic biomes most likely in Central America or Mexico.

In South America, arid-adapted species are primarily distributed in inter-Andean dry valleys in Peru. Multiple, independent colonization events of dry valleys are reconstructed in the South American clade. One shift resulted in the sister pair *C. flexuosum* and *C. weberbaueri*. Both are large, thorny shrubs with hirsute, membranous leaves. *Citharexylum pachyphyllum* is a short, dense shrub with many thorns and small, leathery leaves. This species,

and likely other morphologically similar species not sampled here, represent a separate shift into inter-Andean dry valleys. Each of these clades is inferred to have stem ancestral areas in mesic biomes also in Andean South America.

CONCLUSIONS

Verbenaceae are well represented in arid zones in South America, where species of *Aloysia* and *Junellia* often are community dominants, and several other genera, including *Glandularia* and *Verbena*, are well represented. North American desert floras include a subset of the Verbenaceae genera found in South American deserts, including *Aloysia*, *Glandularia*, and *Verbena*, but also including *Citharexylum*, which does not occur in the deserts of southern South America. Most of these genera, including *Citharexylum*, also are distributed in the Andes. Our results show that the origins of the North American desert Verbenaceae do not all fit a single pattern of origin, but have arisen from ancestors in multiple lineages and biomes. Biogeographic analysis shows that the distributions of *Aloysia* and *Glandularia* in North America are most likely derived from ancestors in southern South American deserts and are consistent with possible long-distance dispersal histories. The geographic ancestry of *Verbena* is less clear, with conflicting signal from nuclear and plastid DNA trees suggesting a southern South American origin or Andean origin, respectively. *Citharexylum* presents a clear case of in situ evolution of desert-adapted species in the deserts of North America. The south-to-north dispersal pattern exhibited by desert disjuncts in Verbenaceae is consistent with that from many other studies of plant disjunctions between the interior deserts of South and North America, in contrast with the more common north-to-south dispersal pattern of coastal disjuncts between the mediterranean zones and coastal deserts of California and Chile (Wen and Ickert-Bond, 2009).

Suggestions that an Andean dispersal corridor is responsible for the disjunct distribution of *Verbena* (and possibly *Aloysia*), indicate that caution is warranted before invoking long-distance dispersal as the cause of the distributions long observed between the deserts of North and South America. Changes in local conditions along the Andes and coastal South America associated with the dramatic climatic and sea-level fluctuations of the past may have created and then eliminated opportunities for stepwise dispersal that could be responsible for existing distributions.

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