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Systematics and Ethnobotany of *Salvia* Subgenus *Calosphace* and Origins of the Hallucinogenic Sage, *Salvia divinorum*

A Dissertation submitted in partial satisfaction
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by

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ABSTRACT OF THE DISSERTATION

Systematics and Ethnobotany of *Salvia* Subgenus *Calosphace* and Origins of the Hallucinogenic Sage, *Salvia divinorum*

by

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Doctor of Philosophy, Graduate Program in Plant Biology
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Dr. Seung-Chul Kim, Chairperson

Salvia subgenus *Calosphace* (*Lamiaceae*), the largest of 5 subgenera with some 500 species and strongly supported as monophyletic, has received no comprehensive systematic research since the initial establishment of 91 taxonomic sections. Representative taxa of 73 sections of *Calosphace* were sampled to investigate the phylogenetic relationships and identify major lineages using chloroplast (intergenic spacer, *psbA-trnH*) and nuclear DNA (ribosomal spacer region, ITS). Phylogenetic analysis of the combined data set established the monophyly of nine sections (*Blakea, Corrugatae, Dusenostachys, Erythrostachys, Hastatae, Incarnatae, Microsphace, Nobiles,* and *Sigmoideae*) and four major lineages (*S. axillaris, "Hastatae* clade", "*Uliginosae* clade", and "Core *Calosphace*") corresponding with the four major stamen types identified within the subgenus. Disjunct sections spanning two or more centers of diversity are not supported by the results; no more than seven dispersal events to South America are required to account for the current disjunct distributions. One member of the subgenus, *Salvia divinorum* is hallucinogenic and used in traditional healing ceremonies by the Mazatecs of Mexico. It was classified within section *Dusenostachys* and hypothesized to be an interspecific hybrid. Multiple DNA regions (ITS, *trnL-trnF,* and *psbA-trnH*) of 52 species representing the major lineages of subgenus *Calosphace* and six accessions of *S. divinorum* were sequenced to test its phylogenetic position and putative hybridity. *Salvia divinorum* should not be classified within *Dusenostachys* nor is it a hybrid according to the results; its closest relative is *S. venulosa*, a Colombian endemic. In addition to *S. divinorum*, there are many other *Calosphace* species that are used medicinally in North and South America. Ethnobotanical data was gathered for 150 species; those sharing the common names, medicinal uses, appearances, and similar compounds associated into medicinal plant complexes. Five new, previously undocumented complexes were identified: *Mirto* (5 spp. used extensively in the treatment of *susto* and other illnesses in Mexico)*, Ñucchu* (7 spp. used as a symbolic element in religious processions and in the treatment of respiratory ailments in Peru)*, Lí'l++* (3 spp. used for food and medicine by the Chianantec), *Cantueso* (2 spp. used for respiratory ailments in Mexico)*,* and *Manga-paqui* (3 spp. used for kidney and liver problems in Ecuador).

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INTRODUCTION

The topic of this dissertation is the largest subgenus of *Salvia*, *Calosphace*, which is comprised of upwards of 500 species. *Calosphace* is strongly supported as monophyletic (Walker *et al*., 2004; Walker and Sytsma, 2007). Epling's (1939) monograph formally described 91 taxonomic sections within the subgenus. Species of *Calosphace* range from the Great Plains of U.S.A to southern South America, and the subgenus has four distinct centers of diversity: Mexico (more than 275 species), the Andean Cordillera (about 155 spp.), eastern Brazil (60 spp.) and the Caribbean Antilles (48 spp.). Morphological, cytological, and chemotaxonomic evidence suggests western Mexico as the origin of *Calosphace* and *Audibertia*, with subsequent dispersal events to South America (Neisess, 1983; Reisfield, 1987; Ramamoorthy *et al*., 1988; Harley and Heywood, 1992). While there has been no paucity of research within *Salvia* subgenus *Calosphace* in recent years, there has been no comprehensive treatment since Epling's (1939) monograph, however recent research (Reisfield, 1987; Torke, 2000) as suggested that Epling's sections are generally artifical, not reflected of the phylogeny. However, despite the large numbers of papers concerning *Calosphace*, there remain many unanswered questions concerning the biogeography, character evolution and relationships within the subgenus. There is clearly a need for a *Calosphace*-wide study of the subgenus to determine natural and well-defined groups — an adequate framework into which further research can confidently be placed — and thus the birth of chapter 1.

Probably the most interesting species within subgenus *Calosphace* is the hallucinogenic sage, *Salvia divinorum* Epling & Játiva-M., a powerful psychoactive plant that is used in healing ceremonies by the Mazatecs of northeastern Oaxaca, Mexico, for its vision-inducing effects (Wasson, 1962). Research on *Salvia divinorum* by pharmacologists and plant chemists has been extensive due to its unique secondary compounds. The chemical pharmacologically responsible for hallucinogenic activity in *S. divinorum* is salvinorin A (Siebert, 1994), a clerodane diterpenoid that functions as a highly selective κ-opioid receptor agonist (Roth *et al.,* 2002; Yan and Roth, 2004). However, in recent years the plant's availability for scientific research has been compromised by sensationalistic and exaggerated reports by the media of its use by young people.

The first botanical observations of *Salvia divinorum* were made by R. Gordon Wasson (1962) who sent flowering specimens to Carl Epling, who described the species, new to science (Epling and Jativa-M, 1962). *Salvia divinorum* was placed in section *Dusenostachys* (Epl.) Epl. and hypothesized to be closely related to *S. cyanea* Benth. (a synonym of *S. concolor* Lamb. ex Benth.) (Epling and Jativa-M., 1962). However, the botany of *S. divinorum* was not thoroughly examined until Reisfield (1987, 1993) who conducted extensive fieldwork and implemented a series of greenhouse breeding experiments. The results of his study led him to conclude that the species is probably and interspecific hybrid and perhaps also self-incompatible and possessed of inbreeding depression. The study that resulted in Chapter 2 was implemented in order to test Epling's classifcation of this species, determine its closest relatives, and to reevaluate

Reisfields conclusions. It was an early interest in and acquaintance with this species in particular that provided the motivation to initiate this *Salvia* project as a whole and in particular to investigate evolutionary origins of *Salvia divinorum*. When I began however, there was no phylogenetic work completed on section *Dusenostachys* nor even subgenus *Calosphace,* and so a dissertation was born.

But it is not simply *S. divinorum* that has a colorful ethnobotanical history; around the globe many species of sage are highly regarded for their usefulness in the treatment of illness and disease and employed in the healing practices and rituals of many indigenous cultures. And nowhere, perhaps, are they used more frequently and abundantly in folk medicine and ritual than in the Americas, the species of subgenus *Calosphace.* Aside, from a few important species whose ethnobotany has been extensively documented, *i.e. chia* (*S. hispanica*) (*e.g.*, Cahill, 2001, 2003, 2005) and *Salvia divinorum* (*e.g.*, Wasson, 1962; Valdéz *et al.*, 1983; Reisfield, 1993;), the extensive medicinal usage of *Calosphace* species has been greatly underrepresented and largely unreported in the literature. The secondary plant metabolites of *Calosphace* species are primarily terpenes and terpenoids. A number of these terpenes have been shown to be biologically or pharmacologically active (*e.g.,* Siebert, 1994; Imanshahidi and Hosseinzadeh, 2006; Topcu, 2006), including compounds active at the benzodiazepine and opioid receptors (Marder *et al.*, 1996; Viola *et al.*, 1997; Roth *et al.*, 2002). This prevalence of bioactive terpenes and the likelihood of others of importance yet to be discovered within *Calosphace* coupled with the common usage of these species in traditional folk medicine renders the subgenus an enticing research subject and the focus of the third chapter.

