

## INVITED PAPER

*For the Special Issue: Patterns and Processes of American Amphitropical Plant Disjunctions: New Insights*

# Amphitropical disjunctions in New World Menthinae: Three Pliocene dispersals to South America following late Miocene dispersal to North America from the Old World<sup>1</sup>

Bryan T. Drew<sup>2,5</sup>, Sitong Liu<sup>2</sup>, Jose M. Bonifacio<sup>3</sup>, and Kenneth J. Sytsma<sup>4</sup>

**PREMISE OF THE STUDY:** The subtribe Menthinae (Lamiaceae), with 35 genera and 750 species, is among the largest and most economically important subtribes within the mint family. Most genera of Menthinae are found exclusively in the New World, where the group has a virtually continuous distribution ranging from temperate North America to southern South America. In this study, we explored the presence, timing, and origin of amphitropical disjunctions within Menthinae.

**METHODS:** Our analyses were based on a data set consisting of 89 taxa and the nuclear ribosomal DNA markers ITS and ETS. Phylogenetic relationships were determined under maximum likelihood and Bayesian criteria, divergence times were estimated with the program BEAST, and ancestral range estimated with BioGeoBEARS.

**KEY RESULTS:** A North Atlantic Land Bridge migration event at about 10.6 Ma is inferred from western Eurasia to North America. New World Menthinae spread rapidly across North America, and then into Central and South America. Several of the large speciose genera are not monophyletic with nuclear rDNA, a finding mirrored with previous chloroplast DNA results. Three amphitropical disjunctions involving North and southern South America clades, one including a southeastern South American clade with several genera, were inferred to have occurred within the past 5 Myr.

**CONCLUSIONS:** Although three New World Menthinae genera occur in both North and South America, none exhibit an amphitropical disjunction. However, three clades exhibit amphitropical disjunctions, all dating to the early Pliocene, and all involve jump dispersals to either southeastern or southwestern South America from southeastern North America.

**KEY WORDS** BEAST; biogeography; BioGeoBEARS; disjunct; jump dispersal; Lamiaceae; mint family

Closely related organisms that exhibit wide gaps in their ranges, or taxa with disjunct distributions, have long puzzled biologists (Darwin, 1859; Wallace, 1880; Baker, 1959; Raven, 1972; Carlquist, 1974; Briggs, 2009). Not all disjunctions are formed equally, however. Taxa can be disjunct within relatively small political boundaries such as a state, province, or country, or across larger natural landscapes such as continents (intracontinental disjunctions), or

between different continents (intercontinental disjunctions; Raven, 1963, 1972; Thorne, 1972; Tripp and McDade, 2014; Wen et al., 2016; Simpson et al., 2017). Intracontinental organismal disjunctions are typically more readily explained and understood than their more perplexing counterpart, intercontinental disjunctions (Thorne, 1972; Axelrod, 1975; Wen, 1999; Givnish and Renner, 2004; Wen and Ickert-Bond, 2009; Simpson et al., 2017). By understanding plate tectonics, past sea level fluctuations, and paleoclimate regimes, some intercontinental disjunctions, such as East Asian/European/Eastern North America, are easily explained, at least in part, by ancient vicariance. The most challenging types of disjunctions to clarify are those that are widely separated spatially, occur in areas that were never in physical proximity nor bridged by a common vegetation type or climatic regime, and hence cannot be

<sup>1</sup> Manuscript received 6 June 2017; revision accepted 5 September 2017.

<sup>2</sup> Department of Biology, University of Nebraska-Kearney, NE Kearney 68849 USA;

<sup>3</sup> Laboratorio de Botánica, Facultad de Agronomía, Casilla de Correos 1238, Montevideo, Uruguay; and

<sup>4</sup> Department of Botany, University of Wisconsin, Madison, Wisconsin 53706 USA

<sup>5</sup> Author for correspondence (e-mail: bdrewfb@yahoo.com); ORCID id 0000-0001-7248-2799 <https://doi.org/10.3732/ajb.1700225>

logically explained by vicariance, ancient or otherwise. Within this narrower subset of disjunctions are species distributions that occur in northern and southern temperate regions but bypass the tropics, known as amphitropical (and bipolar) disjunctions (Raven, 1963; Solbrig, 1972; Wen and Ickert-Bond, 2009; Donoghue, 2011; Baldwin and Wood, 2016; Simpson et al., 2017).

The mint family (Lamiaceae) contains over 7000 species and is diverse on six continents. The mints are divided into 12 subfamilies (Li et al., 2016; Li and Olmstead, 2017), and several of these subfamilies are further subdivided into tribes and subtribes. The largest mint subfamily is the Nepetoideae, which represents almost half (ca. 3400) of the species in the Lamiaceae. Although the Nepetoideae is widely distributed, the tribes and subtribes within it are often confined to relatively narrow geographic regions (excepting *Salvia*). For example, the subtribes Ociminae and Plectranthinae (tribe Ocimae) are extremely diverse in tropical Africa (and to a lesser extent Australia) but virtually absent in the New World and temperate Eurasia. Conversely, subtribe Hyptidinae (also tribe Ocimae) is extremely diverse in tropical and subtropical environments in the New World, but virtually absent in the Old World. The subtribe Menthinae (tribe Menthae) is one of the few subclades within the Nepetoideae (or more broadly the Lamiaceae) that is widespread, although not especially diverse, across five continents, and has striking amphitropical distributions.

Menthinae, with about 35 genera and 750 species (Harley et al., 2004; Bräuchler et al., 2010; Drew and Sytsma, 2012), is one of the largest and most economically important subtribes within the mint family. The group contains familiar culinary spices such as oregano (*Origanum vulgare*), marjoram (*O. majorana*), thyme (*Thymus serpyllum*), peppermint and spearmint (*Mentha × piperita* and *M. spicata*), and summer and winter savory (*Satureja hortensis* and *S. montana*), and various horticulturally important plants. Although most species of Menthinae with widespread culinary applications are native to the Old World, most of the genera of Menthinae (ca. 22/35) are found exclusively in the New World. These genera have radiated into a large and morphologically diverse clade of species (Fig. 1). Previous studies have suggested that the New World Menthinae radiation occurred after a single colonization event, but chloroplast and nuclear ribosomal DNA (nrDNA) give conflicting results as to what lineage is sister to the New World Menthinae (Trusty et al., 2004; Bräuchler et al., 2010; Drew and Sytsma, 2012).

Within Menthinae, two genera, *Mentha* and *Clinopodium*, occur in both the Old and New World. The only native New World species of *Mentha* is *M. arvensis*, which has a circumboreal range. *Clinopodium vulgare* is the only putatively native New World species of *Clinopodium* that shares a most recent common ancestor with Old World *Clinopodium*, but *C. vulgare* may have been introduced recently to the New World (Doroszenko, 1986; Cantino and Wagstaff, 1998). Drew and Sytsma (2012) sampled two accessions of *Clinopodium vulgare*, one from Portugal and one from the upper peninsula of Michigan, and found them to contain identical sequences over >6700 bp of chloroplast DNA and possess only five differences over 800 nucleotides of nuclear ribosomal DNA, suggesting that the species is either genetically homogenous or has been recently introduced to the New World. Other native New World *Clinopodium* do not share a most recent common ancestor with Old World *Clinopodium* (Doroszenko, 1986; Cantino and Wagstaff, 1998; Bräuchler et al., 2010; Drew and Sytsma, 2011, 2012), emphasizing the nonmonophyly of the genus as presently circumscribed.

