



# Phylogeny and biogeography of New World Stachydeae (Lamiaceae) with emphasis on the origin and diversification of Hawaiian and South American taxa



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## ABSTRACT

Due to its unique geological history and isolated location, the Hawaiian Archipelago provides an ideal setting for studies on biogeography, phylogeny and population biology. Species richness in these islands has been attributed to unique colonization events. The Hawaiian mints comprising of three endemic genera represent one of the largest radiations in the island. Previous studies have shown the Hawaiian mints to be nested within the dry-fruited *Stachys*, probably resulting from one or more hybridization events. Stachydeae, the largest tribe in the subfamily Lamioideae (Lamiaceae), is a taxonomically complex and widespread lineage exhibiting remarkable chromosomal diversity. In this paper we attempted at untangling the relationships between the New World and Hawaiian mint taxa, as well as investigate the origin and diversification of the mints in the New World. There seem to have been at least two independent migration events of *Stachys* to the New World during the Middle to Late Miocene and towards the beginning of the Pliocene, respectively. Results indicate incongruence between the rDNA and cpDNA phylogenies suggesting a reticulate, New World origin for the Hawaiian mints, although dispersal to Hawaii appears to have happened only once during the Pliocene. South American *Stachys* diversified from their Mesoamerican relatives around Late Pliocene and may also have arisen from similar reticulate events indicated by their intercalating position among the Mesoamerican *Stachys* species. Further insights into the phylogenetic relationships between the New World mints may be gathered through the study of low copy nuclear loci.

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## 1. Introduction

### 1.1. The Hawaiian Archipelago and its floral composition

Since the time of Darwin's visit to the Galapagos Islands, islands have become an integral part in the study of evolutionary processes (Emerson, 2002). Oceanic island systems can act as "natural laboratories" for studies in evolutionary biology mainly due to their isolated locations, recent ages, and small sizes, which provide opportunities for studying diversification and unique evolutionary histories of the endemic populations inhabiting them (Emerson, 2002; Funk and Wagner, 1995; Fleischer et al., 1998; Roderick and Gillespie, 1998; Price and Clague, 2002; Price and Wagner, 2004; Price, 2004). Due to its unique geological history and isolated geographical location, the Hawaiian Archipelago provides an excellent setting for biogeographic, phylogenetic and population studies. The youngest of the six main high islands, Hawai'i, is

situated in the far south-east, and has been estimated to be 0.43 million years (My) old, whereas, the oldest island of Kaua'i, aged at 5.1 My, is situated in the northwest corner of the island chain (Carson and Clague, 1995; Clague and Dalrymple, 1987). The low eroded atolls located to the northwest of the main islands are sometimes known as the "leeward islands", and form a chain ranging in age from 7 to 28 My (Fleischer et al., 1998). Most of the mid to high elevation Hawaiian flora and fauna evolved in isolation for up to 5 My on the current main islands (Price and Clague, 2002). This isolation led to the production of a unique and distinctive flora. Continued opening of a variety of new habitats accompanied by geographic dispersal through the islands has led to a significantly high level of endemism and species diversification among the Hawaiian flora with approximately 89% of its flowering plant species endemic to the archipelago (Wagner et al., 1999). This is among the highest rates of endemism known for any flora (Carson and Clague, 1995; Price, 2004). The Hawaiian flora seems to represent a characteristically insular nature due to the limited number of original colonizers and their diversity in origin (Wagner et al., 1999; Baldwin and Wagner, 2010), and it has been suggested that the ca. 1000 native angiosperm species were derived from

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only 272 to 282 natural introductions (Wagner et al., 1999). Various studies have shown that the diversity in many major Hawaiian genera has resulted from a single dispersal event to the island with subsequent phylogenetic radiations, including the Hawaiian silver-sword alliance (Asteraceae; Baldwin et al., 1991; Baldwin and Sanderson, 1998), *Hesperomannia* (Asteraceae; Kim et al., 1998), Hawaiian *Geranium* (Geraniaceae; Pax et al., 1997), Hawaiian lobelioids (Lobeliaceae; Givnish et al., 2009), and *Melicope* and *Platydesma* (Rutaceae; Harbaugh et al., 2009). However, other major radiations like *Cyrtandra* (Gesneriaceae; Cronk et al., 2005) and *Scaevola* (Goodeniaceae; Howarth and Baum, 2005) have been shown to be the results of multiple introductions. A high degree of morphological variation, which is observed in many Hawaiian plant groups, is often contrasted by low levels of genetic diversity (Lindqvist and Albert, 2002; Lindqvist et al., 2003, 2006; Carr, 1998; Baldwin and Sanderson, 1998; McGlaughlin and Friar, 2011; Friar et al., 2007, 2008; Lawton-Rauh et al., 2007; Remington and Robichaux, 2007; Givnish et al., 2009). Hybridization and/or polyploidization prior to dispersal to the Hawaiian Islands have been suggested as a major cause for successful colonization and diversification of North American lineages (Crawford and Stuessy, 1997; Carr, 1998; Barrier et al., 1999; Ballard and Sytsma, 2000; Lindqvist and Albert, 2002; Stefanovic and Costea, 2008; Crawford et al., 2009; Havran et al., 2009; Soltis et al., 2009; Baldwin and Wagner, 2010). An increased genetic variability in the colonizers may have aided their survival abilities through extensive recombination and expression of diverse phenotypes, which helped in their successful establishment in the different islands of the Hawaiian Archipelago.

### 1.2. The Hawaiian mints (Lamiaceae)

The Hawaiian mints represent one of the largest plant radiations displaying a wide range of morphological and ecological variations on one hand with extremely low levels of DNA sequence divergence on the other (Lindqvist and Albert, 2002; Lindqvist et al., 2003, 2006, 2007). The Hawaiian mints are comprised of three genera, *Haplostachys*, *Phyllostegia* and *Stenogyne*, consisting of 59 species. *Haplostachys* (consisting of five species with only one extant) was historically distributed mostly in relatively dry habitats in low-mid elevations. The current distribution of the extant species, *Haplostachys haplostachya*, which is federally listed in the United States as endangered, is restricted to small subpopulations in the xerophytic shrub lands between Mauna Loa and Mauna Kea on Hawai'i (Morden and Loeffler, 1999). *Haplostachys* bears fragrant white flowers with a prominent lower corolla lip and is the only species among the Hawaiian mints to bear dry nutlets.

The genus *Phyllostegia* consists of 32 species with two more described from Tahiti and Tonga (Lindqvist and Albert, 2002; Lindqvist et al., 2003). Among the Hawaiian *Phyllostegia* species, *P. variabilis*, which is presumed to be extinct, used to populate the strand and coastal sites in the western end of the Hawaiian chain on Kure and Midway atolls and Laysan Island. The remaining Hawaiian *Phyllostegia* species inhabit all the main islands except Kaho'olawe and Ni'hau with a primary occurrence in wet to mesic forest habitats. *Phyllostegia* also bears white fragrant flowers with expanded lower lips similar to those of *Haplostachys*.

The third genus of Hawaiian mints is *Stenogyne*, which consists of 22 species on all the extant main islands of Hawaii, exhibiting the greatest diversity on Maui and Hawai'i (Lindqvist and Albert, 2002; Lindqvist et al., 2003, 2006). Plants are mostly perennial vines inhabiting mesic wet forests in areas of lower elevation to subalpine woodlands at higher elevation. Flowers in *Stenogyne* are usually odorless with tubular, reduced-lipped corollas.

Studies by Lindqvist and colleagues (Lindqvist and Albert, 2002; Lindqvist et al., 2003) have shown that the Hawaiian mints are a

monophyletic group deeply nested within the dry-fruited *Stachys* and may have resulted from one or more hybridization events involving bird and insect pollinated Western North American parents.

### 1.3. The *Stachys* lineage

The family Lamiaceae has been traditionally considered as one of the most distinctive near-cosmopolitan angiosperm families with more than 7000 species in seven subfamilies, including Lamioidae, the second largest subfamily with over 1250 species in 63 genera (Harley et al., 2004; Scheen et al., 2010; Bendiksbj et al., 2011). Stachydeae, the largest tribe of Lamioidae with about 470 species, is a widespread and taxonomically complex lineage exhibiting remarkable chromosomal diversity (Mulligan and Munro, 1989), including the highly polyploid Hawaiian taxa. Stachydeae comprise of the three Hawaiian endemic genera and at least seven Old World genera (e.g., *Sideritis*, *Prasium*, *Chamaesphacos*, *Suzukia* and *Thuspeinanta*), all of which are nested within the polyphyletic *Stachys*, the largest member in the tribe, giving rise to a wide array of morphological, cytological and biogeographical diversity observed generally in the subfamily (Scheen et al., 2010). As such, Stachydeae provides an ideal system for studies on species diversification.

Phylogenetic analyses of chloroplast DNA (cpDNA) showed the *Stachys* lineage to be subdivided into two strongly supported clades (Scheen et al., 2010). The first clade has its center of diversity in the eastern part of the Mediterranean region migrating in course of time to Western Asia, Western Europe and Macaronesia, and sub-saharan Africa. The second clade, including the Hawaiian mints, *Suzukia*, all New World (NW) *Stachys* species and some Old World species (OW), has shown evidence for an Old World origin with migrations to the Americas and Hawaii (Lindqvist and Albert, 2002).

A high diversity of *Stachys* species is also found in South America (SA), particularly in the Andean region. The powerful effect of the Andean orogeny has led to rapid diversification and radiations in the Andean flora (Luebert et al., 2011), and the Andean mountain ranges with their high altitude flora are the seat of a variety of species (Marx et al., 2010; Tank and Olmstead, 2009; Turchetto-Zolet et al., 2013). The rapid radiation in this region is comparable in many instances to that observed in oceanic islands (Drummond et al., 2012; Hughes and Eastwood, 2006). The uplift of the Andean ranges began in the late Oligocene to early Miocene about 23 My ago (Mya), intensifying and reaching its peak of mountain building during the middle Miocene (about 12 Mya) and early Pliocene (4.5 Mya) (Turchetto-Zolet et al., 2013). Of the 94 New World species, approximately 30 *Stachys* species are found in South America, one-third of which are endemic to Chile. Most others are found in Ecuador, Colombia or Peru. It is possible that South American taxa have a similar reticulate, polyploid origin and mirror the remarkable adaptive radiation seen on the Hawaiian Islands, only at a smaller and continental scale. Comparative studies of the origin of the Hawaiian and SA lineages may therefore provide profound insights into polyploid hybridization and rapid diversification in both oceanic insular and continental settings.

### 1.4. New World mints and aims of our present study

New World lamioide mint taxa are found in two lineages only: the endemic tribe Synandreae (Scheen et al., 2008, 2010) and the *Stachys* lineage (Nelson, 1981; Turner, 1994a). There has been a relatively limited sampling of New World *Stachys* so far to resolve evolutionary relationships within this lineage, and among members of presumed rapid diversification within temperate North America, Mesoamerica (Mexico and Central America), South

America, and Hawaii. Hence, there is strong need for further studies with greater sampling (representing the entire range of biogeographical and morphological diversity), and incorporation of more loci, to shed further light on the relationships within this lineage. An important outcome will be the untangling of biogeographic relationships and migration events to the New World, as well as understanding species formation and diversification in Old vs. New World and Hawaiian Islands contexts.

The nuclear ribosomal DNA (rDNA) external transcribed spacer (ETS) has been frequently used for phylogenetic analysis in the Asterids. Baldwin and Markos (1998) showed that ETS sequences evolve about 1.3 to 2.4 times faster than rDNA ITS (internal transcribed spacer) sequences and can be very useful in determining phylogenetic relationships to show deep divergences both at higher and lower population levels. Another nuclear region that has been previously used to study Stachydeae species (Lindqvist and Albert, 2002) is the 5S non-transcribed spacer (5S-NTS) region of the ribosomal RNA (5SrRNA). Multiple copies of the 5S rRNA gene alternate with non-transcribed spacer regions in tandem arrays, which are physically separate from the ITS and ETS locations (Long and Dawid, 1980), and they may be found in two or more chromosomal regions (Scoles et al., 1988; Schneeberger et al., 1989; Sastri et al., 1992). Chloroplast DNA is thought to be maternally inherited in Lamiaceae (Corriveau and Coleman, 1988). The noncoding chloroplast regions are presumably not under selective pressure and are assumed to evolve at rates higher than those of genic regions (Kelchner, 2000), and thus are used widely in phylogenetic analyses in plants. Comparisons of results from plastid and nuclear analyses have previously been proved to be of immense use in inferring biogeographical histories of various taxa (Marin et al., 2007; Wang et al., 2007; Nauheimer et al., 2012). Hence, our study employed the use of ETS and 5S-NTS, along with three chloroplast non-coding loci, the trnL intron, the trnL-F intergenic spacer region, and the rps16 intron (Shaw et al., 2007). We utilized the phylogenetic frameworks incorporating chloroplast DNA (cpDNA) and nuclear rDNA loci along with fossil calibrations and ancestral area reconstructions to investigate the relationships between New World and Hawaiian mints, including their hypothetical ancestral areas and their migratory routes and divergence times. Since the number of loci and chromosomal arrangements among the rDNA loci can vary greatly in plants (Rogers and Bendich, 1987) and rDNA loci generally are thought to undergo concerted evolution, we also employed fluorescent in situ hybridization to possibly give insight into rDNA evolution in Stachydeae.

Our present study is aimed at broadly characterizing the relationships between the Hawaiian mints and their North and South American relatives through a considerably broader sampling and greater number of loci than previously accomplished, as well as employing advanced analytical tools. Our study seeks to address the following main objectives: (1) Reconstructing phylogenetic relationships between the Hawaiian and North American mints, with emphasis on the role of hybridization and polyploidy in the history of Hawaiian mints; (2) Investigating the position of the South American taxa with respect to the Southwestern US/Mesoamerican *Stachys* species; and (3) Date and interpret the diversification of the New World mints including radiation to the Hawaiian Islands.