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CHAPTER 1:

Phylogenetic Relationships within New World *Salvia* Subgenus *Calosphace* (Benth.) Benth. (*Lamiaceae*) Based on cpDNA (*psbA-trnH*) and nrDNA (ITS) Sequence Data: Major Lineages and Staminal Diversification

Aaron A. Jenks, Jay B. Walker, and Seung-Chul Kim

Salvia subgenus _Calosphace_ (Benth.) Benth. (_Lamiaceae_), the largest of 5 subgenera with some 500 species and strongly supported as monophyletic, has received no comprehensive systematic research since the initial establishment of 91 taxonomic sections. Representative taxa of 73 sections of _Calosphace_ were sampled to investigate the phylogenetic relationships and identify major lineages using chloroplast (intergenic spacer _psbA-trnH_) and nuclear DNA (ribosomal spacer region ITS). Phylogenetic analysis of the combined data set established the monophyly of nine sections (_Blakea, Corrugatae, Dusenostachys, Erythrostachys, Hastatae, Incarnatae, Microsphace, Nobiles,_ and _Sigmoideae_) 4 major lineages (_S. axillaris, “Hastatae_ clade”, “_Uliginosae_ clade”, and “Core _Calosphace_”) corresponding with four major stamen types within the subgenus. Disjunct sections spanning two or more centers of diversity are not supported by our results; rather, relationships hereby supported exhibit significant geographic structure. No more than 7 dispersal events to South America are required to account for the current disjunct distributions observed within _Calosphace_.

Keywords: *Salvia*, *Calosphace*, *Lamiaceae*, sage, taxonomy, phylogeny, staminal evolution

INTRODUCTION

Salvia L. is a genus of approximately 900 species and has a worldwide distribution. It is the largest of an estimated 180 to 200 genera in family Lamiaceae. *Salvia* is placed in subfamily Nepetoideae (Cantino, 1992; Kaufmann and Wink, 1994) which is strongly supported as monophyletic (Cantino and Sander, 1986; Cantino, 1992; Wagstaff *et al.* 1995, 1998). The unique morphological features of *Salvia* androecia are distinctive within the subfamily; only two stamens are present, each of which has two anther cells (thecae) separated by elongated connective tissue, which forms a lever-like pollination mechanism (Sprengel, 1793; Himmelbaur and Stibal, 1933-1935; Claßen-Bockhoff *et al*., 2003). While recent molecular studies support a non-monophyletic *Salvia* and three independent origins of this unique stamen type, subgenus *Calosphace* (Benth.) Benth. was strongly supported as monophyletic and sister to subg. *Audibertia* (Benth.) Epl. ex Walker (Walker *et al*., 2004; Walker and Sytsma, 2007). *Audibertia* is comprised of 18 species (Neisess, 1983) restricted to the California Floristic Province of northwest Mexico and southwestern United States and has been considered closely related to *Calosphace* (Epling, 1938). Morphological analyses have additionally supported the monophyly of subg. *Calosphace* (El-Gazzar *et al*., 1968; Ramamoorthy and Lorence, 1987; Reisfield, 1987).

Calosphace was originally described by Bentham (1832). Although Linnaeus described only 28 species of *Salvia* (1753), by 1832 Bentham had described 266 species divided among 14 sections. In his contribution to DeCandolle's *Prodromus*, Bentham described 407 species of *Salvia*, and of these 243 were placed in section *Calosphace* (Bentham, 1848). In his *Genera Plantarum*, Bentham divided 12 sections among 4 subgenera, the largest of which was *Calosphace,* simply elevated from section to subgenus (Bentham, 1876). In Briquet's (1897) classification, Bentham's sections and subgenera were split into 8 subgenera, however subgenus *Calosphace* was left intact (although the name was changed to *Jungia*). The only comprehensive treatment of the subgenus since Briquet is Epling's revision (1939).

In contrast to the classifications of Bentham (1848) and Briquet (1897) which divided *Calosphace* into 6 and 7 sections respectively, Epling's (1939) monograph formally described 91 sections within the subgenus. Epling additionally described hundreds of new species bringing the total to 480 within *Calosphace.* However, in a series of 10 subsequent, supplementary publications, he expanded both the number of sections to 100 and species to over 500 (Epling, 1940a, 1941, 1944, 1947, 1951; Epling and Mathias, 1957; Epling, 1960; Epling and Játiva-M., 1963, 1966, 1968). Epling's classification is based primarily on flower structure, although habit, geographic distribution, and vegetative pubescence were also considered. While Epling erected his sections to represent natural groups, many of his sections have been criticized as artificial, the seemingly plastic corolla and vegetative morphology apparently being weighted too heavily (Reisfield, 1987; Torke, 2000).

As might be expected of such a large group, *Calosphace* is quite diverse. The species vary from annual herbs to perennial trees; from small, blue corollas to large, red corollas; and from non-specialist mellitophily to specialized ornithophily. The typical stamen morphology of *Calosphace* consists of a connective with elongated anterior and posterior branches but with only the anterior branches expressing fertile thecae. However, within this general stamen type, a wide range of variation is exhibited including multiple degrees of hetero- and homostyly. It may be that staminal variation has driven speciation as well as contributed to the maintenance of species diversity through mechanical isolation of sympatric species. While highly variable amongst species, the precise, reversible lever mechanism of the staminal connectives has been shown to tightly control pollen partitioning, depositing pollen on an exact area of each pollinator (Claßen-Bockhoff *et al*., 2004). This pollination lever mechanism appears to have arisen multiple times in *Salvia* and independently within *Calosphace* (Walker and Sytsma, 2007).

Species of *Calosphace* range from the Great Plains of U.S.A to southern South America, and the subgenus has four distinct centers of diversity (Figure 1.1). The primary center of species diversity is Mexico and Central America with more than 275 species. Mexico, with about 88% endemism, appears to have been a major center for species radiation (Ramamoorthy, 1984c). The second major center of diversity is the Andean Cordillera, with about 155 species ranging from Venezuela, through Colombia, Ecuador, Peru, Bolivia, to the Andes of northern Chile and western Argentina. Two additional centers of diversity are found in eastern South America and the Caribbean Antilles. The first of theses encompasses some 60 species of eastern Brazil, Uruguay, and eastern Paraguay. The Caribbean center of diversity (~48 species) is centered primarily upon the islands of Hispaniola (Haiti and the Dominican Republic) and Cuba.

Morphological, cytological, and chemotaxonomic evidence suggests western Mexico as the origin of *Calosphace* and *Audibertia*, with subsequent dispersal events to South America (Neisess, 1983; Reisfield, 1987; Ramamoorthy *et al*., 1988; Harley and Heywood, 1992). While *Calosphace* is strongly supported as monophyletic in a recent molecular study (Walker *et al*., 2004), the South American species appear deeply embedded within the Mexican lineages lending further support to the hypothesis of a Central American origin and subsequent dispersal event(s) to South America. It has been suggested that an Old World, common ancestor of the strictly New World *Audibertia*/*Calosphace* clade arrived in Mexico sometime in the Neogene (<23 mya) (Reisfield, 1987), probably the result of a single colonization event. Others (Neisess, 1983; Ramamoorthy *et al*., 1988) have argued for an earlier, Paleaogenic origin of *Salvia* in the Americas (45-50 mya), based among other things upon the strong association between *Calosphace* and the pine, oak and fir forest, the modern representatives of the ancient Madrotertiary flora. Fossilized *Salvia* pollen has been found in Alaska (from the upper Miocene) and Mexico (lower Miocene) (Emboden, 1964; Muller, 1981; Graham, 1999). Vicariant speciation is reported to be the most common mechanism accounting for the high levels of species diversity (Ramamoorthy and Lorence, 1987); while the levels of interspecific hybridization or introgressive speciation are reported to be remarkably low (Haque and Ghoshal, 1981; Ramamoorthy and Elliot, 1998), polyploidy is well-documented and appears to be much more frequent in South American lineages than North American (Alberto *et al*., 2003). The trend within *Calosphace* appears to be long-distance dispersal followed by rapid radiation.