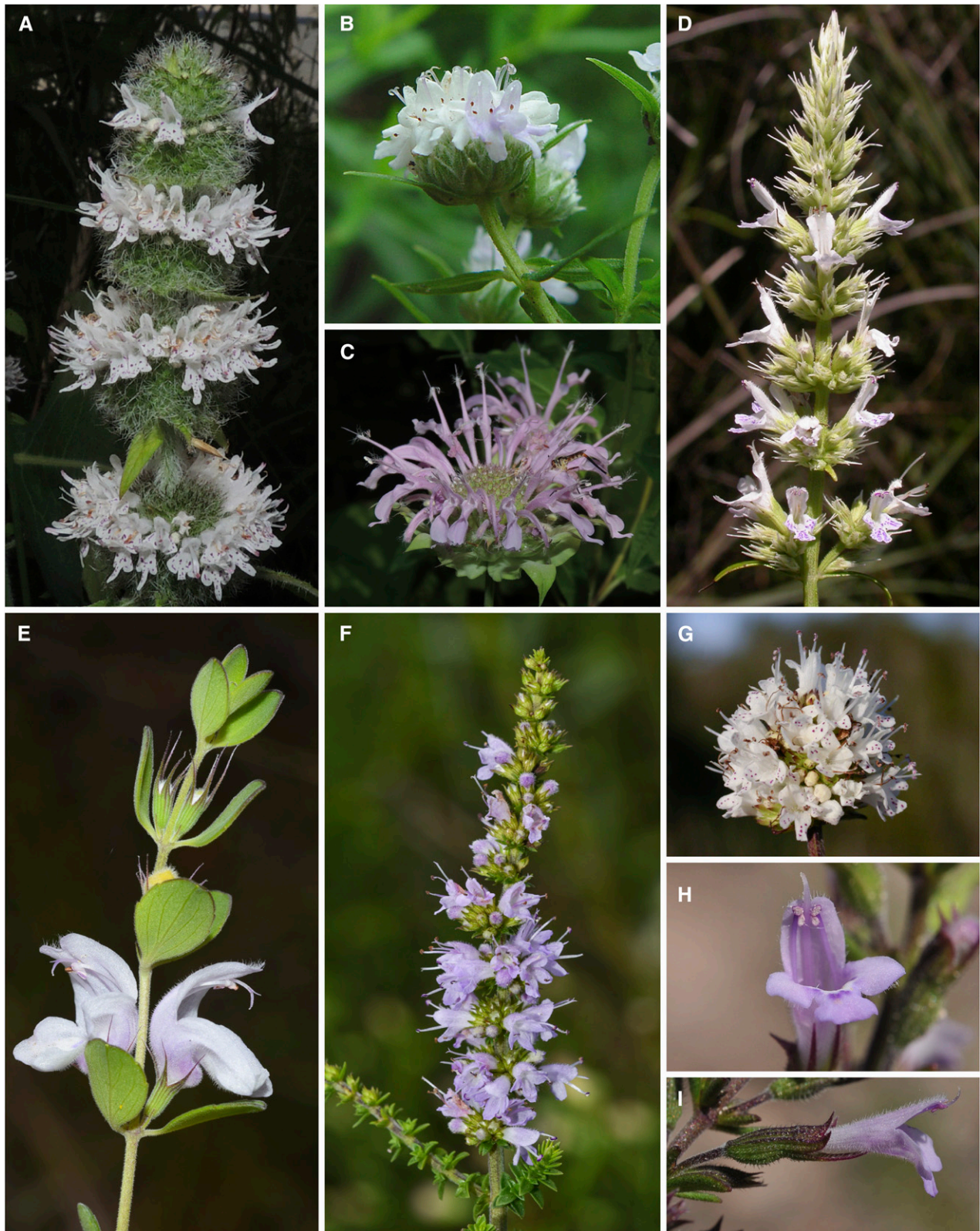
New World representation of Menthinae includes about 23 genera, with three that occur in both the northern and southern hemispheres: *Clinopodium*, *Cunila*, and *Hedeoma*. *Clinopodium* is a taxonomically challenging, nonmonophyletic assemblage of taxa (Doroszenko, 1986; Cantino and Wagstaff, 1998; Bräuchler et al., 2010; Drew and Sytsma, 2011, 2012) that contains about 150 species (The Plant List, 2017) mostly distributed across Eurasia and the New World (Harley et al., 2004). There are about 70 species of New World *Clinopodium* that range from southern Canada to southern Argentina. This group is morphologically heterogeneous and occurs in a wide variety of habitats. *Cunila* contains about 20 species and occurs in the eastern United States (one species) from southern New York to northeastern Texas, mountains of central Mexico to northern Central America, and southeastern South America (northeastern Argentina, southeastern Brazil, southeastern Paraguay, and Uruguay). *Hedeoma* is composed of about 40 species and ranges from northeastern North America to southern South America. Formerly, the genus *Hesperozygis* was considered to occur in Mexico and southeastern South America, but the Mexican taxa are now treated in *Poliomntha* (*P. marifolia*) and *Hedeoma* (*H. ciliolata*).

Thus, the New World Menthinae has a virtually continuous distribution from temperate North America to southern South America. However, no study has explicitly examined the relationships of the three genera that occur in both North and South America nor the temperately distributed Menthinae in a phylogenetic context. Furthermore, no detailed, model-based biogeographic analysis has been performed on the New World Menthinae in the context of defined specific areas, both adjacent and disjunct. Here, we employ two nuclear ribosomal DNA regions (internal and external transcribed spacers) to generate a phylogenetic framework of Menthinae with which to apply model based ancestral region estimation (ARE). We address the following questions: (1) Are there amphitropical disjunct clades in New World Menthinae? (2) If so, when did these disjunctions occur? (3) What was the directionality of movements for these disjunctions? (4) How did these disjunctions arise?

## MATERIALS AND METHODS

**Nomenclature**—Taxonomic descriptions used in this paper generally follow the species descriptions of Epling (Epling, 1937; Irving, 1980; Harley and Paucar, 2000), and the broad taxonomic treatment of Harley et al. (2004), with the following exceptions. *Acinos* (treated within *Clinopodium* by Harley et al., 2004) is treated as a distinct genus here, and *Micromeria* section *Pseudomelissa* (treated as *Clinopodium* by Bräuchler et al., 2006) is retained as *Micromeria*. Subtribal delimitations within Menthae follow Moon et al. (2010) and Drew and Sytsma (2012).

**Sampling and outgroups**—Two nuclear ribosomal DNA regions, the internal and external transcribed spacer (ITS and ETS) regions, were used in this study. A total of 89 accessions were included from within the tribe Menthae and included representatives from all five subtribes. Within the Menthae, four species were sampled from subtr. Salviinae, four species from subtr. Nepetinae, one species from each of subtr. Lycopinae and subtr. Prunellinae, and the remainder (79) of accessions came from subtr. Menthinae. Based upon results of a larger subf. Nepetoideae analysis (Drew and Sytsma, 2012), the subtr. Salviinae was used as an outgroup.



**FIGURE 1** Representatives of New World Menthinae restricted to either temperate North America or South America. (A) *Blephilia hirsuta*; (B) *Pycnanthemum virginianum*; (C) *Monarda fistulosa*; (D) *Rhabdocaulon strictus*; (E) *Glechom marifolia*; (F) *Cunila galioides*; (G) *Cunila microcephala*; (H, I) *Hedeoma multiflora*. Image credits: A and C by Ricardo Kriebel (United States); B by Arthur Haines (United States); D–I by Jose M. Bonifacino (Uruguay).

Sampling for the nrDNA alignment included 67 accessions from Drew and Sytsma (2012), one sequence (*Hedeoma nana*) downloaded from GenBank, and 21 newly sequenced accessions (new sequences labeled with asterisk in Appendix S1 [see the Supplementary Data with this article]). Twenty of the newly sequenced accessions are from within the New World Menthinae. Of the 79 sampled species of Menthinae, 22 taxa are distributed in the Old World, and 57 taxa are endemic to the New World. Within the New World samples, 34 samples have a North American (including Central America) distribution, 21 occur in South America, and *Clinopodium brownei* (two accessions) occurs in both North and South America. We made an effort to sample genera that occur in both North and South America (*Clinopodium*, *Cunila*, *Hedeoma*) more densely. We chose not to include cpDNA analyses here because cpDNA has little variation (relative to nrDNA) within the New World Menthinae (especially within genera), and phylogenies based on cpDNA seem to be influenced by chloroplast capture and/or past introgression, as is evidenced by the clustering of geographically proximate taxa in previous studies on the Menthinae based on cpDNA (Bräuchler et al., 2010; Drew and Sytsma, 2012).

**DNA extraction, amplification, and sequencing**—Leaf samples were obtained from silica-dried, field-collected leaves and herbarium specimens. DNA was extracted from leaf samples using the DNeasy Plant Mini Kit (Qiagen, Valencia, California). We modified the protocol to heat extracts at 65°C for 30 min (instead of 10 min), but otherwise followed the manufacturer specifications. Most New World Menthinae DNA samples were diluted in water (5× or 10×) before sequencing. PCR reactions and thermal cycler settings were similar to those of Sytsma et al. (2002). PCR products, amplified with TaKaRa Ex Taq DNA polymerase (TaKaRa, Otsu, Japan), were diluted 30× in water before cycle sequencing and subsequently cleaned using Agencourt magnetic beads (Agencourt, Beverly, Massachusetts). Cycle sequencing reactions used the ABI PRISM Big-Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California). Samples were electrophoresed on an automated Applied Biosystems 3730xl DNA Analyzer, using 50 cm capillary arrays and POP-7 polymer. Data were analyzed using PE-Biosystems version 3.7 of Sequencing Analysis at the University Wisconsin-Madison Biotechnology Center and the University of Arizona Genetics Core.

We used primers Leu1 (Andreasen et al., 1999) and ITS4 (White et al., 1990) to amplify ITS for most taxa. The internal primers ITS2 and ITS3 (White et al., 1990) were used to amplify material from some older herbarium specimens as well as some recalcitrant silica-dried samples. Combinations of these primers were used for sequencing. ETS was amplified using 18S-IGS (Baldwin and Markos, 1998) and ETS-bdf1 (Drew and Sytsma, 2011). The primers 18S-E (Baldwin and Markos, 1998) and ETS-bdf1 were used for sequencing.

**Phylogenetic analyses and divergence time estimation**—Nuclear ribosomal DNA sequences were edited in the programs Sequencher v.4.7 (Gene Codes, Ann Arbor, Michigan, USA) and Geneious v.9.1.8 (Kearse et al., 2012). The resulting sequences were manually aligned in Mesquite v.3.2 (Maddison and Maddison, 2017). The nrDNA data set was analyzed using two partitions (ITS and ETS). For each of our two data partitions, we used a model of evolution as determined by the Akaike information criterion (AIC) in jModelTest (Darriba et al., 2012) on the Cyberinfrastructure for Phylogenetic

Research (CIPRES) cluster (<http://www.phylo.org/>) (Miller et al., 2010). The GTR+Γ+I model was suggested for both partitions. This model was subsequently used for all analyses below. Gaps were treated as missing data in all analyses.

A maximum likelihood (ML) analysis was conducted using the program RAxML v.8.2.10 (Stamatakis, 2014) on CIPRES. Bayesian analysis was performed in the program MrBayes v.3.2.6 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) as implemented on CIPRES. The analysis was run for 10 million generations, with the first 25% of trees discarded as burnin. Convergence and mixing was assessed visually using the program Tracer v.1.6 (Rambaut et al., 2014) and by assessing whether standard deviation of split frequencies fell below 0.01 (which occurred within 2 million generations).