## 2. Materials and methods

### 2.1. Taxon sampling, DNA extraction, amplification, and sequencing

All taxon names in this present study follow the “World checklist of Lamiaceae and Verbenaceae” (Govaerts et al., 2013). Sequence data were collected for a total of 166 accessions, including 119

species for 5S-NTS, 99 species for ETS and 76 species for cpDNA, from specimens held at the following herbaria: A, AAU, BISH, C, LL, M, NY, RM, TEX, US, UPS, UNA and UTC (abbreviations following Holmgren et al., 1990) (Appendix A). In a few cases, fresh material further dried in silica gel was obtained from Hawaiian mints collected during fieldwork in Hawaii (with permissions from the Department of Land and Natural Resources, State of Hawaii, P.O. Box 4849, Hilo, Hawaii 96720 USA) and of cultivated *Stachys*. The 5S-NTS dataset included a total of 160 accessions with extensive sampling of species from the Old World (OW) (22 species), New World (NW) (46 species), and the Hawaiian Islands (51 species) (Appendix A). The ETS dataset included 106 accessions representing 20 OW species, 41 NW species, and 38 Hawaiian species, and the combined cpDNA dataset included 78 accessions representing 30 OW, 26 NW, and 20 Hawaiian species. Twelve South American species were included in both ETS and 5S-NTS datasets. Sampling of some taxa for the three different datasets was different due to limitations in the availability of material. However, based on previous studies (Lindqvist and Albert, 2002) and the observance of limited variation among ETS and in particular cpDNA sequences, we expect that placement of the missing taxa will be congruent among the different loci. The monotypic genus *Melittis melissophyllum* L., with a distribution ranging from Europe to western Asia, has previously been shown by Scheen et al. (2010) to emerge as sister to the remaining Stachydeae with strong support; hence *M. melissophyllum* was chosen as outgroup for all analyses. DNA was extracted from silica dried leaves or from herbarium specimen leaf fragments using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Several DNA sequences from the 5S-NTS region were used from previous studies (Lindqvist and Albert, 2002; Lindqvist et al., 2003), and a few cpDNA sequences of Old World taxa were obtained from a previous study done by Scheen et al. (2010). The trnL intron and trnL-trnF intergenic spacer (referred from here onwards as trnL-F region) were amplified using the universal primers of Taberlet et al. (1991), either as one fragment using primers c and f or as two separate fragments using primers c and d, and e and f respectively (Scheen et al., 2010). The rps16 intron was amplified using the primers rpsF and rpsR2R (Oxelman et al., 1997). In certain occasions when low quality DNA was used as template the internal primers rpsLF and rpsLR were used in addition to the two mentioned above (Scheen et al., 2010). Amplification of the 5S-NTS region was performed with the 5S\_PI and 5S\_PII universal primers (Cox et al., 1992). Initial amplification of members of Stachydeae was done with the primers 18S-ETS (Baldwin and Markos, 1998) and ETS-B (Beardsley et al., 2003). Based on the sequences derived from this initial amplification, Stachydeae specific primers were developed and the ETS region was amplified using the more specific primers ETS2F (TGACTACTGGCAGGATCAACC) and ETS2R (TGACTACTGGCAGGATCAACC) designed for this study. All loci were amplified using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). For the amplification of the rDNA a touchdown method was used with the following thermocycling profile: hold for 10 min at 95 °C; 10 cycles of 1 min at 95 °C, 1 min at 60 °C and decreasing the temperature by 1 °C every cycle, 1 min at 72 °C; followed by 35 cycles of 1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C; extension for 10 min at 72 °C. PCR products were purified by the QIAquick PCR purification kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. For the amplification of the cpDNA, the following thermocycling program was used: hold for 2 min at 94 °C; 35 cycles of 50 s at 94 °C, 50 s at 50 °C, 1 min at 72 °C; extension for 10 min at 72 °C. All PCR reactions were performed in 25 µl volumes with the AmpliTaq Gold DNA Polymerase (rDNA) and AmpliTaq DNA Polymerase (cpDNA) buffer II kits (Applied Biosystems, Foster City, CA, USA) using 8.5 µl of de-ionized water, 2.5 µl each of buffer, MgCl<sub>2</sub>, Bovine Serum Albumin (BSA), Tetramethylammonium Chloride (TMACl) and Dimethyl sulfoxide



(DMSO), 0.5  $\mu$ l each of the primers (1  $\mu$ M), 0.2  $\mu$ l AmpliTaq Gold and 2  $\mu$ l, unquantified genomic DNA.

All ETS and 5S-NTS PCR products generated were further cloned using the Qiagen PCR cloning kit (Qiagen, Hilden, Germany) following the manufacturer's instructions, except for using 25  $\mu$ l competent cells to transform each ligation reaction. Transformed clones were incubated overnight at 37 °C. Positive clones (eight per plate) were picked and PCR reactions were prepared in 25  $\mu$ l volumes with the AmpliTaq DNA Polymerase buffer II kit (Applied Biosystems, Foster City, CA, USA) using 2.5  $\mu$ l buffer, 2.5  $\mu$ l MgCl<sub>2</sub>, 1.0  $\mu$ l dNTP, 0.6 each of M13F and M13R primers and 0.2  $\mu$ l of AmpliTaq polymerase. All PCR products were examined by gel electrophoresis on 1% agarose gels and positive PCR amplified products were sequenced in one direction using SP6 or T7 primers at the University of Washington High Throughput Genomics Center at Seattle, USA.

## 2.2. Phylogenetic analysis including network analysis

All sequences generated were edited and assembled in the program Sequencher 4.7 (Gene Codes, Ann Arbor, Michigan, USA), aligned with the program ClustalX v.2 (Larkin et al., 2007), and alignments were manually adjusted in the program BioEdit (Hall, 1999). Gaps were treated as missing and indels were not coded. The cpDNA datasets were analyzed separately from the rDNA datasets as described below. The two separate trnL-F fragments generated were concatenated with the program DAMBE (Xia and Xie, 2001). Since the trnL-F region and the rps16 intron give rise to compatible topologies (not shown), the datasets generated from these two regions were concatenated with the program WINCLADA (Nixon, 1999) before running the phylogenetic analyses. In the ETS rDNA dataset all clones from individual accessions grouped together (not shown), showing close relationships with each other, and were converted to consensus sequences by introducing ambiguities at polymorphic sites following the IUPAC ambiguity codes for nucleotides in the program BioEdit (Hall, 1999). The 5S-NTS dataset included sequences from Lindqvist and Albert's (2002) previous study, in which amplification products had not been cloned, and in some cases contained considerable ambiguities representing polymorphic loci. Hence a different approach was adopted for the 5S-NTS dataset. Since new 5S-NTS cloned sequences from individual accessions also formed their own clades (not shown), a single representative clone was selected for each group of clones, and the final 5S-NTS dataset used for all the phylogenetic analyses consists of these single representative clones. We also constructed an ETS dataset with single representative clones in a similar manner as the 5S-NTS in order to test for possible bias or homoplasy introduced with the consensus sequences. The same major clades were retrieved from this analysis, and there were no supported differences (not shown). An additional test with a 5S-NTS dataset constructed from consensus sequences resulted in a polytomy among all the major clades in the Bayesian 50% majority rule consensus tree (not shown) likely caused by introduced homoplasy due to the high level of intra-individual polymorphism. Hence, we went ahead with the 5S-NTS dataset derived from single representative clones and the ETS dataset derived from the consensus sequences for all our future analyses. ETS and 5S-NTS topologies were incongruent as observed from a one-tailed Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) implemented in PAUP\* v.4.0b10 (Swofford, 2002) ( $P = 0.018$ ; refer to Fig. 2 and Supplementary Figs. 1 and 2 for the tree topologies). Hence, the ETS and 5S-NTS data were not combined.

Phylogenetic relationships were examined for the cpDNA, ETS and 5S-NTS datasets separately using Bayesian inference conducted in the program MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2001). We used substitution models that best fit the data as determined by the Bayesian Information Criterion (BIC) using the

program jModeltest v.1.1 (Posada, 2008). The TPM2+G model was favored for the 5S-NTS dataset, TPM2uf+G for the ETS dataset, and TVM+G for the combined cpDNA dataset. However, since these models are not implemented in MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2001) we used the models with the next highest BIC score, which was GTR+G for all our three datasets. The Bayesian analyses were run with two Markov Chain Monte Carlo (MCMC) runs for ten million generations each. Trees were sampled every 500 generations, the program Tracer v.1.5 (Drummond and Rambaut, 2007) was used to check for stationarity, and the burn-in value for obtaining a 50% majority rule consensus tree was set to ignore the first 25% of trees, to include trees only after stationarity was reached. Clade support was determined by Bayesian posterior probabilities (PP; Rannala and Yang, 1996). The 50% majority rule consensus trees were viewed with the program FigTree v.1.3.1 (Rambaut, 2008). Maximum Likelihood (ML) and Parsimony analyses were also conducted with RaxML Blackbox (Stamatakis et al., 2008) and TNT (Goloboff et al., 2008), respectively. In order to investigate reticulation within the mints, a median joining network was created with the program Splitstree v.4 (Huson, 1998); an integrated program for estimating phylogenetic trees and networks. For this analysis, a 5S-NTS data matrix consisting of 114 accessions was pruned as follows: Old World species distantly related to members of the NW clade were highly divergent and therefore removed, and since the relationships between the three Hawaiian genera were poorly resolved and produced a large polytomy, the number of Hawaiian mint accessions was reduced keeping fewer representatives from each of the two genera *Phyllostegia* and *Stenogyne*.

## 2.3. Divergence time estimation

Estimation of divergence times was obtained using the program BEAST v.1.7.2 (Drummond and Rambaut, 2007). This method simultaneously estimates phylogeny and molecular rates using a Markov Chain Monte Carlo strategy. The analyses were run on the pruned 5S-NTS dataset used in the network reconstruction (refer to Section 2.2) in addition to the ETS and concatenated cpDNA datasets. To estimate divergence times, a Yule process speciation prior and an uncorrelated lognormal (UCLN) model of rate change with a relaxed clock (Drummond et al., 2006) was used and the analyses were run for 30 million generations with parameters sampled every 1000 generations. Trace files were loaded into Tracer v.1.5 (Drummond and Rambaut, 2007) to look for an Effective Sampling Size (ESS) greater than 200 for all parameters sampled from the MCMC, and to examine the posterior distributions of all parameters and their associated statistics including 95% highest posterior density (HPD) intervals. Initially to optimize efficiency in BEAST, we undertook several trial runs of 10–20 million generations and analyzed the results using Tracer v.1.5 (Drummond and Rambaut, 2007). These results were then used to determine the number of generations necessary to achieve the desired effective sampling size (ESS) of at least 200 and to optimize the operator settings for our abovementioned final analysis. The program TreeAnnotator v. 1.5.4 (Drummond and Rambaut, 2007) was used to summarize the set of post burn-in trees and their parameters (burn-in set to 3000), to produce a maximum clade credibility (MCC) chronogram showing mean divergence time estimates with 95% HPD intervals. FigTree v.1.3.1 (Rambaut, 2008) was used for visualization of the resulting divergence timings. Lamiales have been elusive in terms of reliable fossil records, and there is scant existence of some old and reliable fossils (Call and Dilcher, 1992; Mai, 2001). Possibly the oldest reliable lamiooid fossils have been sampled from the Seravallian Age of the Middle Miocene flora of Germany and belong to *Stachys laticarpa* (seed/fruit) and *Lamium* sp. (13.8–11.6 Million years ago; Mya) (Mai, 2001). We used the *Stachys laticarpa* fossil as a calibration point

(13.8 Mya) to constrain the crown group of the *Stachys* s.l. clade (Stachydeae excluding *Melittis*). To reflect the uncertainty related to the fossil data, we set lognormally distributed priors for our calibration with the following value for the offset  $O = 13.8$ , with Standard deviation = 0.8 and Mean = 0.5.

#### 2.4. Ancestral area reconstruction

We used the recently developed S-DIVA (Statistical Dispersal-Vicariance Analysis; Yu et al., 2010) as implemented in the program RASP. The program implements a Bayesian approach to dispersal-vicariance analysis (DIVA; Ronquist, 1997), following the method suggested by Nylander et al. (2008), which estimates optimized areas over a set of trees and accounts for uncertainty in the phylogenetic estimate. The distribution range of the Old World, North American, South American, and Hawaiian mints was divided into seven areas based on their present day geographic distributions and the presence of one or more endemic species based on the World Checklist of Lamiaceae and Verbenaceae (Govaerts et al., 2013). These areas are A (Old World), B (Eastern North America), C (Western North America), D (Central US), E (Southern US, Mexico, Central America), F (South America), and G (Hawaii) (Fig. 4). We used the pruned 5S-NTS, ETS and combined cpDNA datasets including only one accession per species for the ancestral area reconstructions to avoid possible overestimation of areas represented by several accessions. For each dataset, the S-DIVA analysis was performed using the tree file generated after the burn-in period from the MrBayes run. This file was converted into a condensed tree file in RASP. We ran S-DIVA with all the default settings except maximum number of areas being set to 4. This was repeated with maximum number of areas being maintained to the maximum that can be possible at each node. The analysis was repeated with the same settings to test for reproducibility. We did not select “allow reconstruction” letting the program calculate the proportions of inferred alternative most-parsimonious ancestral ranges at each node in a tree accounting for topological as well as dispersal-vicariance uncertainties.

#### 2.5. Fluorescence in situ hybridization (FISH) and nucleotide diversity

Fluorescence in situ hybridization (FISH) was performed using 25S (ETS) and 5S rDNA as probes. Since actively growing root tips were needed, fresh material from only five species, *Stachys coccinea*, *S. affinis*, *S. chamissonis*, *S. bullata* and *Phyllostegia vestita*, was available for this study. The chromosome preparation was performed as described by Lan et al. (2006), except that the root tips were treated with enzyme mixture (2% R-10 cellulase and 1% pectolase) at 37 °C for 20 min, and the slides were checked by phase contrast microscope without Giemsa staining. Probe labeling and FISH were carried out as described by Lan and Albert (2011).

Number of polymorphic loci (*S*), haplotype diversity (*H<sub>d</sub>*) and nucleotide diversity ( $\mu$ ) was determined for the complete datasets for ETS and 5S-NTS with the program DnaSP v.5 (Librado and Rozas, 2009). For this purpose, only accessions, which were common to both loci and were cloned, were used. For the 5S-NTS dataset, all the accessions taken from Lindqvist and Albert's previous study (2002) had been directly sequenced and ambiguities in these sequences were coded following the IUPAC ambiguity codes for nucleotides and hence these sequences were excluded here.