While there has been no paucity of research within *Salvia* subgenus *Calosphace* in recent years, there has been no comprehensive treatment since Epling's (1939) monograph. This is due in large part to the daunting size of the subgenus, the sheer numbers of taxa many of which are either difficult to distinguish, poorly described, or only collected once or twice—in short, due to the lack of a good framework for further research. In spite of these handicaps, a number of sectional revisions have been produced (Peterson, 1978; Reisfield, 1987; Santos, 1991; Espejo-Serna and Ramamoorthy, 1993; Santos, 1995, 1996; Turner, 1996; Torke, 2000; Santos, 2004; Santos and Harley, 2004) while even more papers have described new species or made other contributions to our understanding of the group including many floras and geographically restricted treatments (*e.g.*, Epling, 1940b; Nowicke and Epling, 1969; Standley and Williams, 1973; Ramamoorthy, 1984a, 1984b, 1984c, 1984d; Ramamoorthy and Lorence, 1987; Wood and Harley, 1988; Santos, 1993, 1994; Fernández-A., 1995a, 1995b, 1995c; Xifreda, 1999; Fernández-A., 2002, 2003). Recent papers have even elucidated the relationship of *Calosphace* to other members of genus *Salvia* and have proffered clues to the *Calosphace* phylogeny (Walker *et al*., 2004; Walker and Sytsma, 2007). However, despite the large numbers of papers concerning *Calosphace*, there remain many unanswered questions concerning the biogeography, character evolution and relationships within the subgenus. There is clearly a need for an updated treatment of the subgenus based upon natural and well-defined groups, an adequate framework into which further research can confidently be placed, and it is towards these ends that our current paper strives. We hope to more fully describe the major lineages and evolutionary trends within the subgenus. Specifically, we seek to determine the major lineages and investigate the phylogenetic relationships within *Salvia* subg. *Calosphace*, testing previous hypotheses of relationships, specifically Epling's (1939) classification; in addition, this paper will test the hypothesis of Mexican origin for *Calosphace* and subsequent dispersal events to South America and address questions including number of long-distance dispersals, character evolution, disjunct lineages, and speciation.

MATERIALS AND METHODS

Nomenclature:

Species-level nomenclature follows Alziar (1988-1993), while supraspecific classification follows Epling (1939, 1940, 1941, 1944, 1947, 1951; Epling and Mathias, 1957; Epling, 1960; Epling and Játiva-M., 1963, 1966, 1968). Although taxonomic changes have been proposed by later authors (*i.e.*, sections renamed or combined and species moved between sections), Epling's classification remains the only comprehensive treatment of the subgenus. Comment will be made in the discussion concerning subsequent changes in sectional placement or nomenclature published by later authors.

Taxon Sampling and Outgroup Selection:

Sequence data were obtained for 336 accessions; of these, 150 represented duplicate species. Initial, neighbor-joining analysis was performed on all 336 samples to test for intraspecific monophyly. After eliminating duplicates only 175 taxa (including four outgroup taxa) were used in final maximum parsimony (MP) analyses of the combined datasest. Epling originally erected 91 sections (1939) within *Calosphace*, but by the time of his death had expanded this to 102 sections (Epling, 1940a, 1941, 1944, 1947, 1951; Epling and Mathias, 1957; Epling, 1960; Epling and Játiva-M., 1963, 1966, 1968). Our sampling includes taxa from 73 of these sections, representing all of the major sections, *i.e*. none of the sections not sampled were comprised of more than 3 species (except section *Urbania* Epl., endemic to the island of Hispaniola (10 spp), for which we were unable to obtain material). In addition, for 11 sections having disjunct distribution, we have also sampled from multiple centers of diversity.

Outgroups used for *Calosphace*-wide analyses were chosen based upon previous molecular studies (Walker *et al*., 2004; Walker and Sytsma, 2006); these species were *Dorystaechas hastata* Boiss. & Heldr. ex Benth.*, Salvia greatae* Brand.*, S. californica* Brand., and *S. mellifera* Greene*. Dorystaechas hastate* Boiss. & Heldr. ex Benth., a monospecific Asian genus, was strongly supported as sister to the clade comprised of *Audibertia* and *Calosphace*. *S. greatae, S. californica, and S. mellifera* are all placed within *Audibertia*, strongly supported as sister to Calosphace (Walker *et al*., 2004).

All samples were either collected by the authors or obtained from herbarium specimens. Accession information and vouchers for the 175 taxa analyzed in the MP analyses are given (Appendix 1).

DNA isolation, amplification, and sequencing:

Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA). Extractions were made from fresh, dried (including herbarium specimens), or frozen leaf material. Polymerase chain reaction (PCR) amplifications were typically prepared in 25 μl reactions; PCR reactions were performed on a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Watertown, MA). The PCR profile for ITS and *psbA-trnH* was as follows: 2 minutes of initial denaturation at 95° C, 35 cycles with a 1minute denaturation at 94° C, 2 minutes annealing at 52° C, and 2 minutes extension at 72° C, with a final extension of 10 minutes at 72° C. The entire nuclear ribosomal internal transcribed spacer (ITS) region (including ITS1, the 5.8S gene, and ITS2) was amplified with primers ITS5 and ITS4, or alternately in the case of more highly degraded DNA from herbarium specimens, in two parts with primer combinations ITS5-ITS2 and ITS3-ITS4 (from White *et al*., 1990). The noncoding, intergenic spacer region of chloroplast DNA, *psbA-trnH*, was amplified using forward and reverse primers as described previously (Kim *et al*., 1999). All PCR products were purified with the QIAquick PCR purification Kit (Qiagen). Sequencing reactions were carried out for the purified PCR products using Big Dye Terminator Cycle Sequencing reagents (Applied Biosystems, Foster City, California, USA). Sequencing primers used were identical to amplification primers (4 primers for ITS and 2 primers for *psbA-trnH*).

Sequence editing and alignment:

Sequence fragments were assembled and edited using Sequencer version 4.2.2 (Gene Codes, Ann Arbor, Michigan, USA). The corrected consensus sequences were aligned manually in MacClade v4.06 (Maddison and Maddison, 2003).

Phylogenetic analyses:

Insertions or deletions were present in the alignment of the noncoding *psbA-trnH* region (7 indels) and the ITS region (4 indels). Although no gap coding was performed, these unambiguous indels were identified and mapped to the tree topology (Figure 1.2-4).

Initially the ITS and *psbA-trnH* data sets were analyzed independently; however, the cpDNA had very low sequence variability (low % of parsimony informative characters) and a number of the taxa shared identical *psbA-trnH* sequences. As a result, no tests for incongruency between the ITS and cpDNA datasets were performed, however examination of the support values for the cpDNA topology favored by each locus revealed no supported nodes in conflict with ITS. We therefore combined the ITS and *psbA-trnH* datasets for further analysis.

The three data sets were analyzed using an equally weighted, unordered maximum parsimony (MP) approach (Fitch, 1971) implemented in PAUP ver. 4.0 (Swofford, 2002). The MP analyses for each data set included a heuristic search for the most parsimonious trees; starting trees were obtained via stepwise addition. Sequences were added via simple addition with 1 tree held at each step. Branch swapping was performed via tree-bisection-recombination (TBR), and steepest descent and MulTrees options were in effect. Branches were collapsed if maximum branch length was zero, and topological constraints were not enforced. Support for nodes was calculated by bootstrap analysis with 250 simple-addition bootstrap replicates, each with a maximum of 200 trees saved and TBR branch-swapping.

In order to search for multiple islands, we performed another MP heuristic search of the 3 data sets independently, employing random addition instead of simple. The search was performed with 300 random addition sequences; due to the large data set, each replication limited to 5 minutes in order for the analysis to run to completion. TBR, MulTrees and Steepest descent were in effect, while the maximum number of trees held was set to 20,000. Branch-swapping was via stepwise addition and 1 tree was held at each step. Branches were collapsed if their score was zero, gaps were coded as missing data, and characters were unordered. The bootstrap values were generated via an abbreviated analysis that included 100 bootstrap replicates, each with 5 random addition sequences and each of these limited to 120 seconds. No more than 15,000 trees were saved per replicate, while all other parsimony parameters were constant with the heuristic searches.