Divergence time estimation was performed using BEAST v.1.8.4 (Drummond and Rambaut, 2007; Drummond et al., 2012) as implemented on CIPRES. For the BEAST analyses, we used a data set that contained only Menthinae taxa (79 accessions) due to sampling imbalance and obvious sequence rate heterogeneity between taxa of the Menthinae and the other four subtribes. The analyses contained two partitions that corresponded to the ITS and the ETS spacer regions. Each partition was assigned the GTR+Γ+I model of sequence evolution and with partitions unlinked in BEAST. Rate variation across branches was specified as uncorrelated and lognormally distributed, and we used the Yule process tree prior (Yule, 1925; Gernhard, 2008). Posterior tree distributions were gathered by conducting two separate BEAST runs of 40 million generations each and sampling every 2000 generations. Each run started with a randomly generated tree. After checking that ESS values were over 200 in Tracer, we combined the two runs using the program Log-Combiner v.1.8.4, discarding 4 million generations from each run as burnin. The resulting trees were condensed and interpreted by the program TreeAnnotator v.1.8.4.

**Calibration points**—The root of the Menthinae was constrained using a uniform prior distribution that ranged from 17.1 to 25.3 Ma (million years ago). This secondary constraint was based on the 95% confidence interval range for the comparable node from the fossil dating analysis of subf. Nepetoideae (Drew and Sytsma, 2012). This study employed two fossils as calibration points within Nepetoideae, one from the middle Eocene (Kar, 1996) that constrained the Nepetoideae crown, and a *Melissa* fossil from the middle Oligocene (Reid and Chandler, 1926) that constrained the most recent common ancestor of *Melissa* + *Lepechinia*. Since the study of Drew and Sytsma (2012), Manchester et al. (2015) reviewed the *Melissa* fossil and questioned its identity as part of an asterid-wide review of fossils in a phylogenetic context. To our knowledge, no recent critical review has been undertaken to assess the validity of the Kar (1996) fossil. An alternative method of dating the Lamiaceae, which included broad sampling within Lamiaceae and used a range of dates to constrain the Lamiaceae crown (Supplementary Table S3 from Yao et al., 2016), but no fossil constraints within Lamiaceae, yielded similar dates for the Menthinae crown (Drew, 2017; B. Drew, unpublished data).

**Reconstruction of ancestral areas**—Ancestral range estimation (ARE), with a focus on inferring the geographic entry of Menthinae into the New World and subsequent range expansion, separations, and dispersals, employed the nested DEC and DEC+J models in BioGeoBEARS (Matzke, 2013, 2014) in R v.3.3.1. The “J” parameter

allows for a daughter lineage to immediately occupy a new area, via long-distance dispersal, that differs from the parental lineage. The “J” parameter was not supported for within-continental radiation of *Salvia* subg. *Audibertia* (Walker et al., 2015), but considering the disjunct nature of amphitropical distributions the “J” parameter might be especially supported in this analysis of disjunct Menthinae. We identified six broad geographic areas (see Fig. 3) important in the context of the distributions of Menthinae in the New World: (1) temperate eastern North America, extending west to the Rocky Mountains and south to north-central Texas; (2) Mediterranean and temperate western North America, extending east to the Rocky Mountains and south to the border of arid and desert regions of southwestern North America; (3) Mexico north to adjacent arid and desert regions of the United States, Central America, and the Caribbean; (4) northern South America, north and central Andes, and the Amazon Basin; (5) southeast South America, extending from central Brazil to southern Argentina and west to the foothills of the Andes; (6) southwestern South America, extending from the Andes of the northern Chile/Argentina border south along the Andes to southernmost Chile. Our New World geographic area designations were based upon Menthinae species distributions and geological criteria. We identified natural breaks within the distributional ranges of genera (and species within genera) and used this information to guide our geographic delimitations. We scored all other sampled Old World Menthinae as a seventh area. Most of these species occur in Mediterranean Europe, northeast Africa, or southwest Asia and were likewise scored together in the larger biogeographic study of the more inclusive tribe Mentheae (Drew and Sytsma, 2012).

Species distributions were obtained from primary literature (e.g., Epling, 1937; Epling and Játiva, 1964; Irving, 1980; Harley and Paucar, 2000; Harley et al., 2004; González-Gallegos et al., 2014) and from the Global Biodiversity Information Facility (GBIF; www.gbif.org) using protocols to maximize accurate species distributions (Maldonado et al., 2015; Spalink et al., 2016a, b). In BioGeoBEARS, we allowed the inferred ancestors to occupy up to three areas based on the largest area distribution of any extant New World species. Dispersal probabilities between pairs of areas were specified for two time slices to better model possible important geological events affecting the spread of Menthinae to the New World and subsequent vicariant and/or dispersal events as the clade diversified throughout the Americas (e.g., northern hemisphere Land Bridges, Beringia route, formation of the Panamanian Isthmus). The timing of an established overland migration route through the Panamanian Isthmus is of particular interest for amphitropical distributions. Because of the ongoing debate of the timing of this closure based on both geological evidence and time-calibrated phylogenetic trees (Bacon et al., 2013, 2015; Montes et al., 2012, 2015; O’Dea et al., 2016), we explored two sets of time intervals. Following prevailing geological evidence (O’Dea et al., 2016) we invoked a time slice from 0 to 3 Ma in which complete overland migration through the Panamanian Isthmus is permitted and a time slice 3–21 Ma in which long-distance dispersal or at least island-hopping is required. Following emerging phylogenetic evidence pointing to the ease of migration across the isthmus earlier in the Miocene (Bacon et al., 2013, 2015) and countering geological evidence supporting Miocene connections (Montes et al., 2012, 2015), we ran a separate analysis that invoked corresponding times slices of 0–7 Ma and 7–21 Ma.

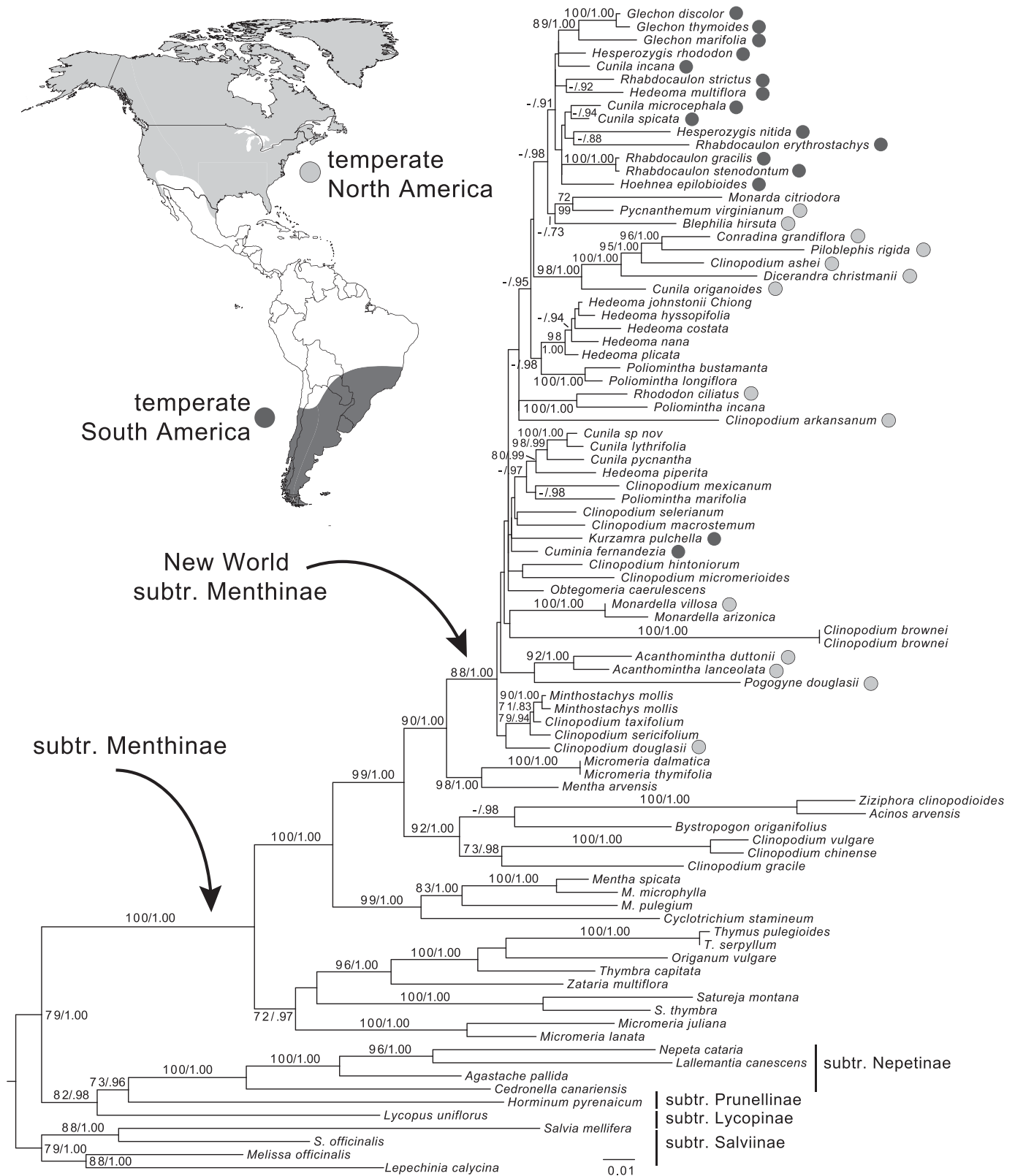
As we wanted to include phylogenetic uncertainty in our ancestral range estimation (especially for early-diverging nodes within

the New World Menthinae), we evaluated ARE across a random subset of 100 trees from the posterior probability (PP) of BEAST trees as well as from the maximum clade credibility (MCC) chronogram. Resulting ML scores for the more parameter-rich DEC+J model were tested for significance against the ML scores of the DEC model using the likelihood ratio test. We also conducted 100 biogeographical stochastic mapping replicates (Matzke, 2016) on the MCC chronogram to evaluate the probability of each class of cladogenetic event (vicariance, sympatry, subset-sympatry, and jump dispersals) given the DEC or DEC+J model, distribution data, and phylogeny. Details regarding model development, geographical regions involved, species coding, temporal stratification, and dispersal probabilities among the geographical regions through time are provided in Appendix S2.