### 3. Results

#### 3.1. Phylogenetic analysis and median joining network

The aligned, combined cpDNA matrix was composed of 1770 nucleotides. The complete aligned ETS consensus dataset was com-

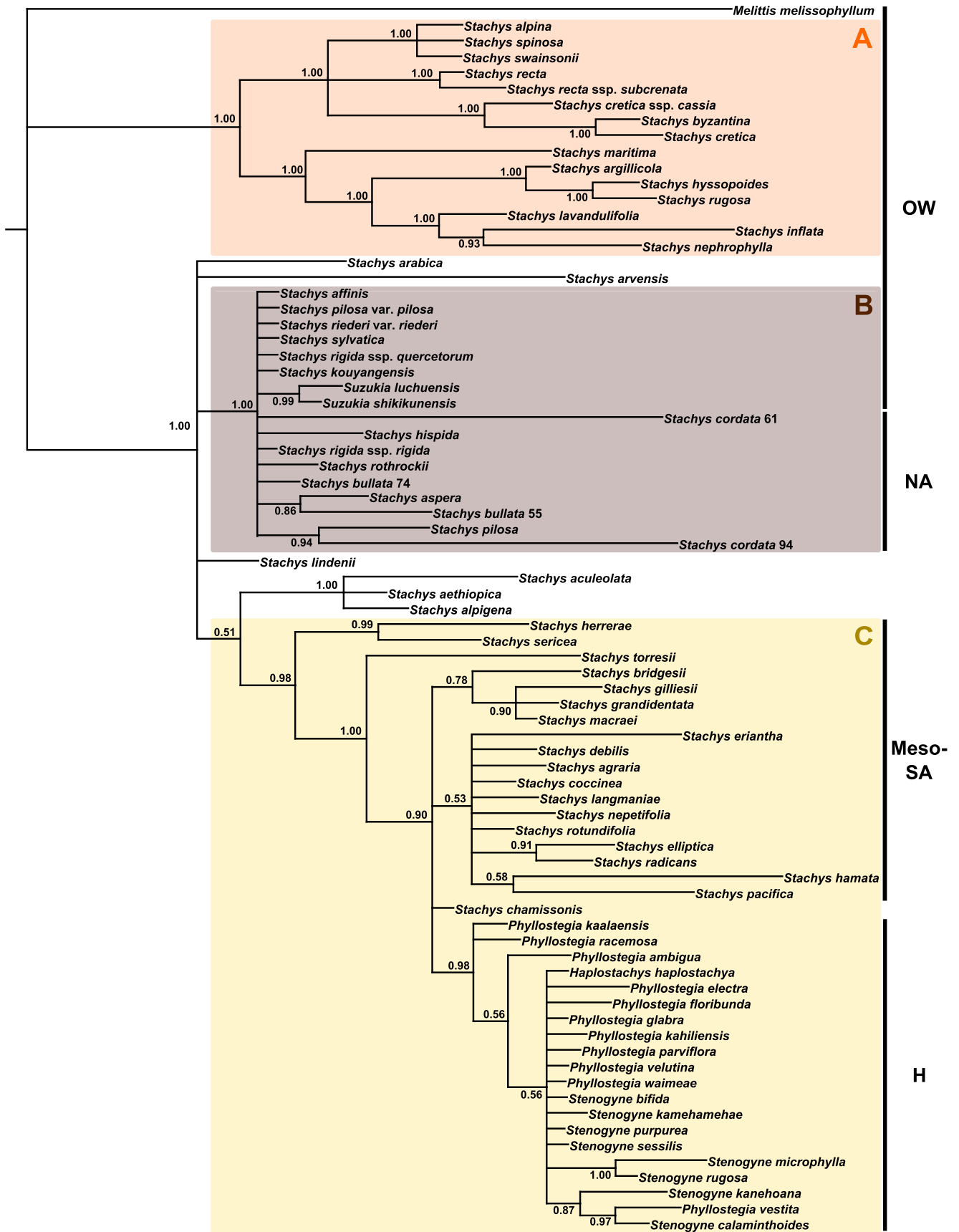
posed of 476 bp, and the complete 5S-NTS dataset was composed of 470 characters. The first approximately 110 bases in the 5S-NTS matrix were highly variable; particularly with respect to the Old World *Stachys* taxa (e.g., *S. lavandulifolia*, *S. cretica*). The overall topologies produced by the majority rule consensus tree from the Bayesian MCMC analysis for cpDNA and rDNA are similar to those reported in previous studies (Lindqvist and Albert, 2002), with three major clades observed from the Old World and the New World taxa. Consequently, the combined cpDNA tree (Fig. 1) is incongruent with the ETS and 5S-NTS rDNA trees (Fig. 2 and Supplementary Figs. S1 and S2). This conflict involves the relationships between the Hawaiian taxa and the Mesoamerican/South American and temperate North American taxa, respectively.

Maximum likelihood and parsimony analyses resolved the same major clades as from the Bayesian analysis (not shown). However, for reasons of clarity and since there were no supported incongruences between the Bayesian, MP and ML consensus trees, only topological details and support values from the Bayesian analyses will be discussed from here onwards.

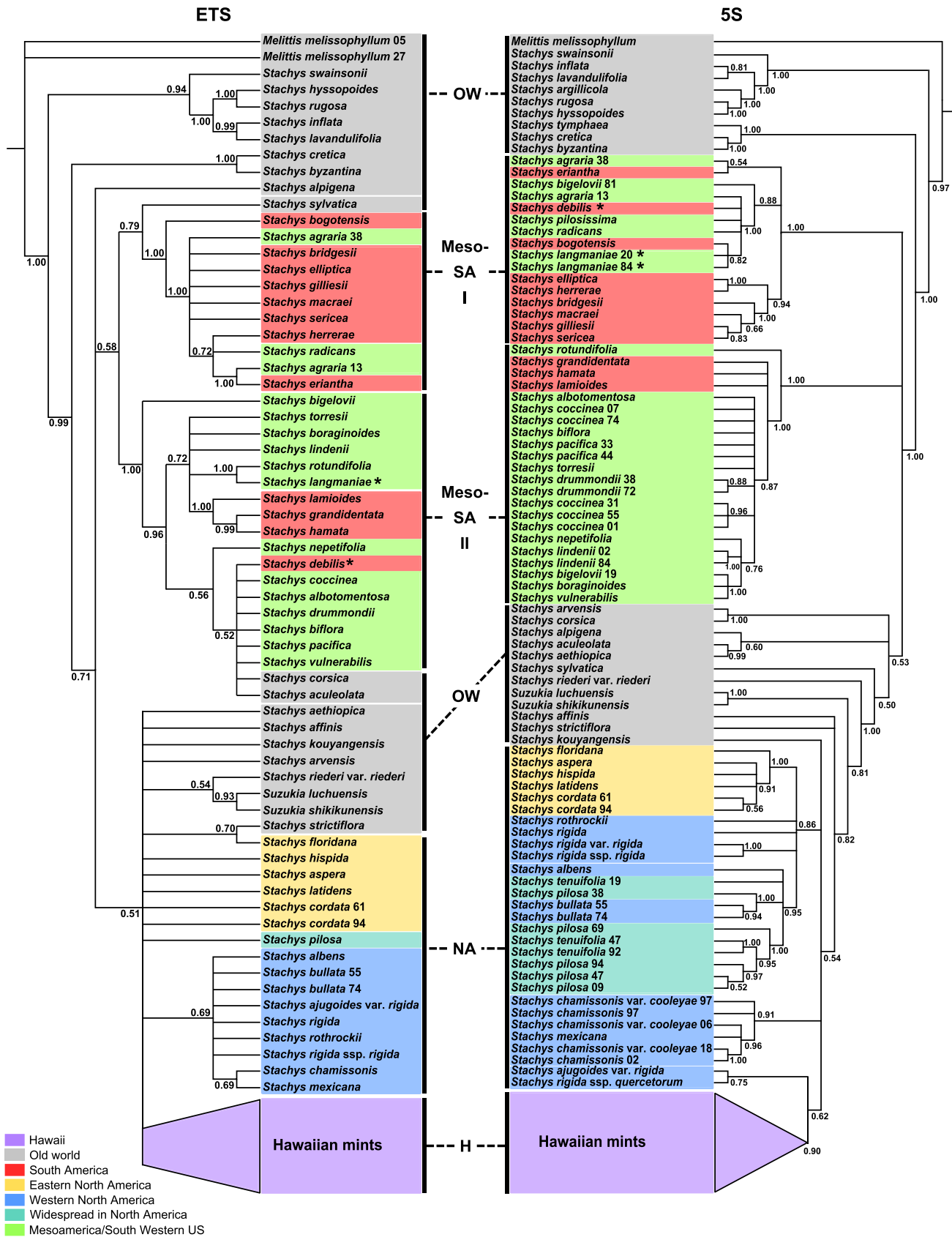
The combined cpDNA majority rule consensus tree from the Bayesian MCMC analysis (Fig. 1) showed strong support values for three broad clades (putative haplogroups A, B and C): one containing only Old World (OW; predominantly Mediterranean) taxa (haplogroup A) (PP = 1.00), another including temperate North American (NA) species, as well as some Old World taxa, including *Suzukia* (haplogroup B) (PP = 1.00), and the third consisting of the well supported Hawaiian mint lineage (H) and the Meso-South American taxa (Meso-SA), including the Californian-Pacific northwestern species *S. chamissonis* (PP = 0.98; haplogroup C; Fig. 1). Within haplogroup B, relationships among the taxa remain largely unresolved. Within haplogroup C we observe some degree of resolution among taxa, e.g., *Stachys herrerae* and *S. sericea* are sister to the rest (PP = 0.99), a clade consisting of exclusively South American *Stachys* (*S. gilliesii*, *S. grandidentata*, and *S. macraei*) is observed (PP = 0.90), *Stachys elliptica* and *S. radicans* resolve as sister to each other (PP = 0.91), and the Hawaiian lineage forms a clade (PP = 0.98). However, we do not find further resolution distinguishing the three Hawaiian genera *Phyllostegia*, *Stenogyne* and *Haplostachys* (Fig. 1).

The overall topology between the ETS and the 5S-NTS majority rule consensus trees derived from the Bayesian MCMC analysis was similar, but in some areas we observe instances of incongruence in the placement of taxa, as well as differences in clade support and resolution (Fig. 2 and Supplementary Figs. S1 and S2). Among the Old World (OW) *Stachys* species, both trees show close relationship between the Mediterranean *S. cretica* and *S. byzantina*, and the two South African species, *S. rugosa* and *S. hyssopoides*, respectively.

The Mesoamerican–South American (Meso-SA) species (accessions ranging from Southwestern United States to Mexico/Central America, and South America) form two broad clades (Meso-SA-I and Meso-SA-II respectively; Fig. 2 and S1 and S2) with robust probability (PP = 1.00 in both clades for ETS and 5S-NTS, respectively). Twelve South American species are present in both clades for ETS and 5S-NTS. In the ETS tree, *Stachys lamiooides*, *S. grandidentata* and *S. hamata* resolve as monophyletic (PP = 1.00), although these species remain unresolved in the 5S-NTS tree (Fig. 2; Meso-SA-II). In the 5S-NTS tree, however, *S. elliptica*, *S. herrerae*, *S. bridgesii*, *S. macraei*, *S. gilliesii*, and *S. sericea* resolve as monophyletic (PP = 0.94), but remain unresolved in the ETS tree (Meso-SA-I; Fig. 2). The rest of the South American taxa are intermixed with the Mesoamerican species. Incongruence is observed in the placement of two Meso-South American *Stachys* species, *S. debilis*, and *S. langmaniae*, between the ETS and 5S-NTS trees (Fig. 2). In the 5S-NTS tree *S. debilis* and *S. langmaniae* group in Meso-SA-I, whereas in the ETS tree these two species group in the Meso-SA-II clade.



**Fig. 1.** Phylogeny based on the 95% majority rule consensus tree obtained from Bayesian phylogenetic analysis of the concatenated cpDNA dataset. Numbers at nodes indicate the posterior probability values (PP). A, B, and C refer to the three major clades (inferred putative haplogroups). OW = Old World; NA = temperate North America; Meso-SA = Meso-South America; H = Hawaii. Different accessions of the same species have been designated by the last two numbers of their collecting number or the year of collection (for those without a number) at the end of taxa names (refer to Appendix A).



**Fig. 2.** Comparative phylogenies of ETS and 5S-NTS datasets based on the 95% majority rule consensus trees obtained from Bayesian phylogenetic analyses. Numbers at nodes indicate the posterior probabilities (PP). OW = Old World; NA = temperate North America; Meso-SA = Meso-South America; H = Hawaii. Different accessions of the same species have been designated by the last two numbers of their collecting number or the year of collection (for those without a number) at the end of taxa names (refer to Appendix A). Color coded boxes follow the geographic distribution of taxa, and a key has been provided with the figure. An asterisk (\*) indicate taxa with incongruent positions between the ETS and 5S-NTS trees.



Members of the *Stachys coccinea* complex described by Turner (1994b), *S. coccinea*, *S. torresii*, *S. pacifica*, *S. lindenii* and *S. albomentosa* group within the Meso-SA-II clade in both ETS and 5S-NTS trees, although they are not resolved as monophyletic (Fig. 2).

Within the 5S-NTS tree, the tropical African species *Stachys aculeolata* is strongly supported as sister to another tropical African species, *S. aethiopica*, and both these species group with African *S. alpigena*, although this is not supported. Within the ETS tree, *S. aculeolata* groups within the strongly supported Meso-SA clade II. *Stachys corsica* seems to also have an incongruent position in the rDNA trees, being placed within Meso-SA clade II in the ETS phylogeny, whereas it is strongly supported as sister to *S. arvensis* in the 5S-NTS phylogeny.

Overall, the ETS phylogeny is poorly resolved with regards to the temperate NA *Stachys* species and their OW and Hawaiian relatives. In the 5S-NTS tree (Figs. 2 and S2), OW *Stachys* species form a paraphyletic grade to the temperate NA and Hawaiian taxa, albeit with poor support, although the Northeastern Asian species *Stachys riederi* var. *riederi* is strongly supported as sister to a clade consisting of five other Central-Eastern Asian *Stachys* (two *Suzukia* species, *Stachys affinis*, *S. strictiflora*, and *S. kouyangensis*), the temperate NA and Hawaiian taxa. Additionally, some clades within this larger lineage are strongly supported (PP = 0.90–1.00), e.g. a group of Central-Eastern NA *Stachys* species (*S. floridana*, *S. aspera*, *S. hispida*, *S. latidens*, and *S. cordata*), a group of predominantly widespread US species (*S. pilosa* and *S. tenuifolia*), the Western US species *S. chamissonis* and *S. mexicana*, and the Hawaiian taxa (Fig. 2). The two *Haplostachys* species form a monophyletic group that is sister to the remaining two Hawaiian genera, *Phyllostegia* and *Stenogyne*, that together form a strongly supported clade (PP = 1.00; Fig. S2).