RESULTS

Phylogenetic relationships:

The ITS data set contained 181 taxa, including 4 outgroup taxa, and 654 total characters (total aligned sequence length) of which 252 were constant (38.5%). Of 402 variable characters (61.5%), 126 were parsimony un-informative (19.3%) and 276 were parsimony informative (42.2% of total). The *psbA-trnH* dataset contained 184 taxa, including 4 outgroup taxa, and 497 characters of which 340 were constant (68.4%). Of 157 variable characters (31.6%), 77 were parsimony informative (15.4% of total). The combined data set of 175 taxa (including 4 outgroup taxa) contained a total of 1151 characters of which 615 were constant (53.4%), 536 were variable (46.6%), and 342 were parsimony informative (39.7% of total). (Table 1.1)

The shortest ITS trees were 1835 steps long and had a consistency index (CI) of 0.3613, homoplasy index (HI) of 0.6387, retention index (RI) of 0.6890, and a rescaled consistency index (RC) of 0.2489. The MP analysis of the *psbA-trnH* dataset was unable to run to completion although it was allowed to run for several days; this was presumably due to the comparatively low percentage of parsimony-informative variable characters and the higher numbers of taxa sharing the same sequence for this region. The shortest *psbA-trnH* trees were 286 steps long and had a CI of 0.7063, HI of 0.2937, RI of 0.7971, and RC of 0.5630. The strict consensus tree from the MP analysis of the chloroplast data result in large numbers of unresolved taxa and only few clades were identified. However the clades that were present in the strict consensus tree were each strongly supported (>99% bootstrap support) and were not in conflict with ITS. (Table 1.1)

In the MP analysis of the combined nuclear and chloroplast dataset, the shortest trees were 2177 steps long and had the following index values: CI 0.3817, HI 0.6881, RI 0.6790, and RC 0.2592 (Table 1.1). In the strict consensus (of 50,000) tree (Fig. 2), *Salvia axillaris* Moc. & Sessé represents the most basal lineage within a monophyletic

Calosphace, 82% MP Bootstrap Probability (BP). The monophyletic "*Hastatae* clade" (97%BP) contains two monophyletic sections, Central American *Blakea* Epl. (66%BP) and Andean *Hastatae* (Benth.) Epl. (77%BP). Within the monophyletic "*Uliginosae* clade" (85%BP) are several monophyletic sections: *Incarnatae* Epl. (78%BP), *Corrugatae* (Benth.) Epl. (86%BP), and *Microsphace* (Benth.) Epl. (78%BP). Other monophyletic lineages identified include: Section *Erythrostachys* (Epl.) Epl., section *Dusenostachys* (Epl.) Epl. (100%BP), core "*Fulgentes* clade" (84%BP), "*Rubescentes* clade" (100%BP), *"Sigmoideae* clade" (95%BP) (within which section *Sigmoideae* is monophyletic (100%BP)), the *S. tubifera* group (75%BP), and the "Andean *Purpurea* clade" (76%BP). The "*Angulatae* clade" is well-supported (84%BP) in the ITS phylogeny.

Clade names in quotations are not formal taxonomic suggestions but rather are named to facilitate ease of referencing, for the purposes of this paper, the monophyletic groups comprised in our results of species from multiple taxonomic sections. Names chosen for these clades are based upon priority (*e.g. Hastatae* has priority over *Blakea* or *Standleyana* Epl. (*S. cacaliifolia* Benth.), hence "*Hastatae* clade") and second, in the case of equal priority amongst two or more sections with species resolved within said clade, that clade is named after the section with the greatest number of species represented within the clade (*e.g.* several sections within the "*Ulignosae* clade" have equal priority, however the clade is named for section *Uliginosae* (Epl.) Epl. since nine species of that section are represented within that clade, more than from any other section represented). In addition, a large clade comprising approximately 2/3 of the sampled taxa, is termed herein “core *Calosphace*”. This clade is the largest and collectively the most derived of the *Calosphace* lineages. It contains a unique, 9 bp deletion (indel psbA1) and was previously identified as a strongly supported monophyletic clade (99%BP) based upon molecular analysis involving a sampling of other taxa and gene regions (Walker, 2006). Our analysis does not provide strong bootstrap support for this clade, however, this group as a whole does exhibit comparatively much less sequence variation and shorter branch-lengths than the basal lineages described above and seems to represent a rapid and more recent radiation of species.

Indel Mapping:

The only unambiguous indel in the ITS sequence (of 4) was a 41 base pair (bp) deletion (ITS1) occurring from character 43 to 83. The clades containing this deletion (Figure 1.2) were *Salvia axillaris* (however in this lineage the deletion event was 59 bp), the more derived members of the “*Uliginosae* clade” (here with a reversal event in section *Corrugatae* species), sections *Erythrostachys* and *Dusenostachys* (in *Dusenostachys* the deletion was only 41 bp).

The first unambiguous indel in the *psbA-trnH* dataset is a 9 bp deletion (psbA1) occurring in “core *Calosphace*” (Fig. 2). The psbA1 deletion is also found in *S. ovalifolia* St. Hil. ex Benth. In the *psbA-trnH* datamatrix, psbA1 occurs from character 233 to 241. The second indel (psbA2) is a 5 bp deletion occurring in only 4 species: *Salvia sinaloensis* Fern*., S. albo-caerulea* Lind*., S. occidentalis* Swarz*,* and *S. misella* Kunth. This indel is found from character 344 to 348. Indel psbA3 is a 6 bp deletion occurring from character 350 to 355 that is synapomorphic for the "*Uliginosae* clade"; however within that clade there are a few indel reversions: *S. ovalifolia* and the four species containing indel psbA2. Indel psbA4 is a 4 bp deletion occurring from character 389 to 392 in four species of the "*Hastatae* clade": *S. cacaliifolia, S. scutellaroides* Kunth., *S. macrophylla* Benth., and *S. sagittata* R.&P. Indels are phylogenetically informative and provide additional evidence supporting the monophyly of the lineages that contain them. (Figure 1.2)

DISCUSSION

The phylogenenetic discussion of subgenus *Calosphace* is based upon the monophyletic clades supported by our molecular analyses. While there was strong support for multiple clades, especially the more basal lineages, there remains but weak support for many others, specifically certain of the internal branches. Furthermore, as staminal morphology has proved pivotal in our understanding of the *Salvia* relationships at large (Walker and Sytsma, 2007), the significant trends staminal evolution within *Calosphace* are discussed. The biogeographic implications of our molecular phylogeny are also hereby described. (We consider relationships >90% MP Bootstrap Probability (BP) strongly supported, >70% BP well-supported, and >50% BP only moderately supported. Phylogenetic relationships presented are based upon the combined dataset MP analysis unless otherwise stated.)

Phylogeny of *Calosphace*:

Salvia axillaris

Salvia axillaris is placed in the monotypic section *Axillares* (Benth.) Epl. and is well-supported as sister to the rest of *Calosphace* in our analysis (Figure 1.2). This small, woody subshrub, native to Central Mexico and having three described varieties (Turner 1996), is morphologically unique within *Calosphace*. *S. axillaris* alone within *Calosphace* expresses two fertile anther sacs per stamen (stamen type 1; Figure 1.5 and Table 1.2 however this trait is shared by species of *Audibertia* (particularly the more basal lineages (Walker and Sytsma, 2007)). The morphological intermediacy of *S. axillaris* and its affinities with *Audibertia* have been noted earlier (Epling, 1938; Ramamoorthy and Elliot, 1998), specifically the primitive staminal morphology (discussed below).