## RESULTS

**Phylogenetic analysis**—The nrDNA alignment consisted of 1264 nucleotides, with ITS containing 748 characters and ETS containing 516 characters. After 75 characters (trimmed ends and ambiguously aligned characters) were excluded, the final data set had 1189 characters (ITS 677, ETS 512). The ML, MrBayes, and BEAST analyses were in general agreement in terms of topology, with nodes along the New World Menthinae backbone in general poorly supported. Subtribe Menthinae was recovered as monophyletic (ML bootstrap [MLBS] = 100%; posterior probability [PP] = 1.00; Fig. 2). Within the Menthinae, a clade of Mediterranean taxa (MLBS = 72%; PP = 0.97) was sister to the remaining Menthinae (MLBS = 100%; PP = 1.00). Within the latter clade, *Cyclotrichium* and *Mentha* was (MLBS = 99%; PP = 1.00) sister to the remaining Menthinae (MLBS = 99%; PP = 1.00). In the remaining Menthinae, a clade containing *Acinos*, *Bystropogon*, *Clinopodium*, and *Ziziphora* (MLBS = 92%; PP = 1.00) was sister to a clade (MLBS = 90%; PP = 1.00) containing two accessions of *Micromeria* sect. *Pseudomelissa* (*Clinopodium* in Bräuchler et al., 2006) + *Mentha arvensis* (MLBS = 98%; PP = 1.00) and a clade composed of New World Menthinae (MLBS = 88%; PP = 1.00).

Within the New World Menthinae clade, there was low resolution along some portions of the backbone, and most genera were not recovered as monophyletic (Fig. 2). *Acanthomintha* (MLBS = 92%; PP = 1.00), *Glechom* (MLBS = 89%; PP = 1.00), *Minthostachys* (MLBS = 100%; PP = 1.00), and *Monardella* (MLBS = 100%; PP = 1.00) were the only genera recovered as monophyletic, but these genera were not comprehensively sampled (especially *Monardella*). The two accessions of *Clinopodium brownei* formed a clade (MLBS = 100%; PP = 1.00) with a very long stem branch; this long branch was a result of scattered mutations throughout the ITS and ETS sequences and was not due to any specific clusters of questionable alignment. Although portions of the Menthinae backbone were not well supported, several clades with varying support were recovered within the New World Menthinae: (1) two Mexican clades, one consisting of Mexican (and northern central American) *Cunila*, *Clinopodium mexicanum*, *Hedeoma piperita*, and *Poliomintha marifolia* (MLBS < 50%; PP = 0.97), the other containing five species of *Hedeoma* + two species of *Poliomintha* (MLBS < 50%; PP = 0.98); (2) a clade of South American taxa containing *Clinopodium* and *Minthostachys* (MLBS = 79%; PP = 0.94); and (3) a clade of mostly southeastern United States taxa (*Clinopodium ashei*, *Cunila origanoides*, *Conradina*, *Dicerandra*, and *Piloblephis*). A clade



**FIGURE 2** Maximum likelihood (ML) tree of tribe Mentheae with emphasis on subtribe Menthinae based on nuclear ribosomal ITS and ETS. The tree is rooted with subtribe Salviinae based on Drew and Sytsma (2012). ML bootstrap values above 70% and Bayesian posterior probability values above 0.80 are shown near corresponding nodes. Species within the New World clade of subtribe Menthinae restricted to temperate regions of either North America or South America, as depicted in the map, are highlighted in the tree.

composed of mostly eastern United States genera (*Blephilia*, *Monarda*, and *Pycnanthemum*; MLBS < 50%; PP = 0.73) was sister (MLBS < 50%; PP = 0.98) to a clade of southeastern South American taxa (*Cunila*, *Glechona*, *Hedeoma*, *Hesperozygis*, *Hoehnea*, and *Rhabdocalyon*; MLBS < 50%; PP = 0.91).

The three New World genera that occur in both North and South America, *Clinopodium*, *Cunila*, and *Hedeoma*, were all found to be nonmonophyletic. Eleven different species of New World *Clinopodium* were sampled, and *Clinopodium sericifolium* and *Clinopodium taxifolium* were the only *Clinopodium* species within the New World Menthinae that grouped together with MLBS > 70% and/or PP values > 0.80. These two species were nested within a clade (MLBS = 79%; PP = 0.94) that also included two accessions of *Mintostachys*. Seven species of *Cunila* were sampled, and these seven accessions were recovered in four separate clades. *Cunila origanoides*, native to the eastern-central United States, was sister to a clade of southeastern United States taxa (*Clinopodium ashei*, *Conradina grandiflora*, *Dicerandra christmanii*, and *Piloblephis rigida*; MLBS = 98%; PP = 1.00). The three sampled Mexican/northern Central American species of *Cunila* formed a clade (MLBS = 98%; PP = 0.99). Of the three South American *Cunila* species sampled, two formed a clade (*C. spicata* and *C. microcephala*; MLBS < 50%; PP = 94), while the third species (*C. incana*) did not have a supported placement within the southeastern South American clade. We sampled five species of *Hedeoma*. *Hedeoma piperita*, native to central Mexico, was sister to the three Mexican species of *Cunila* (MLBS = 80%; PP = 0.99). The other five species of *Hedeoma* native to Mexico formed a clade (MLBS = 98%; PP = 1.00) that was sister to two Mexican *Poliomintha* species (MLBS < 50%; PP = 0.98). The lone South American *Hedeoma* accession, *H. multiflora*, was nested within a clade of southeastern South American taxa.

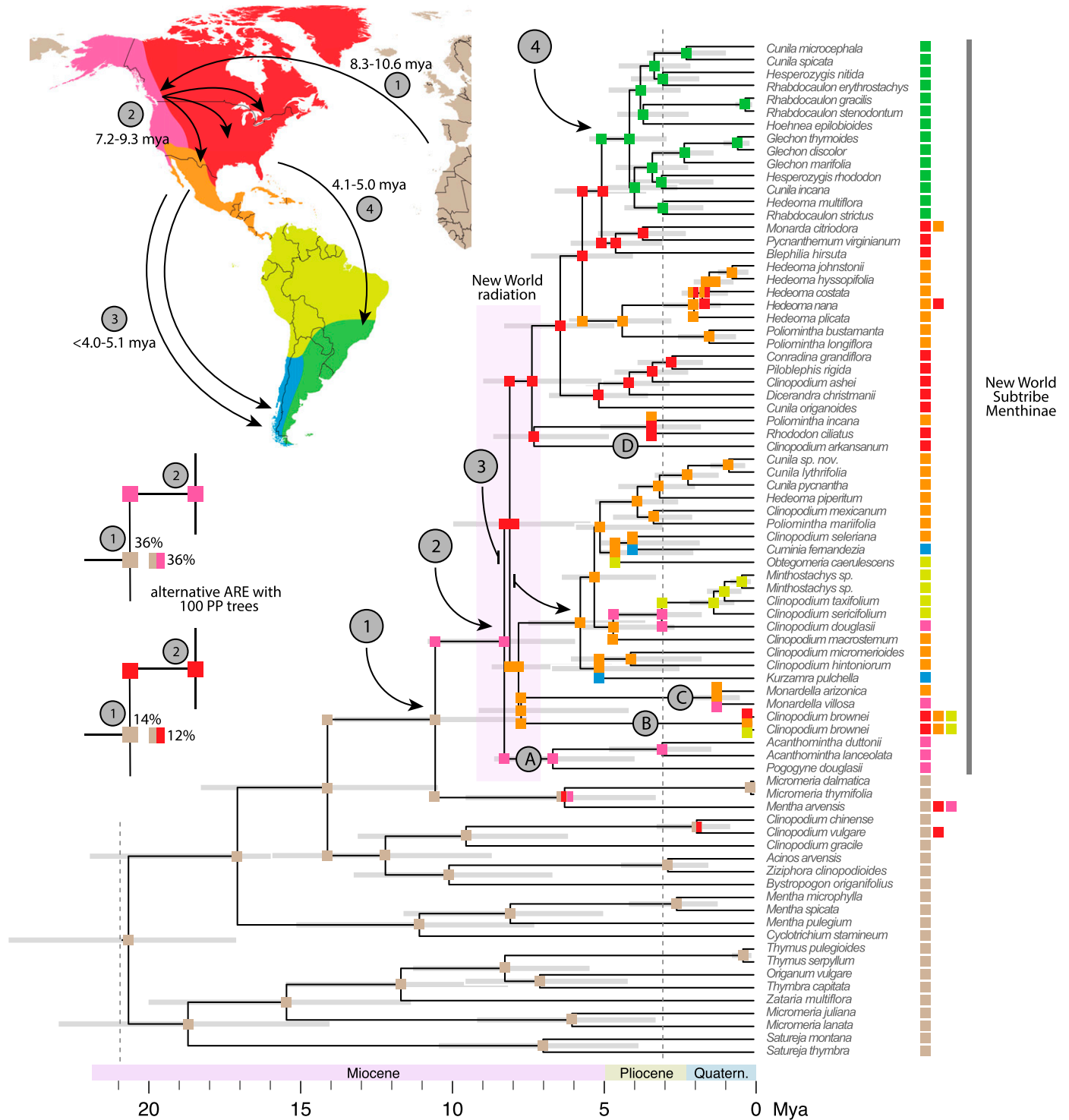
**Divergence time analyses with BEAST**—Our BEAST analyses estimated that the Old World and New World Menthinae diverged in the Late Miocene at about 10.6 Ma (7.5–14 95% highest posterior density [HPD]; Fig. 3, event 1). The crown of the New World Menthinae (Fig. 3, event 2) is estimated to have diversified at about 8.3 Ma (5.9–10.8 HPD). Four clades, singly or in combinations, are sister to the remaining New World lineage in the 100 subset trees from the BEAST PP distribution (clades A, B, C, and D in Fig. 3). Two are centered in the California Floristic Province, *Acanthomintha* + *Pogogyne* (clade A) and *Monardella* (clade C). *Acanthomintha* + *Pogogyne* is the dominant representative of the sister clade to all other New World species (94% of all trees, with 11% as the sole representative). *Monardella* is less representative in this early-diversifying sister clade (26%, with 4% singly). The more widespread (eastern North America, Mexico, to northern South America) *Clinopodium brownei* (clade B) is similarly representative in this early-diversifying sister clade (26%, with 6% singly). The eastern North American *Clinopodium arkansanum* (clade D) is the fourth (24%, with 4% singly).