Although the median joining network for rDNA 5S-NTS dataset (Fig. 3) showed some patterns of reticulation between the Hawaiian taxa and temperate North American *Stachys*, most ingroup clades (i.e., excluding the “OW” clade) from the Bayesian MCMC analysis (Fig. 2) are resolved in the median joining network. For example, the Meso-South American species form two separate clusters with some internal reticulation.

### 3.2. Divergence dates

Our divergence analyses for the three loci gave similar results for some of the major nodes (Fig. 4; Table 1). However, due to the incongruence between cpDNA and rDNA, we have reported only the values for cpDNA nodes, which are also found in the rDNA results. In the following we report the median node age ranges (nodes refer to Fig. 4; values at the nodes are arranged from past to present to match the time-scale) along with the range of the 95% HPD values for the three loci from our BEAST analyses of the individual datasets. The individual median and 95% HPD values for each analysis are listed in Table 1. Our results show a split between the Old World and the predominantly New World clades occurring approximately 11.1–11.9 Mya (95% HPD range 8.3–14.1 Mya; node I) during the Middle Miocene period. Our analysis estimated the initial diversification of the *Stachys* crown group in the New World to begin as early as 8.7–10.6 Mya (95% HPD range 6.2–13.2 Mya; node II) during the Late Miocene period and continuing with further diversification to Mesoamerica/South America between 7.7–9.4 Mya (95% HPD range 5.3–12.0 Mya; node III), and to temperate North America between 7.5–8.5 Mya (95% HPD range 5.4–11.5 Mya; node IV) during the Late Miocene period, although we note that these latter two nodes are not supported in our rDNA Bayesian analyses (Fig. 2). The first well-supported split of the temperate North American mints from their close Asian relatives (e.g., *Stachys riederi* var. *riederi*) appear to have taken place around 4.9–5.7 Mya (95% HPD range 3.4–7.8

Mya; node V) during the late Miocene-early Pliocene period. We particularly mention this node, as this is also well supported in our Bayesian tree for 5S-NTS (Fig. 2). Within the stem group of the temperate North American *Stachys* (including the Hawaiian lineage), further diversification and initial colonization into temperate North America took place around 4.1–5.3 Mya, during the Pliocene period (95% HPD range 2.8–7.6 Mya; node VI), and shortly thereafter possible close relatives of the Hawaiian mints existing in South Western US split from the Hawaiian mints between 3.6–4.7 Mya (95% HPD range 2.3–6.1 Mya; node IX) during the Pliocene period, although again we note that this is poorly supported in the 5S-NTS Bayesian analysis (Fig. 2). Our analysis suggests that colonization and diversification of Hawaii started about 3.4–4.4 Mya (95% HPD range 2.0–6.5 Mya; node X) during the late Pliocene period, when members of *Haplostachys* split from *Phyllostegia* and *Stenogyne*. The two Meso-SA clades diversified around 4.1–4.6 Mya (95% HPD range 2.2–7.3 Mya; node VII) and 3.9–6.0 Mya (95% HPD range 2.2–8.9; node VIII) during the late Miocene-Pliocene period. We also see the diversification of a clade consisting of exclusively SA taxa around 1.6–2.0 Mya (95% HPD range 0.5–3.4 Mya; node XI) during the late Pliocene period for both rDNA loci, although it was not retrieved in the cpDNA analysis.

### 3.3. Historical biogeography reconstruction

The optimal reconstruction of ancestral distribution patterns as inferred from the S-DIVA analyses has been mapped on the BEAST tree (nodes refer to Fig. 4). The S-DIVA results indicate a complex biogeographical history, in which vicariance and dispersal played an important role in shaping the distribution patterns we observe currently in the Old World, New World and Hawaiian mints. S-DIVA postulated 17 dispersals and 14 vicariance events for the 5S-NTS analysis, 18 dispersals and 17 vicariance events for the ETS analysis, and 19 dispersals and 14 vicariance events for the cpDNA analysis for optimal reconstruction. However, vicariance between non-adjacent areas and improbable dispersal scenarios were excluded and will not be considered for further discussion. According to our analysis, the most favored ancestral range for the *Stachys* crown group (node I) was Old World (A) (5S-NTS = 84%; ETS = 100%; cpDNA = 100% probabilities, respectively). S-DIVA suggested Old World to be the most favored ancestral range for the temperate North American/Hawaiian lineage, from our analyses with all the datasets (node IV and V). The ancestral ranges of the Hawaiian mints (CG and BG for 5S-NTS and ETS respectively; node IX), although not recovered with certainty, suggest a possibility of the ancestors of the Hawaiian lineage to be located either in eastern or western North America. However, S-DIVA proposed the ancestral ranges to be EG/FG/EFG (marginal probability of each area being 33%) for the Hawaiian mints from the cpDNA analyses (not shown) showing the probability of the ancestral matriline originating in Meso-SA. The ancestral range for the exclusively South American clade was suggested to be EF/F (marginal probability of each area being 50%) or E (100%) for the 5S-NTS and ETS, respectively (node XI). We did not observe this clade in our cpDNA analysis.

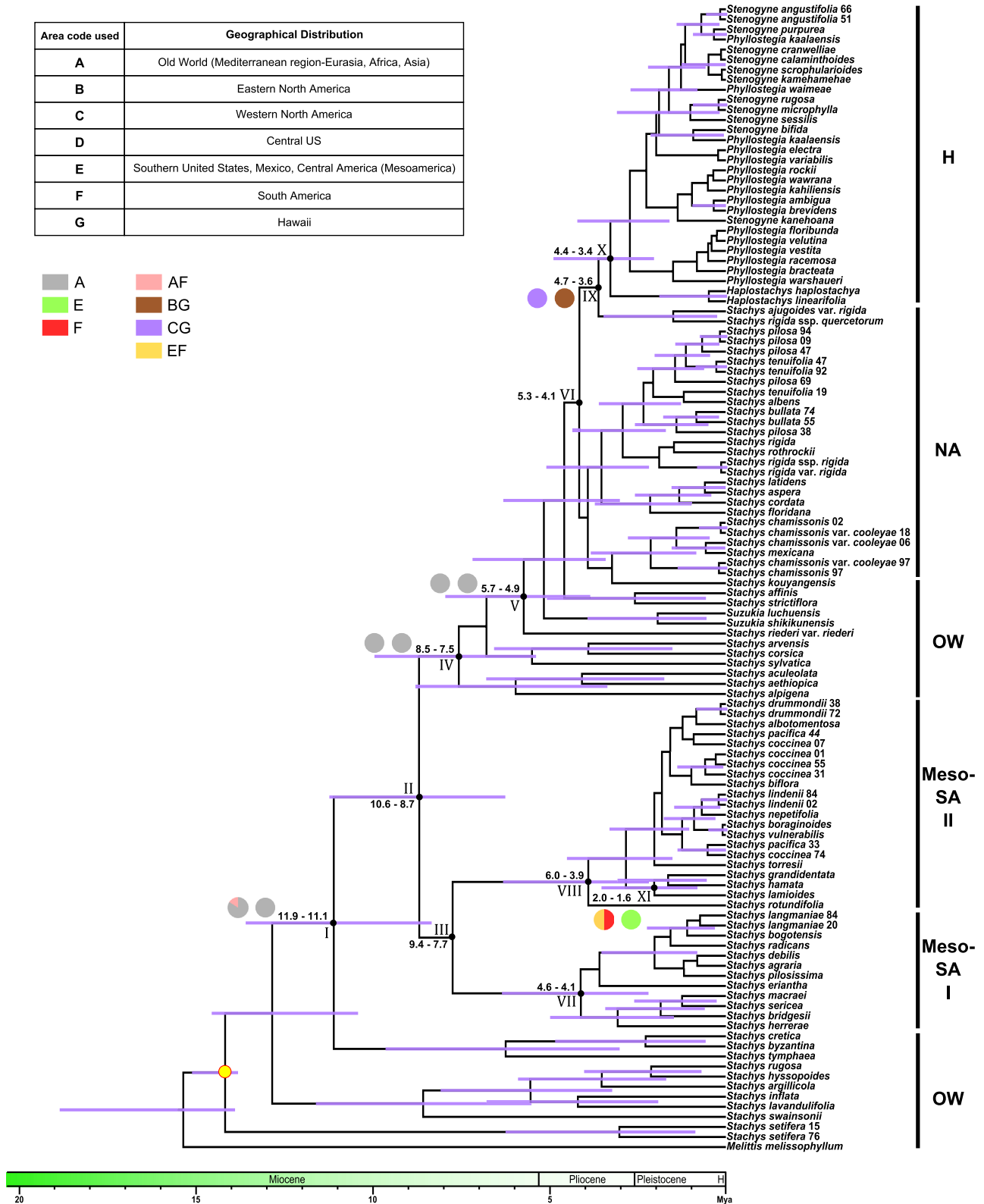
### 3.4. Chromosomal evolution (FISH) and population genetic analysis

The results from the FISH analysis are shown in Table 2 and Fig. S3. The number of 25S (ETS) signals in the five species vary from 4 to 10, while the number of 5S-NTS signals was consistently 4. One pair of chromosomes bears both 25S (ETS) and 5S loci in each of *S. chamissonis*, *S. bullata* and *P. vestita*.

In the ETS dataset, the average number of polymorphic loci was 6.57, average haplotype diversity (Hd) was 0.75 and the average







**Fig. 4.** Maximum credibility chronogram obtained from BEAST (Drummond and Rambaut, 2007) analyses of 5S-NTS, ETS and cpDNA loci, showing the hypothesized biogeographic history of New World Stachydeae. The ranges for the mean divergence time estimates (mean node ages) from the ETS and 5S-NTS loci are shown at the relevant nodes of the cladogram. The purple bars at the nodes represent the 95% HPD (highest posterior density) distributions (refer to Table 1 for the actual node ages and 95% HPD values). The yellow node represents the point of calibration. OW = Old World; NA = temperate North America; Meso-SA = Meso-South America; H = Hawaii. Geological timescale used from Walker and Geissman, 2009 (H = Holocene, Mya = million years ago). Ancestral area reconstructions from S-DIVA (Yu et al., 2010) have been mapped on this chronogram for the relevant nodes. Only the distributions for 5S-NTS and ETS have been shown, and the probabilities of the ancestral areas have been shown in the form of pie charts (5S-NTS left and ETS right). The geographical distribution codes and a color coded legend have been provided for the pie distributions. Different accessions of the same species have been designated by the last two numbers of their collecting number or the year of collection (for those without a number) at the end of taxa names (refer to Appendix A).

**Table 1**

Estimated node ages for selected divergence events under a relaxed clock model for 5S-NTS, ETS and combined cpDNA datasets for the BEAST analyses (Drummond and Rambaut, 2007). Ages are in million years with the 95% HPD (Highest posterior density) within brackets.

Node of interest (Fig. 4)	Age Loci		
	5S-NTS	ETS	cpDNA
I	11.1 (8.3–13.5)	11.9 (9.1–14.1)	***
II	8.7 (6.2–11.1)	10.6 (7.8–13.2)	9.8 (6.8–12.6)
III	7.7 (5.3–10.3)	9.4 (6.6–12.0)	7.4 (5.0–10.1)
IV	7.5 (5.4–9.8)	8.5 (5.6–11.5)	7.0 (4.7–10.8)
V	5.7 (3.8–7.8)	4.9 (3.4–6.7)	***
VI	4.1 (2.8–5.7)	5.3 (3.4–7.6)	***
VII	4.1 (2.2–6.2)	4.6 (2.4–7.3)	***
VIII	3.9 (2.2–6.2)	6.0 (3.5–8.9)	***
IX	3.6 (2.3–5.0)	4.7 (2.9–6.1)	***
X	3.4 (2.0–4.8)	4.4 (2.6–6.5)	3.9 (2.2–5.6)
XI	2.0 (0.8–3.4)	1.6 (0.5–3.1)	***

\*\*\* Represent nodes which were either not retrieved or were incongruent in the cpDNA analysis.

**Table 2**

FISH results for ETS (25S) and 5S-NTS showing number of signals and chromosome numbers (2n).

Taxon name	ETS (25S)	5S-NTS	2n
<i>Stachys coccinea</i>	6	4	80–84
<i>Stachys chamissonis</i>	10	4	64
<i>Stachys affinis</i>	10	4	66
<i>Stachys bullata</i>	4	4	66
<i>Phyllostegia vestita</i>	4	4	66

et al., 2005; Alvarez and Wendel, 2003; Yang and Berry, 2011; Smedmark and Anderberg, 2007).

Incomplete lineage sorting may also have given rise to the cyto-nuclear discordance observed in our current study. Nauheimer et al. (2012) used such chloroplast and nuclear DNA incongruence to investigate phylogenetic relationships among the genus *Alocasia* (Araceae) and dated the divergence of ancestors of *Alocasia* from its sister group originating in the mainland about 24 Mya. In case of the Hawaiian mints, rapid diversification and speciation may have taken place more recently giving insufficient time for the full sorting of ancestral polymorphisms by genetic drift (Carstens and Knowles, 2007; Knowles and Carstens, 2007).

The considerable chromosomal diversity in *Stachys* may shed further light on the observed molecular phylogenetic incongruence. Chromosomal studies by Mulligan and Munro (1989) showed that geographic distribution may be associated with chromosome numbers and that (1) temperate North American species with a predominant eastern distribution (including *S. floridana* and *S. latidens*) and with widespread temperate North American distribution (including *S. pilosa* and *S. tenuifolia* consisting of both diploid and tetraploid populations) are  $2n = 34$  or 68, (2) temperate western North American *Stachys* species (e.g., *S. albens*, *S. bullata*) are  $2n = 64$  or 66, similarly to the chromosome number for the Hawaiian mints, and (3) Mexican *Stachys* species vary from  $2n = 32$  (*S. eriantha* and *S. agraria*) to  $2n = 80–82$  (*S. drummondii*) and  $2n = 84$  (*S. coccinea*) Given these chromosomal patterns, several scenarios seem possible: (1) Ancestors with base chromosome number  $x = 17$  ( $2n = 68$ ) gave rise to the group of temperate western NA *Stachys* species and the Hawaiian mints through chromosomal fusion events during meiosis resulting in  $2n = 64$  and 66 (Mulligan and Munro, 1989), (2) the Hawaiian mints could be of hybrid origin involving parent lineages with  $2n = 68$  (*Stachys* species from eastern

North America) and  $2n = 64$  (western North American taxa), or (3) interbreeding between temperate NA ( $2n = 64$  or 68) and Meso-SA (e.g.,  $2n = 32$ ) parents led to the Hawaiian lineage (and possibly other descendants on the continent of which only *S. chamissonis* has survived). Similar hybrid origins was also suggested by Lindqvist and Albert in their previous study (2002). Based on our data and incongruent phylogenetic position of the Hawaiian taxa (and *S. chamissonis*), the last scenario, involving western NA and Meso-SA parents, appears the most parsimonious. If their ancestors (Meso-SA and temperate NA) were present in sympatry, reticulation may have occurred during ancient times, despite a lack of direct evidence of hybridization in the extant Hawaiian genera.