"Hastatae **clade"**

This strongly supported (97%) monophyletic clade is well supported (81%BP) as sister to the rest of *Calosphace* (all other species except *S. axillaris*) (Figure 1.2). This clade is comprised of three sections: *Blakea*, comprised of 6 Mexican and Central American endemics represented in our study by *S. patens* Cav. and *S. vitifolia* Benth.; *Standleyana*, a monotypic genus containing *S. cacaliifolia* of southern Mexico and Guatemala; and *Hastatae*, a section of 7 species endemic to the northern Andes (Venezuela to Peru) represented in our sampling by *S. scutellaroides S. macrophylla* and *S. sagittata*. As Epling pointed out (1939), *S. cacaliifolia* is morphologically intermediate between sections *Blakea* and *Hastatae*, and this relationship is supported by the molecular phylogeny. The strictly Andean *Hastatae* appears to represent a single, early dispersal event from Central America to South America. While these three sections are geographically and morphologically distinct from each other and find molecular support for their respective monophyly, together they form a natural group deserving further study.

These three sections were recognized as a natural group by Epling (1939), who in his unpublished manuscript (Epling ms) placed them together in series *Dilatae*. Later workers noted that section *Hastatae* was a ‘distinctive’ group with less intra-specific variation than most (Wood and Harley, 1988). The three taxonomic sections present in this clade share common vegetative traits, including the distinctive glandular, hastate leaves for which the section is named. However, while the flowers are frequently showy and the plants often cultivated for their beauty, the corolla morphology varies considerably amongst the sections. The species of *Blakea* have large, arching galea (*S. patens* reported to have the largest in the subgenus (Walker, 2006)) and well-developed, slightly deflexed lower lip, while *S. cacaliifolia* has greatly reduced galea and lower lip. While the corolla size, shape, and even color (all blue-flowered except *S. rubriflora* (*Hastatae*) with its red corollas) vary considerably amongst the three sections represented in this clade, their reproductive structures are conserved. All share a greatly reduced to absent upper stigma lobe, moderately to strongly excerted stamens, and posterior connective branches that are much shorter than the excerted fertile branch. These posterior connectives are widened into a disc-like plate and frequently free (stamen type

2). It is within this group that we see the first functional staminal lever mechanism expressed in *Calosphace* (Figure 1.5, Table 1.2).

"*Uliginosae* clade"

The "*Uliginosae* clade" is well supported as monophyletic (85%BP) and contains a synapomorphic 6 bp deletion (indel psbA3) (Figure 1.2). This clade contains species from sections *Tomentellae* (Epl.)Epl. ("*Tomentellae* clade I"), *Uliginosae, Incarnatae*, *Corrugatae*, *Microsphace*, and the monotypic sections *Cucullatae* Epl. (*S. clinopodioides* Kunth.) and *Fernaldia* Epl. (*S. albo-caerulea* Lind.). It can be generalized that the species of the *"Uliginosae* clade" are perennial subshrubs (except the annual species of section *Microsphace)* with medium-sized blue flowers (except for the ornithophylous, red-flowered species of section *Incarnatae).* However, the most distinguishing feature of the "*Uliginosae* clade" is a unique staminal structure (stamen type 3; Figure 1.5 and Table 1.2); this structure is also found in the species of *Tomentellae* not placed within the "*Uliginosae* clade", *i.e.* "*Tomentellae* clades II & III". However, this synapomorphy is not found in either *S. ovalifolia* or *S. mocinoi* Benth., the first due to a putative hybrid origin and the second due, possibly, to misidentification This staminal synapomorphy is an appendage is a forward-facing or 'assurgent' tooth located on the ventral side of the posterior connective branches. (See discussion of staminal evolution later in the discussion; Figure 1.5, Table 1.2). The "*Uliginosae* clade" is roughly equivalent to Epling's unpublished series *Assurgentes* (Epling, unpublished), named for this assurgent staminal tooth.

The largest number of species found within this clade are members of section *Uliginosae* (9 spp), although one species of that section, *S. villosa* Fern., is strongly supported elsewhere in the "*Fulgentes* clade". Within the "*Uliginosae* clade", section *Uliginosae* is paraphyletic with other lineages arising within it. Section *Uliginosae* is found in two centers of diversity, Mexico and Brazil; the Brazilian species (*S. uliginosa* Benth. and *S. procurrens* Benth.) are sister to the strictly North American *Incarnatae* (*S. elelgans* Vahl. and *S. cinnabarina* Mart.&Gal.). The two Brazilian *Uliginosae* sampled are paraphyletic to *S. ovalifolia* of the Brazilian section *Rudes* Epl. The placement of *S. ovalifolia* within the "*Uliginosae* clade" is unexpected; the other species sampled from *Rudes* form a well-supported monophyletic clade (87%BP) within the "core *Calosphace*" (Figure 1.3). Importantly, *S. ovalifolia* does not contain the cpDNA deletion that the rest of "*Uliginosae* clade" has (psbA3), while it does contain the 9bp deletion found in every species within the "core *Calosphace*" (psbA1). Morphologically, it does not contain the assurgent tooth on the staminal connective distinctive of the "*Uliginosae* clade". In the ITS phylogeny *S. ovalifolia* is strongly supported as sister to *S. procurrens* (100%BP), while in the chloroplast phylogeny *S. ovalifolia* is found outside the "*Uliginosae* clade" in an unresolved polytomy and *S. procurrens* and *S. uliginosa* are strongly supported as sister (100%BP). Finally, *S. ovalifolia* is sympatric with *S. procurrens* and *S. uliginosa*. The evidence therefore suggests that *S. ovalifolia* is likely the result of recent, interspecific hybridization, a probable example of reticulate evolution within *Calosphace*.

Two species of section *Tomentellae* ("*Tomentellae* clade I"), *S. fruticulosa* Benth. and *S. parryi* Gray, are basal to the rest of the "*Uliginosae* clade". The other sampled species of this section are not supported within the "*Uliginosae* clade". There are three sections supported as monophyletic within the "*Uliginosae* clade": *Incarnatae*, *Corrugatae,* and *Microsphace*. *Incarnatae* contains two Central American endemics; both *S. elegans* and *S. cinnabarina* were sampled. This section is anomalous in the "*Uliginosae* clade"; in this clade they are the only ornithophily-specialized species (red flowers, elongated corolla tube, and non-functional staminal lever mechanism). The strictly Andean *Corrugatae* contains 5 species ranging from Colombia to Bolivia and is represented in our study by *S. corrugata* Vahl. and *S. styphelus* Epl. *Corrugatae* represents the second distinct South American lineage originating with the "*Uliginosae* clade", however the other lineage South American lineage, the clade containing *S. uliginosa, S. procurrens,* and *S. ovalifolia*, contains only species from the Brazilian center of diversity. Section *Microsphace* contains five, annual species and is here represented by *S. misella* and *S. occidentalis*. This is significant in that it is represents one of only two or three times annual species have arisen within an otherwise perennial subgenus. Lastly, mention must be made of *S. mocinoi* Benth., the only species of section *Membranaceae* (Benth.) Epl. sampled in our study. This section, with its large, persistent, membranous bracts does not express assurgent teeth on the posterior branch of the staminal connective as do the rest of the species of the "*Uliginosae* clade." Further sampling from *Membranaceae,* including other accessions of *S. mocinoi,* is required to determine whether the loss of assurgency is actually occurring within "*Uliginosae* clade" or whether this collection of *S. mocinoi* was simply misidentified.

To summarize, the “*Uliginosae* clade” is well supported as monophyletic based upon molecular evidence, its possession of a novel indel, and the synapomorphic staminal morphology (the assurgent tooth). Within this clade we see two independent dispersal events to South America: one within section *Uliginosae* to the eastern Brazilian center of diversity (including Uruguay and eastern Paraguay) and the other to the northern Andes represented by the species of section *Corrugatae*. Within the *“Uliginosae* clade” we see an evolution from blue-flowered, generalist mellitophily to the specialized ornithophily (Wester, 2007) of *S. elegans* and *S. cinnabarina* (sect. *Incarnatae*). Finally, the transition from perennial shrubs/subshrubs to annual herbs (sect. *Microsphace*) is seen within the clade.