After the initial migration into the New World in the late Miocene, lineages diversified quickly across central North America, Mexico, and South America either immediately before or very early in the Pliocene. The most recent common ancestor of a clade (PP = 0.62 in BEAST analysis) containing all sampled *Clinopodium* from mainland Mexico and South America, Mexican accessions of *Cunila*, *Cuminia* (from the Juan Fernández Islands off the coast of Chile), *Hedeoma piperita*, *Kurzamra* (from Argentina/Chile border

of the southern Andes), *Mintostachys*, *Obtegomeria*, and *Poliomintha marifolia* was estimated at 5.5 Ma (3.6–7.3 HPD). The most recent common ancestor of the eastern/southeastern United States clade composed of *Clinopodium ashei*, *Conradina*, *Cunila origanoides*, *Dicerandra*, and *Piloblephis* originated 5.1 Ma (3.5–6.9 HPD). A clade of seven Mexican taxa consisting of five species of *Hedeoma* and two species of *Poliomintha* began to diversify 4.4 Ma (2.7–6.1 HPD). The clade of predominantly eastern United States genera (*Blephilia*, *Monarda*, and *Pycnanthemum*) shared a common ancestor with the southeastern South American clade at about 5 Ma (3.5–6.6 HPD).

**Ancestral area estimation**—Biogeographical analyses with BioGeoBEARS yielded very similar results based upon time slices favoring early (7 Ma) or late (3 Ma) closure of the Isthmus of Panama. Results and discussion will follow the results of the 3-Ma analysis, acknowledging that this favors a model with a higher probability of jump dispersal for disjunct clades diverging in the Miocene or Pliocene. Model testing using the MCC chronogram significantly supported DEC+J (Ln L = -104.03) over DEC (Ln L = -115.64) with  $p = 1.4 \times 10^{-6}$ . The resulting parameters of the DEC+J model included: anagenetic dispersal rate  $d = 0.010$ ; extinction rate  $e = 1.0 \times 10^{-8}$ ; cladogenetic dispersal rate  $J = 0.031$ . Biogeographical stochastic mapping, given the parameters of the DECj model, indicated that 73% of all cladogenetic events are sympatric, 11% are subset-sympatry, 5% are vicariance, and 11% involve founder-event dispersals. The separate 100 PP tree analyses gave very similar biogeographical ARE scenarios for the subtribe Menthinae except for the early radiation of the New World clade (see Fig. 3). The PP BEAST tree and the DEC+J ancestral range estimation model portrayed in Fig. 3 best summarizes the most likely ARE scenarios across the different topologies of the 100 PP trees. Alternative ARE scenarios and their probabilities across the 100 PP trees are shown in Fig. 3. Important biogeographical events are summarized in the map inset. DEC+J supports equally (36%) a jump dispersal from the Old World to western North America at 10.6 Ma (Fig. 3, event 1), or a combined area involving Old World + western North America followed by vicariance leading to the crown of the New World Menthinae around 8.3 Ma (Fig. 3, event 2). Alternatively, a jump dispersal from Old World to eastern North America is invoked in 14% of the PP trees, or a vicariance event with these two areas is indicated at 12%. Both eastern North America and the more arid southwestern North America and Central America become important areas immediately after the crown diversification of New World Menthinae via jump dispersals and remain the main biogeographic areas for subsequent diversification of Menthinae throughout the end of the Miocene and into the early Pliocene.

Southwestern North America + Mexico gave rise to a large radiation with subsequent dispersals to northern South America and temperate southwestern South America (Fig. 3, event 3). These events are supported by 95% of the 100 PP BEAST trees. In the latter dispersal to temperate southwestern South America, the monotypic *Kurzamra*, endemic to northeast Chile and northwestern Argentina, exhibits an amphitropical distribution relative to a set of species (e.g., *Clinopodium*, *Cunila*) restricted to Mexico. The crown of this clade dates to the Miocene/Pliocene border (5.5 Ma; 3.6–7.5 HPD). The ditypic *Cuminia*, endemic to the Juan Fernández Islands in the southeast Pacific off the coast of Chile, also exhibits an amphitropical distribution relative to its closest relatives in the temperate southwestern South American clade. In many trees,



**FIGURE 3** Ancestral region reconstruction using BioGeoBEARS for subtribe Menthinae based on BEAST analyses (showing 95% HPD intervals in gray bars). Six designated areas in the New World are indicated in map inset, and each taxon in the tree is color-coded for its area distribution. Inferred ancestral areas (single or combined) are shown under the DECj model and represent the most frequent state (based on model of Panamanian Isthmus closure ca. 3 Ma; dashed lines represent the two time slices used in BioGeoBEARS). The numbers and letters represent nodes or clades of interest and are discussed in the text. Proportion of 100 stochastic mapping events for the four most frequent occurrences for nodes 1 and 2 are provided. The locations and dates (in millions of years) of four jump dispersals are summarized in the map: one from the Old World to North America, one from eastern North America to southeastern South America, and two from southwestern North America/Mexico to southwestern South America.

*Cuminia* is sister to *Clinopodium selerianum* restricted to southern Mexico and Guatemala, with its stem node dated to about 4.8 Ma in the Pliocene. The final amphitropical disjunction (Fig. 3, event 4) is seen between the morphologically heterogeneous clade restricted to southeastern South America (e.g., *Glechon*, *Rhabdocaulon*, see Fig. 1) and a clade restricted to eastern North America (e.g., *Monarda*, *Pycnanthemum*, see Fig. 1). This disjunction, supported by all 100 PP BEAST trees, involved a jump dispersal to South America from eastern North America and is dated between 4.1–5.0 Ma in the early Pliocene.

## DISCUSSION

We present here a phylogenetic framework of the New World Menthinae based on the largest sampling of species and biogeographical areas to date. This study supports but greatly extends the phylogenetic and evolutionary results previously obtained (e.g., Bräuchler et al., 2010; Drew and Sytsma, 2012). These previous studies had identified three key features of the radiation of New World Menthinae: (1) The clade is marked by a rapid radiation in species number and floral morphology (e.g., flower color/size, stamen number) across both North and South America. (2) While nuclear and cpDNA support similar relationships for most Old World lineages and some relationships within the New World clade, cpDNA is relatively phylogenetically uninformative (especially as more taxa are added) within the New World radiation, and cpDNA trees demonstrate a strong geographic signal, suggesting past introgression and/or chloroplast capture may be influencing results and contributing to discordance with nrDNA based phylogeny estimates. (3) Many of the large New World genera are not monophyletic based upon either nuclear or cpDNA evidence. The phylogenetic framework presented here reinforces these previous findings. Thus, to address the biogeographical questions pertaining to the origin of the New World Menthinae, subsequent biogeographic spread (vicariance or dispersal), and the rise of amphitropical distributions, our approach in using 100 PP BEAST trees as well as stochastic mapping in BioGeoBEARS was necessary to identify well-supported biogeographical events as well as alternatives. Reliance on a single MCC chronogram for ancestral range estimation would have been misleading.

**Location and timing of dispersal of Menthinae into the New World**—Previous studies have given conflicting information regarding the Old World sister lineage relative to the New World Menthinae (Trusty et al., 2004; Bräuchler et al., 2010; Drew and Sytsma, 2012). Results based on cpDNA suggested that the Canary Island genus *Bystropogon* is sister to New World Menthinae (Trusty et al., 2004; Bräuchler et al., 2010; Drew and Sytsma, 2012). The timing of the *Bystropogon*/New World Menthinae split based on cpDNA (ca. 10 Ma, late Miocene; Drew and Sytsma, 2012) is similar to the timing of the Old World/New World Menthinae divergence presented here based on nrDNA (Fig. 3, event 1). Morphologically, *Bystropogon* has been considered similar to the South American genus *Minthostachys* (Harley et al., 2004), and *Minthostachys* was placed within *Bystropogon* by Bentham (1832–1836). However, molecular phylogenetic analyses have not indicated a sister (nor particularly close) relationship between the two taxa, and studies based on nrDNA (as presented here) have suggested a relationship between *Bystropogon* and the Old World genera *Acinos*

(treated in *Clinopodium* by Harley et al., 2004) and *Ziziphora*. Needless to say, relationships within this group of Menthinae are complex, but all evidence points to a group of western Eurasian or Mediterranean species (as opposed east Asian) as sister to New World Menthinae.