Often homologous nuclear DNA regions from a population of closely related plants can indicate conflicting evolutionary patterns (McBreen and Lockhart, 2006). Since such incompatibilities may arise from interspecific hybridization and/or recombination, as discussed above, bifurcating phylogenies may not be the best process to evaluate such relationships. In such cases, networks, which show the patterns of haplotype distribution among the different species involved (Ferrerri et al., 2011) should be evaluated to better understand the relationships. Median joining networks have been effectively used for nuclear loci to show a hybrid history in other organisms, e.g., the fungus *Armillaria* (Baumgartner et al., 2012). In our case, only moderate levels of reticulation were observed and mainly within the major clades, although also to some extent between temperate North American, Hawaiian species, and eastern Asian species like *S. affinis*, *S. kuoyangensis* (Fig. 3). This reiterates the possibility that the Hawaiian mints may be products of hybridization involving temperate NA *Stachys* species, which have their closest relatives in eastern Asia. A complex polyploid North American origin similar to the Hawaiian mints has been elucidated in the Hawaiian violets (Marcussen et al., 2012).

The great extent of morphological diversification observed within the Hawaiian genera may be a result of a hybrid origin involving polyploid ancestors from separate lineages (Lindqvist and Albert, 2002). The transgeneric positions of some individuals of *Phyllostegia* and *Stenogyne* may be caused by more recent reticulations between *Stenogyne* and *Phyllostegia* species (Lindqvist et al., 2003, 2006), although it has also been suggested that the unresolved placements of most *Phyllostegia* individuals could be caused by lineage sorting following ancient hybrid lineage formation or from homoplasy alone (Lindqvist et al., 2003).

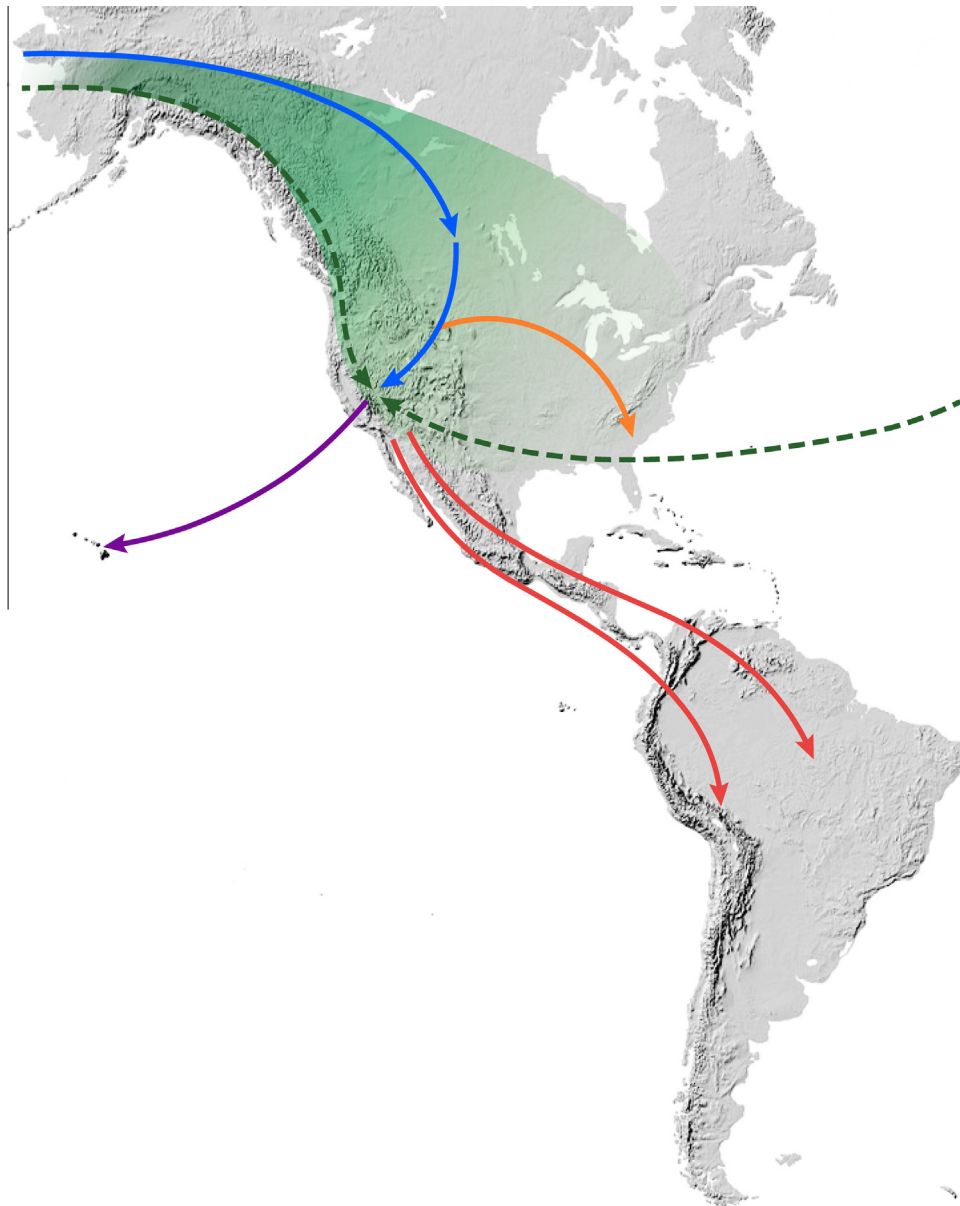
#### 4.2. Evolutionary patterns and resolution of ribosomal DNA

Another aspect of our phylogenetic analyses is revealed in the instances of incongruence and different levels of resolution in the ribosomal DNA ETS and 5S-NTS phylogenies (Fig. 2). Our 5S-NTS data result in a better resolved phylogeny (Fig. 2, Fig. S1 and S2) and exhibit more variation than the ETS data. For example, the average number of polymorphic loci in the 5S-NTS dataset was almost two times higher compared to the ETS dataset (refer to Section 3.4). It is generally thought that the thousands of repeats within 18S–26S and 5S rDNA arrays and other tandemly repeated multigene families retain a high degree of identity due to concerted evolution (Cronn et al., 1996). Concerted evolution, i.e., homogenization of the various copies of sequence repeats or members of the same gene family, takes place throughout the entire genome by means of processes like unequal crossing over or gene conversion. It is possible that such homogenization has occurred at different rates in ETS and 5S-NTS, and that the ETS region underwent a faster rate of homogenization resulting in less variation and resolution in the phylogenetic signals compared to 5S-NTS.

Becerra (2003) also encountered higher variability in the 5S-NTS loci as compared to ITS and ETS in Mexican *Bursera* (Burseraceae) species, and Okuyama et al. (2005), who investigated incongruence between ITS and ETS sequences in *Mitella* (Saxifragaceae) species, concluded that different intensities of concerted evolution after hybridization can lead to differences in phylogenies between the two rDNA loci. Similarly, Morgan et al. (2009) encountered such incongruence between ITS and 5S-NTS regions in the Machaerantherinae.

Information about chromosomal arrangements among the rDNA loci may give further clues into the observed differences between the ETS and 5S-NTS results. As observed from our FISH analysis results (Fig. S3; Table 2), considerable variation of 25S (ETS) loci among the five mint species shows no strong correlation with the chromosome numbers. It has been suggested that such variation might be the results of chromosomal rearrangements, such

as duplication, deletion, translocation, or transpositional events (Lan and Albert, 2011), which may be a prevalent scenario in polyploid plants (Cronn et al., 1996), such as the mints involved in our study. The conservation of 5S loci number among the five species indicates that the 5S rDNA array might undergo fewer chromosomal rearrangements than the 25S rDNA (ETS) arrays in the five species of mints studied (Table 2, Fig. S3). It has been demonstrated that repeats of *Gossypium* (Malvaceae) 18S-26S rDNA arrays evolve under strong inter- and intralocus concerted evolution (Wendel et al., 1995). On the other hand, the predominant homogenizing forces acting on 5S ribosomal genes and spacers operate at the level of the individual array, and interlocus concerted evolution has not been an important factor in the evolution of these arrays (Cronn et al., 1996). The fewer loci but substantially higher intra-individual polymorphisms observed in the mint 5S-NTS sequences as opposed to the highly variable number of loci but fewer



**Fig. 5.** Map showing hypothetical migratory pathways for members of Stachydeae in the New World. Green (dashed) and blue arrows indicate two separate colonization events of North America. The dashed arrows suggest alternative migratory routes. Red arrows indicate colonization and diversifications into Mesoamerica and South America; orange arrow denotes diversification into the different parts of temperate North America, including colonizations into the East; purple arrow indicates migration to Hawaii.



polymorphisms in ETS suggest a similar pattern. Previous studies showing the existence of intralocus and interlocus 5S rDNA diversity in *Paphiopedilum* (Orchidaceae) hypothesized that weak (interlocus) homogenization forces on 5S arrays may be caused by the commonly observed intercalary locations of 5S loci on the chromosomes (Lan and Albert, 2011).

However, it is important to note that the external transcribed spacer (ETS), similar to the internal transcribed spacer (ITS), of the 18S–26S rDNA arrays plays an important function in rRNA maturation (Beltrame et al., 1994; Bena et al., 1998) and hence is expected to be under greater selective constraint than the 5S non-transcribed spacer. Pillier et al. (1990) suggested that there may be two stem-loop structures formed downstream of the site of initiation and major processing site, and these may play a role in regulation of rDNA transcription, and lead to reduced length variation among genera in ETS. Recent studies (Rooney and Ward, 2005) have shown that repetitive elements from a repeat family, more similar to each other than between species, may be evolving through birth-and-death evolution, rather than concerted evolution. However, comparing the different rDNA elements, Ganley and Kobayashi (2007) did not find any such evidence of birth-and-death evolution for lower levels of polymorphisms in a study of fungal genomes. However, in species with complex rDNA arrangements and cryptic variation within repeats, as may be the case with Stachydeae ETS and 5S-NTS, lower levels of polymorphisms may be the result of birth–death evolution. Our data shows more variation and higher resolution among the temperate NA and Hawaiian taxa in the 5S-NTS Bayesian tree, whereas these remain unresolved in the ETS tree (Fig. 2), which may be explained by the conserved nature of the ETS locus possibly evolved through a mixed process of homogenization, selection, and birth-and-death evolution.

#### 4.3. Divergence times and historical biogeography of the New World and Hawaiian taxa

##### 4.3.1. Origin and dispersal of *Stachys* to the New World

Lamiaceae has been placed in a derived phylogenetic position within Lamiales (Bremer et al., 2002; Bendiksby et al., 2011), for which a fossil based stem group of at least 28.4 Million years ago (Mya) has been suggested (Martinez-Millan, 2010). Our molecular dating (Fig. 4, node I; Table 1) suggests that within the New World *Stachys* crown group, cladogenetic events began with an initial split between Old and New World mints during the Middle Miocene period, followed by an initial diversification in the New World during the Late Miocene period. Due to the intercalating of OW species within an otherwise NW lineage, our study suggests that besides this initial colonization event into the New World, a much later, second migration event must have followed towards the beginning of the Pliocene period. Descendants from the first migration event appear to have survived only in Meso-South America (indicated by dashed green and red arrows in Fig. 5), whereas the later event colonized different parts of temperate North America (blue arrows in Fig. 5), including diversification in the eastern region (orange arrow in Fig. 5). Colonization of the Hawaiian Islands may have taken place between the Early to Late Pliocene period (Fig. 4; shown in Fig. 5 by the purple arrow).

Our results from the optimal ancestral area reconstruction (Fig. 4) suggest the Old World to have acted as centers of origin for the New World mints during the Miocene period (Figs. 4 and 5). The sister lineage to the predominantly New World lineage (Fig. 4, node I) are today found in the Mediterranean region (e.g., *Stachys byzantina* and *S. cretica*). Such amphiatlantic distributions featuring tropical and subtropical taxa have been recorded in several other eudicot groups (e.g. Smedmark and

Anderberg, 2007). The breakup of Gondwana and the resulting vicariance events have been hypothesized for such extant distribution patterns in older plant lineages (e.g., Lauraceae, Chanderbali et al., 2001). On the other hand, geological and paleobotanical evidence suggest that Eurasia and North America were connected via the Bering land bridge (BLB) across the north end of the Pacific Ocean connecting Siberia and Alaska throughout most of the Paleogene/Neogene (Li, 1952; Wen, 1999, 2001), and the North Atlantic land bridge (NALB) across the north end of the Atlantic Ocean linking northern Canada to Europe via Greenland in the Paleogene (Tiffney, 1985). Intercontinental connections via these two land bridges led to extensive floristic exchanges between Eurasia and North America (Wolfe, 1975; Tiffney, 1985; Qian, 1999; Qian and Ricklefs, 2000, 2004; Zhou et al., 2006). The BLB, lying close to the Arctic Circle, has been shown as the last Neogene land connection between Eurasia and North America (Tiffney, 1985, 2008; Tiffney and Manchester, 2001). Steep declines in temperatures during the end of the Eocene led tropical and subtropical taxa towards a more equatorial distribution, which was a major cause for the divergence and speciation between North American and Eurasian species of many Tertiary relict genera occupying the Beringia before the breakup of the BLB about 5.4–5.5 Mya (Milne, 2006; Milne and Abbott, 2002; Marincovich and Gladenkov, 1999). Marincovich and Gladenkov (1999) have suggested a minimum age range of 4.8–7.4 million years for the opening of the Bering Strait.