Section *Tomentellae*

In our analyses, species of *Tomentellae* are found within four distinct clades. Two species are sister to the remaining “*Uliginosae* clade” (“*Tomentellae* clade I”), another, *S. candicans* Mart. & Gal., is sister to *S. oaxacana* Fern. and section *Dusenostachys,* while the rest of the sampled species are found within two exclusively *Tomentellae* clades (“*Tomentellae* clade II” and “*Tomentellae* clade III”) (Figure 1.2). The three sampled Andean species, *S. bangii* Rusby, *S. retinerva* Briq., and *S. gilliesii* Benth., form a monophyletic (100%BP) clade (“*Tomentellae* clade II”) and two species from Mexico, *S. ballotaeflora* Benth. and *S. pinguifolia* (Fern.) Wooten & Standley, comprise another monophyletic (60%BP) clade (“*Tomentellae* clade III”). All species of *Tomentellae* share a staminal synapomorphy with the “*Uliginosae* clade”; they all express forward-facing (assurgent) teeth on the ventral side of the elongated staminal connectives. At this point it is unclear whether these two *Tomentellae* clades form a monophyletic group together with the "*Uliginosae* clade" (weakly supported in the ITS phylogeny). Further work is required to clarify the delimitations of the *Tomentellae/Uliginosae* group, including sequencing more gene regions and a more thorough analysis of the morphology.

Sections *Erythrostachys* and *Dusenostachys* and associated taxa

The taxa here discussed are those species found between "*Tomentellae* clade III" and the "core *Calosphace*" clade on the MP tree (Figure 1.2). The species that make up this loose assemblage are *S. candicans, S. oaxacana* and those of sections *Erythrostachys* and *Dusenostachys.* In addition, this intermediate position is held by *S. venulosa* Epl. and *S. divinorum* Epl. & Játiva-M. This group of taxa (except *S. divinorum* and *S. venulosa*) are represented as a monophyletic group in the MP analysis, however the bootstrap support is weak (<50%). The staminal morphology of these intermediate taxa is similar to that of "core *Calosphace*", *i.e.* the lower (posterior) staminal connective branches are either entire or express a late-developing, small, retrorse tooth near the filament attachment point (stamen type 4; Figure 1.5, Table 1.2). However, unlike the "core *Calosphace*" clade, these taxa do not contain the 9 base-pair deletion, psbA1 (Figure 1.2-4).

Section *Erythrostachys* is comprised of 5 arborescent species, four species of Mexico (*S. regla* ranges into the far South of Texas) and another, not sampled, from Colombia. These large, beautiful plants are also notable for their large, red corollas ensconced in flaring campanulate calyces. The shared morphological characters would suggest monophyly, and the molecular evidence generally supports this. *S. regla* Cav., *S. betulaefolia* Epl., and *S. pubescens* Benth. are all well-supported as monophyletic (86%BP), however, the support for the inclusion of *S. sessei* Benth. in this clade is weak. *S. sessei* is placed as sister to *Tomentellae* species, *S. ballotaeflora* and *S. pinguifolia,* in the ITS phylogeny but that relationship is also only weakly supported (<50%BP). A relationship between sections *Tomentellae* and *Erythrostachys* is again suggested by the placement of *S. candicans* (*Tomentellae)* sister to *Erythrostachys*.

In the ITS phylogeny, *S. oaxacana* (sect. *Conzatiana* Epl.) is strongly supported as sister to *S. candicans* (99%BP), while in the combined analysis they together form a monophyletic group with section *Dusenostachys*. The other sampled member of section *Conzatiana*, *S. aspera* Mart. & Gal., is placed in an unresolved polytomy within the "core *Calosphace*" in our analysis. Section *Conzatiana*, comprised of only three species, appears polyphyletic. The unsampled species, *S. pexa* Epl., is more closely allied with *S. oaxacana* and would probably be found near that species rather than *S. aspera.* While all three species are small, low-lying woody perennials of the Tehuacan valley, Oaxaca, the large, red, bird-pollinated flowers of *S. oaxacana* and *S. pexa* distinguish them from *S. aspera,* with its small, yellow, primarily bee-pollinated flowers. The floral morphology of *S. oaxacana* is highly suggestive of section *Erythrostachys.*

Section *Dusenostachys* is represented in our study by *S. recurva* Benth. (syn. *S. atrocaulis* Fern.), *S. concolor* Lamb. ex Benth., *S. flaccidifolia* Fern., and *S. divinorum* Epl. & Játiva-M. The section as originally circumscribed contained *S. madrensis* Seem.

(also sampled), however this species was removed from *Dusenostachys* to be placed in a newly erected section *Longipes* Epl. (Epling, 1944). The results of our analysis support the removal of *S. madrensis* from *Dusenostachys;* our results also suggest that *S. divinorum* is not closely related to the other members of *Dusenostachys. S. flaccidifolia* was removed from section *Dusenostachys* and placed within section *Angulatae* (Epl.)Epl. near species *S. arthrocoma* Fern. and *S. cyanantha* Epl. (Epling, 1941). While *S. flaccidifolia* occurs sympatrically with *S. arthrocoma,* our analysis supports a relationship with the other species of *Dusenostachys* (*S. concolor* and *S. recurva*) rather than species of *Angulatae*. The four sampled species of *Dusenostachys* are grow only in or on the banks of streams and waterways (uniquely hydrophytic within *Calosphace*), have fragile, hollow stems, and large, deep blue-to-purple corollas (*S. divinorum* has violet to white corollas). Reisfied (1987) concluded that *Dusenostachys* was largely artificial and that *S. divinorum* was especially misplaced in *Dusenostachys*. While *S. divinorum* appears not to be correctly placed in this section, the remaining sampled species are strongly supported as monophyletic (100%BP) in our study. The lack of support and long branch-length call into question this section's association with section either *Erythrostachys*, *S. candicans,* or *S. oaxacana.* Further studies on this section, including *S. divinorum,* are currently underway. (Of the other, unsampled species of *Dusenostachys, S. biserrata* Mart.&Gal. of Pico Orizaba appears related but is probably extinct; *S. monclovensis* Fern., endemic to the desert mountains of Coahuila, appears unrelated based on morphology and partial sequence data (not included in final analysis); and *S. oligantha* Dusen. (syn. *S. lepida* Epl.) of Brazil is also unlikely to be closely related.)

Salvia divinorum and *S. venulosa* are strongly supported (100%BP) as sister to each other. A close relationship between *S. divinorum* and the other sampled species of section *Dusenostachys* is not supported by our results. *S. venulosa*, a narrow endemic of northern Colombia, was placed in the rather variable section *Tubiflorae* (Epl.)Epl. The other sampled species of *Tubiflora* are *S. tortuosa* Kunth. and *S. tubifera* Cav., however morphologically *S. tortuosa* does not appear to be closely related to the rest of the section (Wood and Harley, 1988). Our results indicate that section *Tubiflorae* is highly polyphyletic. Further sampling of the Colombian members of *Tubiflorae* is needed to determine if any of these species might be closely allied with *S. divinorum* and *S. venulosa.*

***“Fulgentes* clade”**

The clade, comprised solely of Mexican and Central American endemics, includes all sampled members of sections *Fulgentes* Epl. and *Cardinalis* Epl., and some of the species assigned to sections *Nobiles* Epl. and *Flocculosae* (Epl.)Epl. (Figure 1.3). Sampled members of *Fulgentes* were *S. pulchella* D.C., *S. microphylla* (var. *microphylla)* Kunth., *S. microphylla* var. *neurepia* (Fern.)Epl., *S. microphylla* var. *wislizeni* Gray (syn. *S. lemmoni* Gray), *S. lineata* Benth. and *S. fulgens* Cav. We were able to sample section *Cardinalis* essentially in its entirety including species: *S. dorisiana* Standl., *S. karwinskii* Benth., *S. wagneriana* Polak., *S. involucrata* Cav. (syn. *S. puberula* Fern.), *S. holwayi* Blake, *S.* (cf.) *stolonifera* Benth., and *S. cardinalis* Kunth. The members of section *Flocculosae* found within this clade are *S. lycioides* Gray, *S. muelleri* Epl., *S.*

coahuilensis Fern. and the commonly cultivated ornamental *S. greggii* Gray. *S. gesneraeflora* Lindl. & Paxton, *S. adenophora* Fern. and *S. disjuncta* Fern. all of section *Nobiles* were found within the '*Fulgentes* clade' as were *S. madrensis* (*Longipes*) and *S. villosa* Fern. (*Uliginosae*).