The nrDNA analyses presented here suggest that the most recent common ancestor of *Micromeria* (sect. *Pseudomelissa*) and *Mentha arvensis* may be sister to the New World Menthinae. This is an intriguing idea based upon the current distribution of *Mentha arvensis*, which has a more or less circumboreal distribution, and the European/Mediterranean distribution of other species most closely related to the New World Menthinae, and would support migration across the North Atlantic Land Bridge (NALB) in the late Miocene (and not Beringia). The fact that *Micromeria* sect. *Pseudomelissa* is quite similar to Old World *Clinopodium* (Bräuchler et al., 2005, 2006) and that *Mentha arvensis* is a polyploid species (Harley et al., 2004; Edwards et al., 2006) whose cpDNA history differs markedly from its nrDNA history (Drew and Sytsma, 2012) certainly complicate matters. This scenario is more likely than a long-distance dispersal event of *Bystropogon* (or a *Bystropogon* ancestor) from the Canary Islands across the Atlantic Ocean. The NALB in the late Miocene was especially suitable geologically and climatically for temperate migrations (Tiffney and Manchester, 2001; Milne, 2006; Brikiatis, 2014, 2016). The likely NALB role in the expansion of the Menthinae to the New World would not be unusual, as the NALB has been implicated in similar late-Miocene migrations for a diverse set of plant clades (e.g., *Cercis*: Fritsch and Cruz, 2012; *Di-oscarea*: Viruel et al., 2015; *Quercus*: Denk et al., 2010; *Smilax*: Denk et al., 2015; *Sparganium*: Sulman et al., 2013).

## Biogeography and diversification within New World Menthinae—

It is unclear what lineage diverged first within the New World Menthinae (Figs. 2, 3). The nrDNA evidence presented here suggests that three clades, singly or in combination, are the most likely candidates as sister to all other New World Menthinae (clades A–D, Fig. 3). Two of these are restricted to the California Floristic Province, *Acanthomintha* + *Pogogyne* and *Monardella*, whereas the other two are either restricted to eastern North America (*Clinopodium arkansanum*) or extend farther south from eastern North America (*Clinopodium brownei*). Although a precise ancestral range estimate within North America for the initial dispersal from western regions of the Old World, likely via the NALB, is unknown, the New World Menthinae quickly (within a few million years) radiated throughout North and South America via multiple pathways. By the beginning of the Pliocene, the New World Menthinae had already achieved their present areas of distribution. Furthermore, in terms of floral form and habit, the New World Menthinae had additionally acquired greater diversity than their Old World counterparts (Drew and Sytsma, 2012; Drew et al., 2016).

Potential mechanisms that may explain both the relatively rapid spread of the New World Menthinae in terms of species numbers and morphology include polyploidy, a favorable climate, and pollinator interactions. Polyploidy has been shown to be a driver of diversification at several stages in organismal evolution in general (Van de Peer et al., 2009b) and specifically in flowering plants (Van de Peer et al., 2009a). Edwards et al. (2006), in a study that focused on a southeastern clade of New World Menthinae using the *GapC* gene region, found at least one and possibly two genome duplications within the clade. In a study that included broader sampling within the Menthinae (both Old World and New World) and cloning of

the nuclear *PPR-AT3G09060* gene, one or possibly two gene duplications were found across the New World Menthinae, whereas Old World taxa possessed only a single copy (Drew et al., 2016; B. Drew, unpublished data). Almost all taxa within the Menthinae prefer open, usually xeric environments, and this type of habitat has steadily increased from the late Miocene onward in both North and South America in concert with worldwide Tertiary cooling and aridification (Zachos et al., 2008; Töpel et al., 2012; De Vleeschouwer et al., 2017). The subtribe Menthinae may have benefited from this cooling/drying trend by arriving in the New World as it was starting, thereby having an advantage over the competition. Finally, a number of genera of New World Menthinae have species that are pollinated by hummingbirds (e.g., *Clinopodium*, *Hedeoma*, *Monarda*, *Monardella*, *Poliomnitha*). Hummingbirds are absent from the Old World and diversified in the New World coincident with the timing of the Menthinae diversification (Chaves et al., 2011; McGuire et al., 2014). The ability of various lineages of New World Menthinae to co-opt hummingbirds as pollinators may have spurred diversification (Schmidt-Lebuhn et al., 2007; Abrahamczyk and Renner, 2015; Serrano-Serrano et al., 2017), particularly in concert with a cooling climate.

**Amphitropical disjunctions in New World Menthinae mints**—The three New World Menthinae genera that occur in both North and South America, *Clinopodium*, *Cunila*, and *Hedeoma*, are non-monophyletic, and none (at least given our current sampling) qualify as amphitropical disjunctions. However, our analyses uncover three clades exhibiting amphitropical distributions that are consistent across almost all the trees examined. All three appear to correspond to the same time frame, namely, the very beginning of the Pliocene about 4–5 Ma. The first two amphitropical distributions occur in a largely southwestern North American and Central America clade; both involve a disjunction with a mono- or ditypic genus in southern South America. The monotypic *Kurzamra*, endemic to Chile and northwestern Argentina, is embedded within this clade, and all sampled BEAST trees indicate a dispersal from the north to southwestern South America, even though its exact placement is not clear. The second is the ditypic *Cuminia*, endemic to Robinson Crusoe Island, one of the Juan Fernández Islands off the coast of Chile. Little is known about its relationships, timing of arrival into the Juan Fernández Island archipelago, and continental source of propagules (Bernardello et al., 2006; Stuessy et al., 2014). Bernardello et al. (2006) simply indicated that *Cuminia* likely arrived from the “pantropics” via nutlets ingested internally by birds. Speculation based on morphology, physiognomy, and habitat suggested a relationship to either *Bystropogon* of the Canary Islands or South American *Minthostachys* (Ruiz et al., 2000). Based on sequence data, a relationship of *Cuminia* to *Monardella* and *Minthostachys* was suggested by Trusty et al. (2004) or more broadly to the New World *Clinopodium* group (Bräuchler et al., 2010). Although the exact placement of *Cuminia* within the southwestern North American and Central America clade is not clear based on our results, it is sister to *Clinopodium selerianum* in just under 40% of the 100 PP BEAST trees examined in BioGeoBEARS. Notably, 13% of the PP BEAST trees recover the southern endemic genera, *Kurzamra* and *Cuminia*, as monophyletic, with a most recent common ancestor ca. 5 Ma. This relationship was also found with cpDNA (Drew and Sytsma, 2012).

The third amphitropical disjunction involves a larger clade of southeastern South American genera (*Cunila*, *Glechon*, *Hedeoma*,

*Hesperozygis*, *Hoehnea*, and *Rhabdocaulon*) arising out of eastern North America via a jump dispersal in the early Pliocene. This clade is sister to a North American temperate clade composed of *Blephilia*, *Monarda*, and *Pycnanthemum*. This relationship is similar with cpDNA (fig. 2 of Drew and Sytsma, 2012). However, cpDNA results indicated that all the temperate South American taxa (that were sampled in that less taxon-dense study) were sister to an eastern North American clade plus *Cunila* (from Mexico) and *Hedeoma costata*. These two sister clades, with a few exceptions (e.g., *Pycnanthemum*, *Dicerandra*) possess the derived character of two as opposed to four stamens (Drew and Sytsma, 2012). Outside of the aforementioned clades, the two-stamened condition is unusual within Menthinae.

These amphitropical members of the New World Menthinae also share other features considered correlated with this disjunct pattern. First, all these species prefer open habitats, a characteristic that has been implicated in amphitropical disjunctions previously (Raven, 1963). Second, most Lamiaceae (if not almost all) are self-compatible (Uberta-Jiménez and Hidalgo-Fernández, 1992; Harley et al., 2004), a characteristic Raven (1963) considered crucial for successful long-distance colonization. Third, at least *Glechon* and *Hesperozygis* possess mericarps that are mucilaginous when wetted. The distribution of these taxa is part of the migratory pathway for several bird species (Zimmer, 1938; Raven, 1963; Park, 2016), and bird migration seems the most likely mechanism of dispersal for generating amphitropical distributions. Although none of the potential North American ancestral clades exhibit this trait today, the dispersal of mericarps in the Menthinae (reviewed by Boumann and Meeuse, 1992; Harley et al., 2004) has been effective in allowing a limited amount of intercontinental dispersals coupled with extensive overland migrations (Drew and Sytsma, 2012).

While not often addressed in discussions of amphitropical or bipolar distributions in the New World, the closure of the Isthmus of Panama may have an important role in facilitating the overland migration of Menthinae mints. Prevailing geological evidence has argued for an early Pleistocene closure (O’Dea et al., 2016), although there is evidence that at least “stepping-stone” islands, if not direct connections, existed between North America and South America earlier in the Miocene (Wegner et al., 2011; Montes et al., 2012, 2015). On the other hand, testing geological models of the Isthmus based on phylogenetic frameworks for plant clades provides evidence that many dispersals are late Miocene or Pliocene in origin (Bacon et al., 2013, 2015). In this context, the dating of all three amphitropical disjunctions to late Miocene or early Pliocene is noteworthy and would add to the growing list of plant clades that point to easier, earlier dispersal across the Isthmus of Panama. Future research in Menthinae (and other Lamiaceae) should address the importance and mode of long-distance dispersal via their mericarps vs. overland migrations.

## ACKNOWLEDGEMENTS

We thank the many collectors who aided in this study (see Appendix S1). In particular, the UC Berkeley Botanical Garden, the Denver Botanical Garden, and the Historic Bok Sanctuary (Bok Tower Gardens) were helpful in the procurement of plant specimens. We gratefully thank F. MEXU, MO, OS, and WIS for allowing us to use their resources and for allowing the destructive sampling of specimens included in this study. Sarah Friedrich helped with graphics. Special thanks to Ivalú Cacho, Mark Elvin, Holly Forbes,

Jesús Guadalupe González-Gallegos, Jason Singhurst, and Jay Walker for collecting assistance and advice. Thanks also to Nicole Pauley and Isabella Gomez for help in the laboratory, Ricardo Kriebel for assistance with R scripts, and two anonymous reviewers whose suggestions improved this article. This study was partially funded by NSF DDIG DEB-0910336, DEB-1655611, and the Hofmeister Fund through the Botany Department, University of Wisconsin, Madison.