The timing of migration events in the New World mints suggests that movement through the North Atlantic Land Bridge (NALB) was unlikely since it was closed and biotic connections were lost probably sometime about 15 Mya in the Middle Miocene (Milne, 2006). There was widespread aridification and drastic cooling of the climate during the transition from the Eocene to Oligocene, which is probably another reason that it is highly unlikely that tropical and subtropical taxa travelled across the NALB to expand their ranges between Eurasia and North America (Smedmark and Anderberg, 2007) later than this period. The BLB has been postulated to be the migratory route for a variety of gymnosperm and angiosperm families during the late Miocene, including Hamamelidaceae (Xie et al., 2010), Brassicaceae (Dobes et al., 2004), Asteraceae (Thompson and Whitton, 2006); Saxifragaceae (Oliver et al., 2006), and Pinaceae (Anderson et al., 2006).

Since our divergence dating suggests an initial diversification of *Stachys* in the New World during the Late Miocene period (Fig. 4, Table 1; Fig. 5 shown by green dashed arrow), dispersal across the BLB until the opening of the Bering Strait seems likely. Similarly, the temperate NA-Hawaiian lineage diversified from Central-East Asian species during Early and Late Pliocene (node V, Fig. 4; and shown by blue arrow in Fig. 5), also suggesting a Beringian origin.

On the other hand, since the sister group to the NW *Stachys* lineage in both rDNA analyses are predominantly Mediterranean in origin, with apparently no Central-East Asian nor temperate NA lineages left behind, long distance dispersal across the Atlantic (Givnish and Renner, 2004; Clayton et al., 2009) can be posited as an equally likely scenario and a major driving force for the initial migration to the New World (indicated by green dashed arrow in Fig. 5). Givnish and Renner (2004) suggested the presence of strong westward ocean currents leading to water dispersal across the tropical Atlantic for introduction of taxa from Africa to South America. However, another means of introduction to the New World could have been via long distance dispersal over the Atlantic through zoochory by birds (Gillespie et al., 2012).

##### 4.3.2. Origin and diversification of the South American *Stachys*

The Mesoamerican/South American taxa, including species that span into southwestern United States, form two strongly

supported rDNA clades (Meso-SA clades I and II, Fig. 2). It should be noted here that five members of the *Stachys coccinea* complex (*S. albotomentosa*, *S. coccinea*, *S. lindenii*, *S. pacifica*, and *S. torresii* (Turner, 1994a), although not resolved as monophyletic, group within the Meso-SA-II clade in both rDNA trees (Fig. 2), suggesting a close relationship among these species. Except for *S. debilis* and *S. langmaniae*, which group in different ETS and 5S-NTS clades, respectively, the two clades show similar species composition. Hence, South American, as well as Mesoamerican, species are found in both clades. As such, similarly to the Hawaiian lineage, the origin of the South American mints may involve reticulation events implicating species from different lineages, in this case species from regions covering Mexican/Central America and the southwestern United States. Conflicting signals in the various datasets may be the result of hybridization events between such taxa. The median joining network (Fig. 3) also shows some reticulation within the Mesoamerican/South American clades, further supporting that the South American *Stachys* species may have derived from hybridization events (and in some cases polyploidization, e.g., *S. coccinea* and *S. drummondii*) between *Stachys* from Mexican-Central American and southwestern United States.

Diversification into two separate Mesoamerican/South American lineages may have occurred in the Late Miocene/Early Pliocene (Fig 4 and 5 and Table 1). Within these lineages we observe a clade consisting exclusively of South American taxa to have diverged from the Mesamerican species around the Late Pliocene. It is possible that migration and diversification to South America occurred in the Late Pliocene through long distance dispersal by birds as mentioned above, or through vicariance when the Isthmus of Panama closed approximately 3 Mya, as part of the Great American Biotic Exchange (Cody et al., 2010). Such pattern of movement involving dispersal and vicariance events from Central/East Asia to North and South America has been elucidated in New World *Dryopteris* (Dryopteridaceae) (Sessa et al., 2012). Similar to our results, the South American species of *Valeriana* (Valerianaceae) do not form a clade and there seems to have been multiple introductions during the Early Miocene (Bell et al., 2012). Luebert et al. (2011) pointed to a Paleocene or earlier diversification of Heliotropiaceae in the New World, especially South America. In contrast, movement from South America to North America and Hawaii during Miocene and Pliocene is observed in *Portulaca* (Portulacaceae) (Ocampo and Columbus, 2012).

#### 4.3.3. Dispersal to the Hawaiian Island: the Hawaiian mints revisited

Our results corroborate Lindqvist and Albert's (2002) findings suggesting a single introduction to Hawaii (Figs. 4 and 5; Table 1). The MRCA of the Hawaiian lineage arrived to the islands approximately during the Late Pliocene (Table 1). This timing event corresponds to the presence of Hawaiian endemic mints in all of the extant main islands, including the oldest island Kaua'i, which is approximately 5.1 Million years old (Carson and Clague, 1995). In comparison, it has been suggested that the Hawaiian lobeliads colonized the Hawaiian-Emperor chain ca. 13 Mya (Givnish et al., 2009). The 5S-NTS results and the current species sampling suggest that the extant closest relatives of the Hawaiian genera may be located in southwestern NA (e.g., *S. ajugoides* and *S. rigida*, both of which have an extant predominantly southwestern US distribution). However, this topology is not strongly supported and given the patterns of cpDNA and rDNA incongruence, discussed above, southwestern NA may have been the subject of hybrid introgressions between descendants of the two separate colonizations of the New World (green and blue arrows in Fig. 5). Our ancestral area reconstruction also points to a Hawaiian ancestral matriline originating from Meso-SA, although this is

ambiguous and not well resolved (refer to Section 3.3). The incongruent phylogenetic position of *S. chamissonis*, another western US species and perhaps a continental survivor of this hybrid introgression, may support this hypothesis. We must note, though, that the cpDNA data point to a Meso-SA maternal/seed parent and hence, we cannot rule out the possibility that the Hawaiian lineage originated from further south on the American continent.

The hybrid ancestors of the extant Hawaiian lineage in all probability arrived through long distance dispersal via seeds carried by birds (zoochory). Baldwin and Wagner (2010) reviewed the origin of the Hawaiian endemic flora from temperate North America and showed that a vast majority had arrived to the archipelago via similar dispersal by birds. Tiffney and Manchester (2001) noted that if we assume that there was a uniformity of meteorological principles over the last 65 million years ago, abiotic dispersal above 60°N happened from an East to West direction.

## 5. Future directions

With the help of more loci and considerably increased sampling, our research has been able to elucidate the possible origins and diversification of the New World mints, including South American, and the Hawaiian species. However, we have observed that maternally inherited cpDNA loci, as well as rDNA loci like ETS, have not been able to fully resolve the relationships among and within the temperate NA and the Hawaiian mints. Hence, current efforts involving the investigation of low copy nuclear loci may shed further light towards untangling the origin of the Hawaiian lineage and their relationships with New World mints, since such nuclear genes have independent evolutionary histories, are free from biased concerted evolution, and may be useful in generating sufficient variability necessary for reliable phylogenetic analysis (Sang, 2002; Curto et al., 2012).

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## Appendix A

See Table A1.

## Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2013.05.023>.

**Table A1**

List of taxa, voucher information, and GenBank accession numbers for 5S-NTS, ETS, trnL intron, trnL-F spacer and rps16 intron for the mints used in this study. The collecting year has been listed for specimens without a collecting number; n.a. = sequence not available.

Taxa list	Voucher information	Geographic distribution/collecting locality	Genbank accession numbers				
			5S-NTS	ETS	trnL intron	trnL-F spacer	rps16 Intron
<i>Haplostachys haplostachya</i> (A. Gray) St. John	S. Perlman 14328 (NY)	Hawaii/Hawai'i	AF308173	n.a.	n.a.	n.a.	n.a.
	V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen HI03-33 (NTBG)	Hawaii/Hawai'i	n.a.	KF235784	KF235643	KF235696	KF235589
<i>Haplostachys linearifolia</i> (Drake) Sherff	J. F. Rock 14025 (NY)	Hawaii/West Moloka'i	AF308174	KF235785	n.a.	n.a.	n.a.
	M. E. Steiner, E. Andersson, K. Apelgren & J. Nitare 1127 (UPS)	W. & S.W. Europe to Turkey/Hungary	KF235749	KF235786	EF546929	EF54849	FJ854051
<i>Phyllostegia ambigua</i> (A. Gray) Hillebr.	S. Vautier & J. P. Bersier 2896805 (US)	W. & S.W. Europe to Turkey/France	n.a.	KF235787	n.a.	n.a.	n.a.
	R. Hobdy 3023 (BISH)	Hawaii/East Maui	AF308176	n.a.	n.a.	n.a.	n.a.
	G. Clarke 688 (BISH)	Hawaii/Hawai'i	AF308175	n.a.	n.a.	n.a.	n.a.
<i>Phyllostegia bracteata</i> Sherff	V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen HI03-59 (Volcano Rare Plant Facility)	Hawaii/Hawai'i	n.a.	KF235788	KF235644	KF235697	KF235590
	B.H. Gagne s.n. 1981 (BISH)	Hawaii/Maui	AF308177	KF235789	n.a.	n.a.	n.a.
<i>Phyllostegia brevidens</i> A. Gray	K. Wood 3200 (BISH)	Hawaii/East Maui	AF308179	n.a.	n.a.	n.a.	n.a.
	J. Griffin s.n. 1985 (BISH)	Hawaii/Hawai'i	AF308178	KF235790	n.a.	n.a.	n.a.
<i>Phyllostegia electra</i> C. Forbes	K. Wood 2967 (BISH)	Hawaii/Kaua'i	AF308180	KF235791	KF235645	KF235698	KF235591
<i>Phyllostegia floribunda</i> Benth.	J. D. Jacobi 1326 (BISH)	Hawaii/Hawai'i	AF308181	n.a.	n.a.	n.a.	n.a.
	V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen HI03-63 (Volcano Rare Plant Facility)	Hawaii/Hawai'i	n.a.	KF235792	K35F2646	KF235699	KF235592
<i>Phyllostegia glabra</i> (Gaudich.) Benth	K. Wood 3962 (NY)	Hawaii/Maui	n.a.	KF235794	AF502031	FJ854182	FJ854065
<i>Phyllostegia glabra</i> var. <i>glabra</i>	O. Degener 24160 (BISH)	Hawaii/Lana'i	AF308183	n.a.	n.a.	n.a.	n.a.
	W. L. Wagner 5761 (BISH)	Hawaii/Moloka'i	AF308184	n.a.	n.a.	n.a.	n.a.
	J. Obata s.n. 1990 (BISH)	Hawaii/O'ahu	AF308182	KF235793	n.a.	n.a.	n.a.
<i>Phyllostegia glabra</i> x <i>grandiflora</i>	J. Lau 3538 (BISH)	Hawaii/O'ahu	AF308186	n.a.	n.a.	n.a.	n.a.
<i>Phyllostegia grandiflora</i> (Gaud.) Benth.	P. Welton 801 (BISH)	Hawaii/O'ahu	AF308187	KF235795	n.a.	n.a.	n.a.
<i>Phyllostegia helleri</i> Sherff	S. Perlman 15690 (BISH)	Hawaiian Is. (Kauai: Wai'alea Valley)/Kaua'i	KF235750	n.a.	n.a.	n.a.	n.a.
<i>Phyllostegia hirsuta</i> Benth.	E. Hosaka s.n. 1933 (NY)	Hawaii/O'ahu	AF308189	n.a.	n.a.	n.a.	n.a.
	J. Obata s.n. 1993 (BISH)	Hawaii/O'ahu	AF308190	KF235796	n.a.	n.a.	n.a.
<i>Phyllostegia hispida</i> Hillebr.	L. Stemmerman 3973 (BISH)	Hawaii/Moloka'i	AF308191	n.a.	n.a.	n.a.	n.a.
<i>Phyllostegia haliakalae</i> Wawra	D. Herbst 4048 (BISH)	Hawaii/Laua'i	KF235751	n.a.	n.a.	n.a.	n.a.
<i>Phyllostegia kaalaensis</i> St. John	S. Perlman 6117 (BISH)	Hawaii/O'ahu	AF308194	KF235798	KF235648	KF235701	KF235593
	W. Takekuchi & Paquin 3440 (BISH)	Hawaii/O'ahu	AF308195	n.a.	n.a.	n.a.	n.a.
	W. Takekuchi 941 (BISH)	Hawaii/O'ahu	AF308196	n.a.	n.a.	n.a.	n.a.
<i>Phyllostegia kahiliensis</i> St. John	W. L. Wagner 5217 (BISH)	Hawaii/Kaua'i	AF308197	KF235797	KF235647	KF235700	KF235594
<i>Phyllostegia knudsenii</i> Hillebr.	K. Wood 2583 (BISH)	Hawaii/Kaua'i	AF308198	KF235799	n.a.	n.a.	n.a.
<i>Phyllostegia lantanoides</i> Sherff	J. Obata 86-624 (BISH)	Hawaii/O'ahu	AF308199	KF235800	n.a.	n.a.	n.a.
<i>Phyllostegia macrophylla</i> (Gaud.) Benth.	F. R. Warshauer 2862 (BISH)	Hawaii/East Maui	AF308200	n.a.	n.a.	n.a.	n.a.
	S. Perlman 14184 (NY)	Hawaii/Hawai'i	AF308201	KF235801	n.a.	n.a.	n.a.
<i>Phyllostegia mannii</i> Sherff	F. R. Warshauer 2418 (BISH)	Hawaii/Moloka'i	AF308205	KF235802	n.a.	n.a.	n.a.
<i>Phyllostegia mollis</i> Benth.	O. Degener 20866 (NY)	Hawaii/O'ahu	AF308203	KF235803	n.a.	n.a.	n.a.
<i>Phyllostegia racemosa</i> Benth.	F. R. Warshauer 1447 (BISH)	Hawaii/Hawai'i	AF308205	n.a.	n.a.	n.a.	n.a.
	V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen HI03-57 (Volcano Rare Plant Facility)	Hawaii/Hawai'i	n.a.	KF235805	KF235650	KF235703	KF235596
<i>Phyllostegia parviflora</i> (Gaud.) Benth. var. <i>lydgatei</i> (Sherff.) W.L. Wagner	J. Obata s.n. 1990 (BISH)	Hawaii/O'ahu	AF308204	KF235804	KF235649	KF235702	KF235595
<i>Phyllostegia</i> cf. <i>renovans</i>	S. Perlman 13256 (BISH)	Hawaii/Kaua'i	AF308206	KF235806	n.a.	n.a.	n.a.
<i>Phyllostegia rockii</i> Sherff	C. N. Forbes 199 (BISH)	Hawaii/East Maui	AF308207	KF235807	n.a.	n.a.	n.a.
<i>Phyllostegia stachyoides</i> A. Gray	J. S. Meidell 111 (BISH)	Hawaii/West Maui	AF308208	n.a.	n.a.	n.a.	n.a.
	K. R. Wood 6280 (BISH)	Hawaii/Moloka'i	AF308209	n.a.	n.a.	n.a.	n.a.
<i>Phyllostegia variabilis</i> Bitter	F. R. Warshauer 1856 (BISH)	Hawaii/Hawai'i	AF308210	n.a.	n.a.	n.a.	n.a.
	C. Lamoureux 1926a (BISH)	Hawaii/Kure	AF308211	n.a.	n.a.	n.a.	n.a.
<i>Phyllostegia velutina</i> (Sherff) St. John	W. A. Brian 1903 (BISH)	Hawaii/Laysan Island	n.a.	KF235808	n.a.	n.a.	n.a.
	J. Griffin s.n., 1992 (BISH)	Hawaii/Hawai'i	AF308212	n.a.	n.a.	n.a.	n.a.
	V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen HI03-	Hawaii/Hawai'i	n.a.	KF235809	KF235651	KF235704	KF235597