When Ramamoorthy (1984d) placed *S. cardinalis* (the type of sect. *Cardinalis*) as synonym under *S. fulgentes*, he erected section *Holwaya* to include the remaining members of what had been section *Cardinalis.* However, our results do not support either of these sections as monophyletic, the species of each intermingled with each other. The relationship between these two sections was noted previously based upon secondary metabolites (Rodriquez-Hahn *et al*., 1988). In a revision of section *Nobiles,* dos Santos (1991) recognized that the three Mexican species assigned to the otherwise Brazilian section were better placed in section *Holwaya*; these three species are *S. gesneraeflora, S. adenophora* and *S. disjuncta*. In our analyses, these species are indeed placed within the *"Fulgentes* clade". *S. cardinalis* was placed as a synonym under *S. fulgens* (Ramamoorthy, 1984d), and it was further suggested that *S. gesneraeflora* be placed as synonym with *S. fulgens* (Santos, 1991). These three taxa do form a monophyletic clade (97%BP) in our analysis, thus seeming to support at some level these assertions. However, while morphologically these taxa certainly appear conspecific, we are hesitant to offer full support of synonymy until a more thorough morphological analysis is performed due to sequence variability amongst these taxa. *S. microphylla* Kunth and its two described varieties, *S. m.* var. *neurepia* and *S. m.* var. *wislizeni,* is not supported as monophyletic by our results; variety *wislizeni* is placed within the *Flocculosae* of the *'Fulgentes* clade' strongly supported as sister to *S. coahuilensis* Fern. (96%BP).

Salvia madrensis while first placed in section *Dusenostachys* (Epling 1939) and later placed into section *Longipes* (Epling 1944) appears unrelated to the other species of either section and is placed here within the *'Fulgentes* clade'. The other sampled members of *Dusenostachys* (*S. recurva*, *S. concolor,* and *S. flaccidifolia*) form a monophyletic clade (100%BP) outside of the 'core *Calosphace',* while the other sampled species of *Longipes* (*S. gravities* Epl. and *S. erythrostoma* Epl.) form a strongly supported monophyletic (100%BP) clade ("*Rubescentes* clade") with *S. rubescens* Kunth. (sect. *Rubescentes* Epl.). Morphologically, *S. madrensis* appears closely allied with *S. subhastata* Epl. (of monotypic section *Sphacelioides* Epl., not sampled in our current molecular analysis), while the current molecular results suggest a relationship between *S. madrensis, S. adenophora,* and *S. disjuncta.*

In summary, the "*Fulgentes* clade" represents another departure from the pleisiomorphic blue-flowered condition and a radiation of large, red-flowered, ornithophilous Central American species. While this monophyletic clade contains all sampled members of sections *Fulgentes* and *Cardinalis*, neither section is monophyletic within this clade. Section *Nobiles* as proposed by Epling is clearly polyphyletic, and our results support the removal of the three Mexican members from *Nobiles* as proposed by Santos (1991) their subsequent association here with the '*Fulgentes* clade'. Section *Flocculosae* appears at the least paraphyletic in our results with members found not only within this clade but elsewhere. While the '*Fulgentes* clade' appears to be a natural group, additional research is needed to clarify its delimitations and inter-specific relationships, as well as to ascribe new nomenclature to this seemingly monophyletic assemblage.

South American Lineages

Several groups deserve mention in an otherwise rather poorly resolved "core *Calosphace*" (Figure 1.3,4). The monotypic section *Discolores* (Epl.)Epl. (the distinctive *S. discolor* Kunth.) is strongly supported sister (100%BP) to *S. leucocephala* Kunth. (sect. *Leucocephalae* (Epl.)Epl.); both of these species have fairly narrow ranges within the central Andes. The two sampled species of section *Cylindriflorae* (Epl.)Epl., *S. rusbyi* Britton ex. Rusby and *S. haenkei* Benth., together with the monotypic section *Tuberosae* (Epl.)Epl. (*S. oxyphora* Briq.) and *S. platystoma* Epl. form a largely Bolivian, monophyletic clade (57%BP). *S. platystoma* is placed in section *Malacophyllae* Epl. *Malacophyllae* appears to be at least paraphyletic (if not polyphyletic), with the other sampled species of that section (*S. sophrona* Briq., *S. stachydifolia* Benth. and *S. rypara* Briq.) occurring elsewhere in "core *Calosphace*". This section like so many within "core *Calosphace"* awaits further work for elucidation and circumscription.

One section from the Brazilian center of diversity that is supported as monophyletic in our analysis are *Nobiles* Epl. *Salvia hilarii* Benth. and *S. articulata* Epl. (the two sampled Brazilian species of *Nobiles*) are strongly supported as sister (92%BP); while, as mentioned above under the discussion of the '*Fulgentes* clade', our results support the previous removal of the three Mexican taxa from this section (Santos, 1991). Three species were sampled from another Brazilian section, *Rudes. Salvia cerradicola*

Santos and *S. brevipes* Benth. are well supported as sister species (86%BP) by our results, with the third sampled species, *S. ovalifolia*, anomalously placed in the '*Uliginosae* clade' (as described under that clade, possibly due to inter-specific hybridization or other form of reticulate evolution). Another species placed originally (Epling, 1939) in *Rudes*, *S. sphacelaefolia* Epl. of Mexico, and later (Santos, 1996) removed from the otherwise strictly Brazilian section, shows no phylogenetic affinity to the other sampled species of *Rudes* in our results (see discussion of "*Sigmoideae* clade" below). The species of sections *Rudes* and *Nobiles* are placed in a clade with the Brazilian species *S. tomentella* Pohl (sect. *Albolanatae* (Epl.)Epl.) and *S. grewiifolia* S. Moore (sect. *Hoehneana* (Epl.)Epl.), however, an association with the other Brazilian taxa such as *S. confertiflora* Pohl, *S. scabrida* Pohl and *S. guaranitica* St.-Hil. ex. Benth. remains unknown and unsupported by our analysis. *Salvia guaranitica* (section *Coeruleae* Epl.) and *S. scabrida* (of monotypic section *Asperifoliae* (Epl.)Epl.), both of Brazil, find support as sister species (60%BP), while the other sampled species of *Coeruleae,* (*S. atrocyanea* Epl. of Bolivia) occurs elsewhere, indicating the non-monophyly of *Coeruleae*.

The monophyletic "*Rubescentes* clade" contains three species from the northern Andes: *S. rubescens* Kunth., *S. gracilipes* Epl., and *S. erythrostoma* Epl. *Salvia gracilipes* and *S. erythrostoma* are placed in section *Longipes*, however, the type species of section *Longipes*, the yellow-flowered, Mexican endemic, *S. madrensis*, is placed within the "*Fulgentes* clade" rather than here, indicating the highly artificial nature of *Longipes*.