## LITERATURE CITED

- Abrahamczyk, S., and S. S. Renner. 2015. The temporal build-up of hummingbird/plant mutualisms in North America and temperate South America. *BMC Evolutionary Biology* 15: 104.
- Andreasen, K., B. G. Baldwin, and B. Bremer. 1999. Phylogenetic utility of the nuclear rDNA ITS region in subfamily Ixoroideae (Rubiaceae): Comparisons with cpDNA *rbcl* sequence data. *Plant Systematics and Evolution* 217: 119–135.
- Axelrod, D. I. 1975. Evolution and biogeography of Madrean-Tethyan sclerophyll vegetation. *Annals of the Missouri Botanical Garden* 62: 280–334.
- Bacon, C. D., A. Mora, W. L. Wagner, and C. A. Jaramillo. 2013. Testing geological models of evolution of the Isthmus of Panama in a phylogenetic framework. *Botanical Journal of the Linnean Society* 171: 287–300.
- Bacon, C. D., D. Silvestro, C. Jaramillo, B. T. Smith, P. Chakrabarty, and A. Antonelli. 2015. Biological evidence supports an early and complex emergence of the Isthmus of Panama. *Proceedings of the National Academy of Sciences, USA* 112: 6110–6115.
- Baker, H. G. 1959. The contribution of autecological and genecological studies to our knowledge of the past migrations of plants. *American Naturalist* 93: 255–272.
- Baldwin, B. G., and S. Markos. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: Congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* 10: 449–463.
- Baldwin, B. G., and K. R. Wood. 2016. Origin of the Rapa endemic genus *Apostates*: Revisiting major disjunctions and evolutionary conservatism in the *Bahia* alliance (Compositae: Bahieae). *Taxon* 65: 1064–1080.
- Bentham, G. 1832–1836. *Labiatarum genera et species*. Ridgeway & Sons, London, UK.
- Bernardello, G., G. J. Anderson, T. F. Stuessy, and D. J. Crawford. 2006. The angiosperm flora of the Archipelago Juan Fernandez (Chile): Origin and dispersal. *Canadian Journal of Botany* 84: 1266–1281.
- Boumann, F., and A. D. J. Meuse. 1992. Dispersal in Labiatae. In R. M. Harley and T. Reynolds [eds.], *Advances in Labiate science*, 193–202. Kew Botanical Gardens, Kew, UK.
- Bräuchler, C., H. Meimberg, T. Abele, and G. Heubl. 2005. Polyphyly of the genus *Micromeria* (Lamiaceae)—Evidence from cpDNA sequence data. *Taxon* 54: 639–650.
- Bräuchler, C., H. Meimberg, and G. Heubl. 2006. New names in Old World *Clinopodium*—The transfer of the species of *Micromeria* sect. *Pseudomelissa* to *Clinopodium*. *Taxon* 55: 977–981.
- Bräuchler, C., H. Meimberg, and G. Heubl. 2010. Molecular phylogeny of Menthinae (Lamiaceae, Nepetoideae, Mentheae) – Taxonomy, biogeography and conflicts. *Molecular Phylogenetics and Evolution* 55: 501–523.
- Briggs, J. C. 2009. Darwin's biogeography. *Journal of Biogeography* 36: 1011–1017.
- Brikiatis, L. 2014. The De Geer, Thulean and Beringia routes: Key concepts for understanding early Cenozoic biogeography. *Journal of Biogeography* 41: 1036–1054.
- Brikiatis, L. 2016. Late Mesozoic North Atlantic land bridges. *Earth-Science Reviews* 159: 47–57.
- Cantino, P. D., and S. J. Wagstaff. 1998. A reexamination of North American *Satureja* s.l. (Lamiaceae) in light of molecular evidence. *Brittonia* 50: 63–70.
- Carlquist, S. J. 1974. *Island biology*. Columbia University Press, New York, New York, USA.
- Chaves, J. A., J. T. Weir, and T. B. Smith. 2011. Diversification in *Adelomyia* hummingbirds follows Andean uplift. *Molecular Ecology* 20: 4564–4576.
- Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9: 772.
- Darwin, C. R. 1859. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. John Murray, London, UK.
- De Vleeschouwer, D., M. Vahlenkamp, M. Crucifix, and H. Pälke. 2017. Alternating Southern and Northern Hemisphere climate response to astronomical forcing during the past 35 m.y. *Geology* 45: 375–378.
- Denk, T., F. Grimsson, and R. Zetter. 2010. Episodic migration of oaks to Iceland: Evidence for a North Atlantic “land bridge” in the latest Miocene. *American Journal of Botany* 97: 276–287.
- Denk, T., D. Velitzelos, H. T. Guner, and L. Ferrufino-Acosta. 2015. *Smilax* (Smilacaceae) from the Miocene of western Eurasia with Caribbean biogeographic affinities. *American Journal of Botany* 102: 423–438.
- Donoghue, M. J. 2011. Bipolar biogeography. *Proceedings of the National Academy of Sciences, USA* 108: 6341–6342.
- Doroszenko, A. 1986. Taxonomic studies on the *Satureja* complex (Labiatae). Ph.D. dissertation, University of Edinburgh, Edinburgh, UK.
- Drew, B. T. 2017. Phylogenetics and divergence times in Lamiaceae [abstract]. Advances in Lamiaceae Science Symposium, 2017, Antalya, Turkey.
- Drew, B. T., D. Spalink, and K. J. Sytsma. 2016. A single dispersal event led to diversification in New World Menthinae. Botany 2016: Annual Meeting of the Botanical Society of America, Savannah, Georgia, USA [online abstract]. Available at <http://2016.botanyconference.org/engine/search/index.php?func=detail&id=907>.
- Drew, B. T., and K. J. Sytsma. 2011. Testing the monophyly and placement of *Lepechinia* in the tribe Mentheae (Lamiaceae). *Systematic Botany* 36: 1038–1049.
- Drew, B. T., and K. J. Sytsma. 2012. Phylogenetics, biogeography, and staminal evolution in the tribe Mentheae (Lamiaceae). *American Journal of Botany* 99: 933–953.
- Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214.
- 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: 1969–1973.
- Edwards, C. E., D. E. Soltis, and P. S. Soltis. 2006. Molecular phylogeny of *Conradina* and other scrub mints from the southeastern USA: Evidence for hybridization in Pleistocene refugia? *Systematic Botany* 31: 193–207.
- Epling, C. 1937. Synopsis of South American Labiatae. *Repertorium Specierum Novarum Regni Vegetabilis. Beihefte* 85: 1–341.
- Epling, C., and C. Játiva. 1964. Revisión del género *Satureja* en América del Sur. *Brittonia* 16: 393–416.
- Fritsch, P. W., and B. C. Cruz. 2012. Phylogeny of *Cercis* based on DNA sequences of nuclear ITS and four plastid regions: Implications for transatlantic historical biogeography. *Molecular Phylogenetics and Evolution* 62: 816–825.
- Gernhard, T. 2008. The conditioned reconstructed process. *Journal of Theoretical Biology* 253: 769–778.
- Givnish, T., and S. Renner. 2004. Tropical intercontinental disjunctions: Gondwana breakup, immigration from the boreotropics, and transoceanic dispersal. *International Journal of Plant Sciences* 165 (S4): S1–S6.
- González-Gallegos, J. G., A. Castro-Castro, V. Quintero-Fuentes, M. E. Mendoza-López and E. de Castro-Arce. 2014. Revisión taxonómica de Lamiaceae del occidente de México. *Ibguana* 7: 3–545.
- Harley, R. M., S. Atkins, A. L. Budanste, P. D. Cantino, B. J. Conn, R. Grayer, M. M. Harley, et al. 2004. Flowering plants, dicotyledons. In K. Kubitzki [ed.], *The families and genera of vascular plants*, vol. 6, 167–275. Springer Verlag, Berlin, Germany.
- Harley, R. M., and A. G. Paucar. 2000. List of species of tropical American *Clinopodium* (Labiatae), with new combinations. *Kew Bulletin* 55: 917–927.
- Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics (Oxford, England)* 17: 754–755.
- Irving, R. S. 1980. The systematics of *Hedeoma* (Labiatae). *Sida* 8: 218–295.