<i>Phyllostegia vestita</i> Benth.	61 (Volcano Rare Plant Facility) St. John 22360 (NY) V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen HI03-62 (Volcano Rare Plant Facility)	Hawaii/Hawai'i Hawaii/Hawai'i	AF308213 n.a.	n.a. KF235810	n.a. KF235652	n.a. KF235705	n.a. KF235598
<i>Phyllostegia waimeae</i> Wawra.	V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen H103-27 (NTBG)	Hawaii/Kaua'i	KF235752	KF235811	KF235653	KF235706	KF235599
<i>Phyllostegia warshaueri</i> St. John	S. Perlman 10830 (BISH) S. Perlman 14185 (BISH) V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen H103-30 (NTBG)	Hawaii/Kaua'i Hawaii/Hawai'i Hawaii/Hawaii	AF308214 AF308215 n.a.	n.a. n.a. KF235812	n.a. n.a. n.a.	n.a. n.a. n.a.	n.a. n.a. n.a.
<i>Phyllostegia wawrana</i> Sherff	S. Perlman 13690 (NY)	Hawaii/Kaua'i	AF308216	KF235813	n.a.	n.a.	n.a.
<i>Stachys aculeolata</i> Hook f.	Y. B. Harvey, G. Mungai & K. Vollesen 7 (C)	Tropical Africa/Kenya	AF501924	KF235814	FJ854305	FJ854199	FJ854084
<i>Stachys aethiopica</i> L.	B. Petterson 2146 (UPS)	Tropical to S. Africa/Mocambique	KF235753	KF235815	FJB54307	FJ854201	FJ854086
<i>Stachys affinis</i> Bunge	C. Lindqvist and V. A. Albert 359 (UNA), from Companion Plants	China to N. Myanmar/Cultivated	AF501925	KF235816	AF502041	FJ854202	FJ854087
<i>Stachys agraria</i> Schlttdl. & Cham	G. Nesom 6113 (TEX) B. Ertter 5638 (UTC)	Texas to Guatemala/Mexico, Tamaulipas Texas to Guatemala/Texas Nueces Co.	AF501926 AF501947	KF235818 KF235817	n.a. KF235654	n.a. KF235708	n.a. KF235610
<i>Stachys ajugoides</i> var. <i>rigida</i> Jeps. & Hoover	C. Lindqvist 11-02 (UB)	W. USA to Mexico (Baja California)/Cultivated	KF235754	KF235819	n.a.	n.a.	n.a.
<i>Stachys albens</i> A. Gray	G. Baird 1630 (RM) C. Lindqvist 11-06 (UB)	California/Utah, Washington Co. California to Utah/Cultivated	AF501928 n.a.	n.a. KF235820	n.a. n.a.	n.a. n.a.	n.a. n.a.
<i>Stachys albotomentosa</i> Ramamoorthy	H. Rubio 1984 (TEX)	N.E. Mexico/Mexico, Landa Mun.	AF501929	KF235821	n.a.	n.a.	n.a.
<i>Stachys alpina</i> L.	P. Frost-Olsen 4239 (AAU)	Europe to N. Iran/Bulgaria	n.a.	n.a.	FJ854310.1	FJ854205.1	FJ854090.1
<i>Stachys alpigena</i> T.C.E.Fr.	O. Ryding 2133 (UPS)	Ethiopia to Rwanda/Ethiopia	KF235755	KF235822	FJ854309	FJ854204	FJ854089
<i>Stachys arabica</i> Hornem.	I. Gruenberg 685 (UPS)	S. Turkey to Israel/Israel	n.a.	n.a.	FJ854312	FJ854207	FJ854092
<i>Stachys argillicola</i> Sebsebe	I. Friis, M. Tadesse & K. Vollesen 3104 (C)	E. Tropical Africa/Ethiopia, Neghelle	AF501930	n.a.	AF502044	FJ854208	FJ854093
<i>Stachys arvensis</i> (L.) L.	O. Ryding 2394 (C)	Macaronesia to Taiwan/ Tenerife Island	AF501931	KF235823	KF235655	KF235709	KF235601
<i>Stachys aspera</i> Michx.	J. B. Nelson 1326 (UNA)	N.C to E. USA/ Florida, Leon Co.	AF501932	KF235824	KF235656	KF235710	KF235602
<i>Stachys biflora</i> Hook. & Arn.	G. B. Hinton et al. 24399 (UB) G. B. Hinton et al. 24561 (TEX)	Mexico/Mexico, Nueva Leon Mexico/Mexico, Nuevo Leon	AF501953 n.a.	n.a. KF235825	n.a. n.a.	n.a. n.a.	n.a. n.a.
<i>Stachys bigelovii</i> A. Gray	A. McDonald & G. Nesom 2519 (TEX) G. B. Hinton et al. 19781 (TEX) G. B. Hinton et al. 19781 (TEX)	S. Texas to N. Mexico/Mexico, Chihuahua S. Texas to N. Mexico/Mexico, Chihuahua	AF501935 KF235756	KF235826 n.a.	n.a. n.a.	n.a. n.a.	n.a. n.a.
<i>Stachys bogotensis</i> Kunth	J. Cuatrecasas and R. Romero Castenada 25056 (US)	Columbia/Colombia	KF235757	KF235827	n.a.	n.a.	n.a.
<i>Stachys boraginoides</i> Schlecht. & Cham	P. Cruz M. s.n.1982 (LL)	Mexico/ Mexico, Vera Cruz	AF501936	KF235757	KF235827	n.a.	n.a.
<i>Stachys bridgesii</i> Benth.	F. Claude Joseph 4354 (US)	C. and S. Chile/Chile	KF235758	KF235829	KF235657	KF235711	KF235604
<i>Stachys bullata</i> Benth.	M. R. Crosby & N. Morin 14355 (RM)	W. California/California, Monterey Co.	AF501937	KF235831	KF235659	KF235713	KF235605
<i>Stachys byzantina</i> C. Koch	A. L. Moldenke & H. N. Moldenke 25574 (AAU) C. Lindqvist and V. A. Albert 356 (UNA), from Companion Plants	W. California/California Krym, N. Turkey to N. Iran and Greece/ Cultivated	KF235759 AF501938	KF235830 KF235832	KF235658 AF502046	KF235712 FJ854211	KF235606 FJ854096
<i>Stachys chamissonis</i> Benth.	C. Lindqvist 10-02 (UB), received from R. Olmstead	W. Canada to W. USA/Cultivated	KF235760	KF235833	KF235660	KF235714	KF235608
<i>Stachys chamissonis</i> var. <i>cooleyae</i> (A. Heller) G.A. Mulligan & D.B. Munro	A.L. Moldenke & H. N. Moldenke 32097 (LL) R. R. Halse 2906 (UTC) S. Jennings 218 (UTC)	W. Canada to W. USA/Oregon, Marion Co. W. Canada to W. USA/Washington, Mason Co. W. Canada to W. USA/Washington, Skamania Co.	AF501944 AF501945 AF501946	n.a. n.a. n.a.	n.a. n.a. n.a.	n.a. n.a. n.a.	n.a. n.a. n.a.
<i>Stachys coccinea</i> Ort.	M. A. Franklin 5407 (RM) R. A. Bye 5331 (RM)	Arizona to Texas and C. America/Arizona, Santa Cruz Co. Arizona to Texas and C. America/Mexico, Chihuahua	AF501942 AF501943	n.a. n.a.	n.a. n.a.	n.a. n.a.	n.a. n.a.
<i>Stachys cordata</i> Riddell	C. Lindqvist and V. A. Albert 355 (NY) J. Ricketson & L. Raechal 4274 (TEX) C. Lindqvist 10-01 (UB), from High Country Gardens K. I. Matthews 745 (AAU) J. B. Nelson 14361 (UNA) A. L. Moldenke & H. N. Moldenke 27394 (AAU)	Arizona to Texas and C. America/Cultivated Arizona to Texas and C. America/Arizona, Santa Cruz Co. Arizona to Texas and C. America/Cultivated Arizona to Texas and C. America/USA, Arizona N.C. and E. USA/South Carolina, Richland Co. N.C. and E. USA/USA, Ohio, Coshocton Co.	AF308172 AF501941 KF235761 n.a.	n.a. n.a. n.a. KF235834	n.a. n.a. n.a. KF235661	n.a. n.a. n.a. KF235715	n.a. n.a. n.a. KF235609
<i>Stachys corsica</i> Pers.	J. Lambinon 81/sa/191 (AAU)	Corsica, Sardinia/Sardinia	KF235762	KF235836	n.a.	n.a.	n.a.
<i>Stachys cretica</i> subsp. <i>cassia</i> (Boiss.) Rech.f.	S. B. Snogerup 14974 (UPS)	Balkan Pen. to S. Turkey/Pellis, Greece	n.a.	n.a.	KF235665	KF235745	KF235607
<i>Stachys cretica</i> L.	A. Strid et al. 42603 (C)	Mediterranean to W. Asia/Greece, Thasos	AF501948	KF235838	KF235664	KF235747	KF235611
<i>Stachys debilis</i> Kunth.	C & E. Franquemont 106 (AAU)	Equador/Ecuador, Chulkunag, Punin, Chimborazo	KF235763	KF235839	KF235666	KF235718	KF235612



Table A1 (continued)

Taxa list	Voucher information	Geographic distribution/collecting locality	Genbank accession numbers				
			5S-NTS	ETS	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rps16</i> Intron
<i>Stachys drummondii</i> Benth.	B. Ertter 5530 (UTC)	Texas to N.E. Mexico/ Texas, Cameron Co.	AF501950	KF235840	n.a.	n.a.	n.a.
	C.J. Ferguson 72 (TEX)	Texas to N.E. Mexico/ Texas, Cameron Co.	AF501949	n.a.	n.a.	n.a.	n.a.
<i>Stachys elliptica</i> Kunth.	B. Ollgaard et al. 91045 (AAU)	Ecuador/Ecuador, Prov. Loja	KF235764	KF235841	KF235667	KF235719	KF235613
<i>Stachys eriantha</i> Benth.	A. McDonald & G. Nesom 2495 (TEX)	Mexico, Colombia to NW. Venezuela and Ecuador/Mexico, Chihuahua	AF501951	KF235842	KF235668	KF235720	KF235614
<i>Stachys floridana</i> Shuttlw. Ex. Benth.	M. Kortright 102 (UNA)	Florida/Alabama, Tuscaloosa Co.	AF501952	n.a.	n.a.	n.a.	n.a.
	R. Dale Thomas & D. Moreland 28392 (AAU)	Florida to Louisiana/Louisiana, Ouachita	n.a.	KF235843	n.a.	n.a.	n.a.
<i>Stachys gilliesii</i> Benth.	S. Pfanzelt 241 (M)	W. South America to Chile and Brazil/Chile, Region Metropolitana	KF235765	KF235844	KF235669	KF235721	KF235615
<i>Stachys grandidentata</i> Lindl.	W. J. Eyerdam 10081 (US)	N. and C. Chile/Chile	KF235766	KF235845	FJ854313	FJ854217	FJ854102
<i>Stachys hamata</i> Epling	B. Lojtnant et al. 11835 (AAU)	Colombia to Ecuador, NE. Venezuela/Ecuador, Prov. Carchi, La Esperanza	KF235767	KF235846	KF235670	KF235722	KF235616
<i>Stachys herrerae</i> Epling	F. L. Herrera 3499 (US)	Peru/Peru	KF235768	KF235847	KF235671	KF235723	KF235617
<i>Stachys hispida</i> Pursh.	S. R. Zeigler & M.F.Leykern 1963 (AAU)	C. & E. Canada, N.C. & E. USA/Wisconsin, La Crosse	KF235769	KF235848	KF235672	KF235724	KF235618
<i>Stachys hyssopoides</i> Burch. Ex Benth.	E. Retief 1080 (US)	S. Africa/ South Africa, Pretoria	KF235770	KF235849	FJ854319	FJ854218	FJ854103
<i>Stachys inflata</i> Benth.	J. S. Andersen and I. C. Petersen 68 (AAU)	N.E. Turkey to Iran/Iran	KF235771	KF235850	KF235673	KF235748	KF235619
<i>Stachys kouyangensis</i> (Vaniot) Dunn	B. Bartholomew et al. Sino Amer. Bot. Exp. 1362 (US)	Tibet, SC. China to Indo-China/China, Yunnan	KF235772	KF235851	KF235674	KF235725	KF235620
<i>Stachys lamioides</i> Benth.	L. Holm-Nielsen & J. Jaramillo 23323 (AAU)	Colombia to Ecuador/ Ecuador, Prov. Imbabura	KF235773	KF235852	n.a.	n.a.	n.a.
<i>Stachys langmaniae</i> Rzed. and Calderon	McDonald 1620 (TEX)	N.E. Mexico/Mexico, Nuevo Leon	AF501955	KF235853	KF235675	KF235726	KF235621
	J. A. Villarreal w/ M. A. Carranza et al. 5084 (TEX)	N.E. Mexico/Mexico, Nuevo Leon	AF501956	n.a.	n.a.	n.a.	n.a.
<i>Stachys latidens</i> Small	J. A. Churchill 83034 (RM)	N.C. and E. USA/N. Carolina, Mitchell Co.	AF501956	KF235854	n.a.	n.a.	n.a.
<i>Stachys lavandulifolia</i> Vahl.	J. S. Andersen & I. C. Petersen 31 (AAU)	S. & E. Turkey to Iran/ Iran, Semnan	AF501957	KF235855	KF235676	KF235746	KF235622
<i>Stachys lindenii</i> Benth.	R. Torres C. 4602 (TEX)	S. Mexico to Guatemala/Mexico, Oaxaca	AF501959	n.a.	n.a.	n.a.	n.a.
	P. Tenorio L. 11084 (TEX)	S. Mexico to Guatemala/Mexico, Oaxaca	AF501958	KF235856	KF235677	KF235727	KF235623
<i>Stachys macraei</i> Benth.	B. Claude Joseph 3628 (US)	C. & S. Chile/Chile	KF235774	KF235857	KF235678	KF235728	KF235624
<i>Stachys maritima</i> Gouan	I. Gergely 3362 (US)	Mediterranean to W. Caucasus/Romania	n.a.	n.a.	FJ854321	FJ854222	FJ854107
<i>Stachys mexicana</i> Benth. (cf. chamissonis)	R. E. Brooks 20248 (RM)	Alaska to California/Oregon, USA	AF501960	KF235858	n.a.	n.a.	n.a.
<i>Stachys nepetifolia</i> Desf.	I. Diaz V. 44 (TEX)	N.E & C. Mexico/Mexico, Hidalgo	AF501961	KF235859	KF235679	KF235729	KF235625
<i>Stachys nephrophylla</i> Rech.f.	K. H. Rechinger 1150 (WU)	N. Iraq/Iraq	n.a.	n.a.	FJ854322	FJ854223	FJ854108
<i>Stachys pacifica</i> B. L. Turner	M. Fishbein et al. 2133 (TEX)	W. Mexico/Mexico, Sonora	AF501964	n.a.	n.a.	n.a.	n.a.
	G. Flores F. 2344 (TEX)	W. Mexico/Mexico, Nayarit	AF1963	KF235860	KF235680	KF235730	KF235627
<i>Stachys pilosa</i> Nutt.	G. A. Wheeler 11047 (AAU)	Canada to USA/Minnesota, Traverse Co.	KF235776	KF235861	KF235681	KF235707	KF235628
	M. Curto 1494 (AAU)	Canada to USA/Utah, Box Elder Co	AF501965	n.a.	n.a.	n.a.	n.a.
	G. E. Larson 10569 (UTC)	Canada to USA/S. Dakota	AF501966	n.a.	n.a.	n.a.	n.a.
	H. Hapeman s.n 1938 (UPS)	Canada to USA/S. Dakota	KF235775	n.a.	FJ854311	FJ854206	FJ854091
	T. Cramer w/J. T. Kellett 1909 (RM)	Canada to USA/Wyoming, Sublette Co.	AF501968	n.a.	n.a.	n.a.	n.a.
<i>Stachys pilosissima</i> Mart. & Gal.	M. H. Mayfield 2086 (TEX)	Mexico (Veracruz to Oaxaca)/Mexico, Hidalgo	AF501969	n.a.	n.a.	n.a.	n.a.
<i>Stachys radicans</i> Epling	D. E. Breedlove 51924(TEX)	Mexico, Colombia/Mexico, Chiapas	AF501970	KF235862	KF235682	KF235731	KF235629
<i>Stachys recta</i> subsp. <i>subcrenata</i> (Vis.) Briq	G. Schneewis et al. 6268 (WU)	S.E. Europe to W. Turkey, Transcaucasus to NW. Iran/Croatia	n.a.	n.a.	FJ854330	FJ854233	FJ854118
<i>Stachys recta</i> L.	P. Schonswetter 2517 (WU)	Europe to NW. Iran/Austria	n.a.	n.a.	FJ854323	FJ854226	FJ854111
<i>Stachys riederi</i> var. <i>riederi</i> .	H. Takahashi 2950 (C)	Siberia to Japan/Japan, Hokkaido	AF501933	KF235863	KF235683	KF235732	KF235603
<i>Stachys rigida</i> Nutt. Ex Benth.	H. N. Moldenke et al. 32116 (LL)	W. USA/Oregon, Lane Co.	AF501972	KF235865	n.a.	n.a.	n.a.
<i>Stachys rigida</i> subsp. <i>quercetorum</i> (A. Heller) Epling	R. F. Thorne w/ S. Boyd, E. Lathrop 61281 (RM)	Oregon to S. California/California, Riverside Co.	KF235778	n.a.	n.a.	n.a.	n.a.
	G. K. Helmkamp et al. 2153 (UTC)	Oregon to S. California/ California, Mendocino Co.	n.a.	n.a.	AF502042	FJ854225	FJ854110
<i>Stachys rigida</i> subsp. <i>rigida</i>	A. Tiehm 12609 (UTC)	Washington to N. California/Sierra Nevada, Washoe Co.	AF501971	KF235864	KF235684	KF235733	KF235631
<i>Stachys rothrockii</i> A. Gray	E. Neese et al. 15716 (TEX)	Utah to New Mexico/Utah Cane Co.	AF501974	KF235866	KF235685	KF235734	KF235633
<i>Stachys rotundifolia</i> Moc & Sesse ex Benth.	D. E. Breedlove 55575 (TEX)	C. & S. Mexico/Mexico, Chiapas	AF501975	KF235867	KF235686	KF235735	KF235632
<i>Stachys rugosa</i> Aiton	W. J. Hanekom 2487 (US)	S. Africa/ South Africa, Pretoria	KF235779	KF235868	FJ854325	FJ854228	FJ854113
<i>Stachys sericea</i> Cav.	F. C. Joseph 4327 (US)	C. Chile/Chile	KF235780	KF235869	KF235695	KF235744	KF235634
<i>Stachys spinosa</i> L.	A. C. Scheen & M. Bendiksbj 0422 (O)	S. Aegean Is./Crete, Samaria Gorge	n.a.	n.a.	FJ854329	FJ854232	FJ854117

<i>Stachys strictiflora</i> C.Y. Wu	C. Lindqvist 10-07 (UB)	China, Yunnan/Cultivated	KF235783	KF235870	n.a.	n.a.	n.a.
<i>Stachys swainsonii</i> Benth.	A. Strid et al. 39692 (C)	Greece/Greece	AF501977	KF235871	AF502062	FJ854234	FJ854119
<i>Stachys sylvatica</i> L.	C. Lindqvist & V. A. Albert 358 (UNA)	Macaronesia, Europe to W. Himalaya/ Cultivated	AF501978	KF235872	AF502063	FJ854235	FJ854120
<i>Stachys tenuifolia</i> Willd.	T. W. Nelson & J. P. Nelson 6719 (RM)	E. Canada, C. & E. USA/South Dakota, Pennington Co	AF501981	n.a.	n.a.	n.a.	n.a.
	R. L. Mc.Gregor 31847 (RM)	E. Canada, C. & E. USA/Kansas, Montgomery Co.	AF501980	n.a.	n.a.	n.a.	n.a.
	P. H. Raven & T. E. Raven 27692(TEX)	E. Canada, C.& E. USA/Missouri, Jefferson Co.	AF501979	n.a.	n.a.	n.a.	n.a.
<i>Stachys torresii</i> B. L. Turner	A. McDonald 2927 (TEX)	Mexico, Oaxaca/Mexico, Oaxaca	AF501982	KF235873	KF235687	KF235736	KF235635
<i>Stachys tymphaea</i> Hausskn.	A. Strid 33777 (C)	S.E. Germany to S.E. Europe/Greece	AF501983	n.a.	n.a.	n.a.	n.a.
<i>Stachys vulnerabilis</i> Rzed. & Calderon	G. B. Hinton et al. 24774 (TEX)	N.E. Mexico/Mexico, Nueva Leon	AF501984	KF235874	n.a.	n.a.	n.a.
<i>Stenogyne calycosa</i> Sherff	R. Hobdy 2553 (BISH)	Hawaii/East Maui	AF308223	KF235878	n.a.	n.a.	n.a.
<i>Stenogyne angustifolia</i> A. Gray	O. Degener 20866 (BISH)	Hawaii/Hawai'i	AF308213	n.a.	n.a.	n.a.	n.a.
	R. Hobdy 2451 (BISH)	Hawaii/Hawai'i	AF308218	n.a.	n.a.	n.a.	n.a.
	F. R. Warshauer 2171(BISH)	Hawaii/Hawai'i, Pohakuloa	n.a.	KF235875	n.a.	n.a.	n.a.
<i>Stenogyne bifida</i> Hillbr.	F. R. Warshauer 2377 (BISH)	Hawaii/Moloka'i	AF308219	n.a.	n.a.	n.a.	n.a.
	K. Wood 6284 (BISH)	Hawaii/Moloka'i	AF308221	n.a.	n.a.	n.a.	n.a.
	V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen HI03-32 (NTBG)	Hawaii/Hawaii	n.a.	KF235876	KF235688	KF235737	KF235636
<i>Stenogyne calaminthoides</i> A. Gray	C. Lindqvist & V. A. Albert 42 (NY)	Hawaii/Hawai'i	AF308222	n.a.	n.a.	n.a.	n.a.
	V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen HI03-04	Hawaii/Hawai'i	n.a.	KF235877	KF235689	KF235738	KF235637
<i>Stenogyne campanulata</i> Weller & Sakai	K. Wood 1790 (BISH)	Hawaii/Kaua'i	AF308224	n.a.	n.a.	n.a.	n.a.
<i>Stenogyne cranwelliae</i> Sherff	J. Davis 945 (BISH)	Hawaii/Hawai'i	AF308225	KF235879	n.a.	n.a.	n.a.
<i>Stenogyne haliakalae</i> Wawra	G. E. Olson 5 (BISH)	Hawaii/Maui	AF308226	n.a.	n.a.	n.a.	n.a.
<i>Stenogyne kaalae</i> Wawra	K. Nagata 1617 (NY)	Hawaii/O'ahu	AF308227	KF235880	n.a.	n.a.	n.a.
<i>Stenogyne kamehamehae</i> Wawra	W. L. Wagner 4801 (BISH)	Hawaii/West Maui	AF308229	n.a.	n.a.	n.a.	n.a.
	P. K. Higashino 9461 (BISH)	Hawaii/Moloka'i	AF308228	n.a.	n.a.	n.a.	n.a.
	S. Perlman 6933 (BISH)	Hawaii/Hawaii	n.a.	KF235881	KF235690	KF235739	KF235638
	W. L. Wagner 5888 (BISH)	Hawaii/East Maui	AF308230	n.a.	n.a.	n.a.	n.a.
<i>Stenogyne kanehoana</i> Degener & Sherff	J. Obata 356 (BISH)	Hawaii/O'ahu	AF308232	KF235882	KF235691	KF235740	KF235639
<i>Stenogyne macrantha</i> Benth.	W. Mull & M. Mull 1980 (BISH)	Hawaii/Hawai'i	AF308233	n.a.	n.a.	n.a.	n.a.
<i>Stenogyne microphylla</i> Benth.	C. Lindqvist & V. A. Albert 35 (NY)	Hawaii/Hawai'i	AF308235	n.a.	n.a.	n.a.	n.a.
	F. R. Warshauer 2682 (BISH)	Hawaii/Maui	AF308234	KF235883	KF235692	KF235741	KF235640
<i>Stenogyne microphyllaxrugosa</i>	C. Lindqvist & V. A. Albert 38 (NY)	Hawaii/Hawai'i	AF308236	n.a.	n.a.	n.a.	n.a.
<i>Stenogyne purpurea</i> H. Mann	K. Wood 1772 (BISH)	Hawaii/Kaua'i	AF308237	n.a.	n.a.	n.a.	n.a.
	V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen HI03-40 (Volcano Rare Plant Facility)	Hawaii/Kaua'i	n.a.	KF235884	KF235693	KF235742	KF235641
<i>Stenogyne rotundifolia</i> A. Gray	F. R. Warshauer 2545 (BISH)	Hawaii/Maui	AF308238	n.a.	n.a.	n.a.	n.a.
<i>Stenogyne rugosa</i> Benth.	C. Lindqvist & V. A. Albert 40 (NY)	Hawaii/Hawai'i	AF308238	n.a.	n.a.	n.a.	n.a.
	B. H. Gagne s.n. 1975 (BISH)	Hawaii/Maui	AF308239	n.a.	n.a.	n.a.	n.a.
	V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen HI03-65 (Volcano Rare Plant Facility)	Hawaii/Hawai'i	n.a.	KF235885	AF502067	FJ854236	FJ854121
<i>Stenogyne scrophularioides</i> Benth.	W. L. Wagner 5954 (BISH)	Hawaii/Hawai'i	AF308241	n.a.	n.a.	n.a.	n.a.
	V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen HI03-58 (Volcano Rare Plant Facility)	Hawaii/Hawai'i	n.a.	KF235886	n.a.	n.a.	n.a.
<i>Stenogyne sessilis</i> Benth.	S. G. Weller 821 (BISH)	Hawaii/Hawai'i	AF308242	n.a.	n.a.	n.a.	n.a.
	O. Degener 33639 (NY)	Hawaii/Hawai'i	AF308244	n.a.	n.a.	n.a.	n.a.
	S. Perlman 15398 (BISH)	Hawaii/Maui	AF308243	n.a.	n.a.	n.a.	n.a.
	V. A. Albert, M. Bendiksby, C. Lindqvist, A. C. Scheen HI03-67 (Volcano Rare Plant Facility)	Hawaii/Hawai'i	n.a.	KF235887	KF235694	KF235743	KF235642
<i>Suzukia luchuensis</i> Kudo	S. Tawada & S. Hatusima 18179 (US)	C. & S. Nansei-shoto to Taiwan (Lü Tao)/ Okinawa island, Is. Kumejima	KF235781	KF235888	FJ854331	FJ854237	FJ854122
<i>Suzukia shikikunensis</i> Kudo	Chii-Cheng Liao et al. 564 (A)	C. & E. Taiwan/Taiwan Pingtung, Hsien	KF235782	KF235889	FJ854332	FJ854238	FJ854123

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