- Kar, R. K. 1996. On the Indian origin of *Ocimum* (Lamiaceae): A palynological approach. *Palaeobotanist* 43: 45–50.
- Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, et al. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)* 28: 1647–1649.
- Li, B., P. D. Cantino, R. G. Olmstead, G. L. Bramley, C. L. Xiang, Z. H. Ma, Y. H. Tan, and D. X. Zhang. 2016. A large-scale chloroplast phylogeny of the Lamiaceae sheds new light on its subfamilial classification. *Scientific Reports* 6: 34343.
- Li, B., and R. G. Olmstead. 2017. Two new subfamilies in Lamiaceae. *Phytotaxa* 313: 222–226.
- Maddison, W. P., and D. R. Maddison. 2017. Mesquite: a modular system for evolutionary analysis, version 3.2. Website <http://mesquiteproject.org>.
- Maldonado, C., C. I. Molina, A. Zizka, C. Persson, C. M. Taylor, J. Albán, E. Chilquillo, N. Ronsted, and A. Antonelli. 2015. Estimating species diversity and distribution in the era of Big Data: To what extent can we trust public databases? *Global Ecology and Biogeography* 24: 973–984.
- Manchester, S. R., F. Grimsson, and R. Zetter. 2015. Assessing the fossil record of asterids in the context of our current phylogenetic framework. *Annals of the Missouri Botanical Garden* 100: 329–363.
- Matzke, N. J. 2013. BioGeoBEARS: BioGeography with Bayesian (and likelihood) evolutionary analysis in R Scripts, CRAN: The Comprehensive R Archive Network, Vienna, Austria. Website <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: 951–970.
- Matzke, N. J. 2016. Stochastic mapping under biogeographical models. Website [http://phylo.wikidot.com/biogeobears#stochastic\\_mapping](http://phylo.wikidot.com/biogeobears#stochastic_mapping).
- McGuire, J. A., C. C. Witt, J. V. Remsen Jr., A. Corl, D. L. Rabosky, D. L. Altshuler, and R. Dudley. 2014. Molecular phylogenetics and the diversification of hummingbirds. *Current Biology* 24: 910–916.
- Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic tree. Proceedings of the Gateway Computing Environments Workshop (GCE), 1–8. New Orleans, Louisiana, USA.
- Milne, R. I. 2006. Northern hemisphere plant disjunctions: A window on Tertiary land bridges and climate change? *Annals of Botany* 98: 465–472.
- Montes, C., A. Cardona, C. Jaramillo, A. Pardo, J. C. Silva, V. Valencia, C. Ayala, et al. 2015. Middle Miocene closure of the Central American Seaway. *Science* 348: 226–229.
- Montes, C., A. Cardona, R. McFadden, S. E. Moron, C. A. Silva, S. Restrepo-Moreno, D. A. Ramirez, et al. 2012. Evidence for middle Eocene and younger land emergence in central Panama: Implications for Isthmus closure. *Geological Society of America Bulletin* 124: 780–799.
- Moon, H. K., S. P. Hong, E. Smets, and S. Huysmans. 2010. Phylogeny of tribe Menthaeae (Lamiaceae): The story of molecules and micromorphological characters. *Taxon* 59: 1065–1076.
- O'Dea, A., H. A. Lessios, A. G. Coates, R. I. Eytan, S. A. Restrepo-Moreno, A. L. Cione, L. S. Collins, et al. 2016. Formation of the Isthmus of Panama. *Science Advances* 2: e1600883–e1600883.
- Park, M. S. 2016. Plant dispersal bias from North to South America facilitated by shorebird migration routes. Botany 2016: Annual Meeting of the Botanical Society of America, Savannah, Georgia, USA [online abstract]. Available at <http://2016.botanyconference.org/engine/search/index.php?func=detail&id=882>.
- Rambaut, A., M. Suchard, W. Xie, and A. Drummond. 2014. Tracer v. 1.6. Institute of Evolutionary Biology, University of Edinburgh. Available at <http://beast.bio.ed.ac.uk/>.
- Raven, P. H. 1963. Amphitropical relationships in the floras of North and South America. *The Quarterly Review of Biology* 38: 151–177.
- Raven, P. H. 1972. Plant species disjunctions: A summary. *Annals of the Missouri Botanical Garden* 59: 234–246.
- Reid, E. M., and M. E. J. Chandler. 1926. Catalogue of Cainozoic plants in the Department of Geology, vol. 1. The Brembridge Flora. British Museum (Natural History), London, UK.
- Ronquist, F., and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, England)* 19: 1572–1574.
- Ruiz, E., C. Marticorena, D. Crawford, and T. Stuessy. 2000. Morphological and ITS sequence divergence between taxa of *Cuminia* (Lamiaceae), an endemic genus of the Juan Fernandez Islands, Chile. *Brittonia* 52: 341–350.
- Schmidt-Lebuhn, A. N., M. Kessler, and I. Hensen. 2007. Hummingbirds as drivers of plant speciation? *Trends in Plant Science* 12: 329–331.
- Serrano-Serrano, M. L., J. Rolland, J. L. Clark, N. Salamin, and M. Perret. 2017. Hummingbird pollination and the diversification of angiosperms: An old and successful association in Gesneriaceae. *Proceedings. Biological Sciences* 284: 20162816.
- Simpson, M. G., L. Johnson, T. Villaverde, and C. M. Williams. 2017. American amphitropical disjuncts: Perspectives from vascular plant analyses and prospects for future research. *American Journal of Botany* 104: 1600–1650.
- Solbrig, O. T. 1972. Floristic disjunctions between Monte in Argentina and Sonoran Desert in Mexico and United States. *Annals of the Missouri Botanical Garden* 59: 218–223.
- Spalink, D., B. T. Drew, M. C. Pace, J. G. Zaborsky, P. Li, K. M. Cameron, T. J. Givnish, and K. J. Sytsma. 2016a. Evolution of geographical place and niche space: Patterns of diversification in the North American sedge (Cyperaceae) flora. *Molecular Phylogenetics and Evolution* 95: 183–195.
- Spalink, D., B. T. Drew, M. C. Pace, J. G. Zaborsky, J. R. Starr, K. M. Cameron, T. J. Givnish, and K. J. Sytsma. 2016b. Biogeography of the cosmopolitan sedges (Cyperaceae) and the area–richness correlation in plants. *Journal of Biogeography* 43: 1893–1904.
- Stamatakis, A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics (Oxford, England)* 30: 1312–1313.
- Stuessy, T. F., C. König, and P. L. Sepúlveda. 2014. Paraphyly and endemic genera of oceanic islands: Implications for conservation. *Annals of the Missouri Botanical Garden* 100: 50–78.
- Sulman, J. D., B. T. Drew, C. Drummond, E. Hayasaka, and K. J. Sytsma. 2013. Systematics, biogeography, and character evolution of *Sparganium* (Typhaceae): Diversification of a widespread, aquatic lineage. *American Journal of Botany* 100: 2023–2039.
- Sytsma, K. J., J. Morawetz, J. C. Pires, M. Nepokroeff, E. Conti, M. Zjhra, J. C. Hall, and M. W. Chase. 2002. Urticalean rosids: Circumscription, rosid ancestry, and phylogenetics based on *rbcL*, *trnL-F*, and *ndhF* sequences. *American Journal of Botany* 89: 1531–1546.
- The Plant List. 2017. Version 1.1. Published on the Internet; <http://www.theplantlist.org/> [accessed 8 May 2017].
- Thorne, R. 1972. Major disjunctions in the geographic ranges of seed plants. *The Quarterly Review of Biology* 47: 365–411.
- Tiffney, B. H., and S. R. Manchester. 2001. The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere Tertiary. *International Journal of Plant Sciences* 162: S3–S17.
- Töpel, M., A. Antonelli, C. Yesson, and B. Eriksen. 2012. Past climate change and plant evolution in Western North America: A case study in Rosaceae. *PLoS One* 7: e50358.
- Tripp, E. A., and L. A. McDade. 2014. A rich fossil record yields calibrated phylogeny for Acanthaceae (Lamiales) and evidence for marked biases in timing and directionality of intercontinental disjunctions. *Systematic Biology* 63: 660–684.
- Trusty, J. L., R. G. Olmstead, D. J. Bogler, A. Santos-Guerra, and J. Francisco-Ortega. 2004. Using molecular data to test a biogeographic connection of the Macaronesian genus *Bystropogon* (Lamiaceae) to the New World: A case of conflicting phylogenies. *Systematic Botany* 29: 702–715.
- Ubera-Jiménez, J. L., and P. J. Hidalgo-Fernández. 1992. Temporal gynodioecy in *Rosmarinus officinalis*. In R. M. Harley and T. Reynolds [eds.], *Advances in Labiate science*, 281–289. Royal Botanic Gardens, Kew, UK.
- Van de Peer, Y., J. A. Fawcett, S. Proost, L. Sterck, and K. Vandepoel. 2009a. The flowering world: A tale of duplications. *Trends in Plant Science* 14: 680–688.

- Van de Peer, Y., S. Maere, and A. Meyer. 2009b. The evolutionary significance of ancient genome duplications. *Nature Reviews. Genetics* 10: 725–732.
- Viruel, J., J. G. Segarra-Moragues, L. Raz, F. Forest, P. Wilkin, I. Sanmartín, and P. Catalán. 2015. Late Cretaceous–Early Eocene origin of yams (*Dioscorea*, Dioscoreaceae) in the Laurasian Palaeartic and their subsequent Oligocene–Miocene diversification. *Journal of Biogeography* 43: 750–762.
- Walker, J. B., B. T. Drew, and K. J. Sytsma. 2015. Unravelling species relationships and diversification within the iconic California Floristic Province sages (*Salvia* subgenus *Audibertia*, Lamiaceae). *Systematic Botany* 40: 826–844.
- Wallace, A. R. 1880. *Island life*. McMillan, London, UK.
- Wegner, W., G. Worner, R. S. Harmon, and B. R. Jicha. 2011. Magmatic history and evolution of the Central American Land Bridge in Panama since Cretaceous times. *Bulletin of the Geological Society of America* 123: 703–724.
- Wen, J. 1999. Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. *Annual Review of Ecology and Systematics* 30: 421–455.
- Wen, J., and S. M. Ickert-Bond. 2009. Evolution of the Madrean–Tethyan disjunctions and the North and South American amphitropical disjunctions in plants. *Journal of Systematics and Evolution* 47: 331–348.
- Wen, J., Z. L. Nie, and S. M. Ickert-Bond. 2016. Intercontinental disjunctions between eastern Asia and western North America in vascular plants highlight the biogeographic importance of the Bering land bridge from late Cretaceous to Neogene. *Journal of Systematics and Evolution* 54: 469–490.
- White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], *PCR protocols: A guide to methods and applications*, 315–322. Academic Press, New York, New York, USA.
- Yao, G., B. T. Drew, T.-S. Yi, H.-F. Yan, Y.-M. Yuan, and X.-J. Ge. 2016. Phylogenetic relationships, character evolution and biogeographic diversification of *Pogostemon* s.l. (Lamiaceae). *Molecular Phylogenetics and Evolution* 98: 184–200.
- Yule, G. U. 1925. A mathematical theory of evolution, based on the conclusions of Dr. J. C. Willis, F. R. S. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 213: 21–87.
- Zachos, J. C., G. R. Dickens, and R. E. Zeebe. 2008. An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. *Nature* 451: 279–283.
- Zimmer, J. T. 1938. Notes on migrations of South American birds. *The Auk* 55: 405–410.