“*Sigmoideae* clade”

The three sampled species of section *Sigmoideae* Epl., *S. chapalensis* Briq., *S. inconspicua* Benth. and *S. alamosana* Rose, are strongly supported as monophyletic (Figure 1.4). In a recent revision, two subsections within *Sigmoideae* were described while the section as a whole was considered a distinctive and natural group (Espejo-Serna and Ramamoorthy, 1993). In our analysis, *S. tepicensis* Fern. and *S. sphacelaefolia* are strongly supported as sister species (100%BP), and in turn together form a strongly supported sister clade (95%BP) to section *Sigmoideae. Salvia tepicensis* was placed by Epling in section *Scorodonia* Epl. while *S. sphacelaefolia* was placed in section *Rudes*. In her treatment of section *Rudes,* Santos (1996) removed *S. sphacelaefolia* from the otherwise strictly Brazilian section and suggested a putative relationship with *S. tepicensis*. The removal of *S. sphacelaefolia* from section *Rudes* is supported by our results as is the relationship between *S. sphacelaefolia* and *S. tepicensis*. A relationship between sections *Sigmoideae* and *Scorodonia* has been suggested previously (Espejo-Serna and Ramamoorthy, 1993). However, *S. tepicensis* is the only species of *Scorodonia* supported by our results as having a close association with *Sigmoideae*, furthermore *S. tepicensis* does not appear to be closely related to the other species of *Scorodonia*.

“*Angulatae* clade”

Section *Angulatae* (Epl.) Epl., with upwards of 50 species is the largest of Epling's *Calosphace* sections; all three centers of diversity are represented within the section. The monophyletic clade here discussed includes *S. amarissima* Ort. (sect. *Scorodonia*), *S. angulata* Benth. (sect. *Angulatae*), *S. languidula* Epl. (*Angulatae*), *S. longispicata* Mart.&Gal. (*Angulatae*), *S. mexicana* L. (sect. *Briquetia*), two morphotypes of *S. mexicana* var. *minor* Benth. (*Briquetia*), and *S. xalapensis* Benth. (*Angulatae*). The clade containing these taxa is well-supported as monophyletic (84%BP) in the ITS phylogeny but has less support in the combined analysis (<50%BP). The sampled species of *Angulatae* are not monophyletic nor placed exclusively within the "*Angulatae* clade"; species are in both the "*Angulatae* clade" and the "Andean *Purpurea* clade". The sampled species of Section *Briquetia* Epl. are all supported within the "*Angulatae* clade". *Briquetia* is separated from *Angulatae* by primarily by its considerably larger, invaginate corollas, however in his unpublished manuscript, Epling had placed the species of *Briquetia* within the larger *Angulatae.* A relationship between *Briquetia* and *Angulatae* is supported in our analysis, however *Angulatae* appears highly polyphyletic (Figure 1.4).

Salvia mexicana, a wide-ranging and variable species, has one described variety, *S. mexicana* var. *minor* which has significantly smaller corollas which can be either blue or white. Three accessions of *S. mexicana* were sampled, the blue and white morphotypes of variety *minor* and the large blue-flowered standard variety, but our results do not support the monophyly of this species. Another species of section *Briquetia* was sampled, *S. atropaenulata* Epl., however only *psbA-trnH* data was obtained. In the MP analysis of the cpDNA data, *S. atropaenulata* was strongly supported (100%BP) as sister to *S.*

longispicata, implicating this species within the "*Angulatae* clade". Of biogeographic interest, one species of the southern Andes, *S. angulata* (*Angulatae*), is supported within this clade indicating yet another long-distance dispersal event. In summary, a close relationship supported between sections *Briquetia* and *Angulatae* within the "*Angulatae* clade".

"Central American *Purpureae* clade"

The "*Purpureae/Polystachyae* clade" is not strongly supported in our analysis (Figure 1.4), however, in a previous study (Walker, 2006) which included reduced sampling from these sections, including only *S. purpurea* Cav., *S. polystachya* Ort. and *S. iodantha* Fern., these three species were strongly supported as monophyletic. The morphological similarities between *S. polystachya* and *S. purpurea* have been noted previously (Standley and Williams, 1973). This clade, although not well resolved here, includes the Mexican and Central American species of sections *Polystachyae* Epl. (two morphotypes of *S. polystachya*), *Iodanthae* Epl. (*S. iodantha* and *S. arbuscula* Fern.), *Purpureae* (Epl.)Epl. (*S. purpurea*, *S. littae* Vis. and *S. areolata* Epl.), *Maxonia* Epl. (*S. chiapensis* Fern.), *Carnea* (Epl.)Epl. (*S. ionocalyx* Epl.), and *S. gravida* Epl. While sections *Iodanthae* and *Polystachyae* contain no South American species, the Andean species sampled from sections *Purpurea, Maxonia,* and *Carnea* were placed together in another, well-supported clade (see discussion below under "Andean *Purpurea* clade"). This is interesting because there appears to be more geographical structure to these clades than is present in Epling's sections, with evolutionary trends and speciation events occurring in parallel but independently between these two centers of diversity. Of further note, *S. polystachya* appears to be polyphyletic with one form sister to *S. purpurea* (72%BP) and the other weakly supported as sister to *S. gravida*. *Salvia gravida* (syn. *S. vazquezii* Iltis & Ramamoorthy [invalid]) is a large and showy plant, which was placed in section *Skeptostachys* (Epl.) Epl. with the Brazilian species *S. regnelliana* Briq. (Epling 1940).

***Salvia tubifera* group**

Within the "core *Calosphace*" is a monophyletic (75%BP) clade comprised of *S. tubifera* Cav., *S. curtiflora* Epl. and *S. nervata* Mart.&Gal., with the latter two forming a strongly supported sister relationship (92%BP) (Figure 1.4). *S. tubifera* is placed in section *Tubiflorae* while *S. curtiflora* and *S. nervata* are placed in *Curtiflorae.* Standley and Williams (1973) placed *S. nervata* as a synonym under *S. excelsa* Benth. (also sect. *Curtiflorae*). Ramamoorthy later (1984c) placed *S. excelsa* as a synonym under *S. tubifera,* without mention of *S. nervata.* Our results do not support Ramamoorthy's conclusion with *S. nervata* appearing more closely related to *S. curtiflora* than *S. tubifera.* However, a relationship between at least some of the taxa of these two sections is supported by our analysis. Sections *Tubiflorae* and *Curtiflorae* both appear to be polyphyletic. Other sampled members of *Tubiflorae* are *S. venulosa* (strongly supported as sister to *S. divinorum*) and *S. tortuosa* (strongly supported as sister to *S. florida*)*,* while *S. longistyla* Benth. was also sampled from *Curtiflorae.* While our results support the monophyly of *S. tubifera, S. curtiflora,* and *S. nervata,* further sampling and research is required to determine the affinities and placements of other members of these two polyphyletic sections.

“Andean *Purpureae* clade”

This well-supported monophyletic clade (76%BP) (Figure 1.4) contains eight Andean taxa, ranging from Argentina to Venezuela, and the Central American *S. pansamalensis* J.D. Smith (of monotypic section *Insignifoliae* Epl.). *S. pansamalensis* is found in Guatemala and Chiapas and could represent a back-dispersal from the Andes to Central America. The sections represented in this well-resolved lineage include *Carnea* (here represented by *S. carnea* var. *carnea* (syn. *S. killipiana)*), *Angulatae*, *Maxonia, Purpureae, Tubiflorae, Floridae,* and as mentioned *Insignifoliae.* The two Andean species of section *Angulatae* found within this clade, *S. cyanicalyx* Epl. and *S. amplifrons* Briq., are strongly supported as sister (100%BP) as are the two species of *Purpureae, S. tolimensis* Kunth. and *S. rufula* Kunth. (94%BP). *S. florida* Benth. (sect. *Floridae* (Epl.)Epl.) is strongly supported sister to *S. tortuosa* (sect. *Tubiflorae* which see below).

It should also be noted that a single Caribbean species, *S. tuerckheimii* Urb. (sect. *Ekmania* Epl.) was sampled (for ITS only) and well-supported within this clade as sister to *S. cyanicalyx* and *S. amplifrons* (87%BP). Unfortunately, the Caribbean species were underrepresented in our study. There are 46 species found on the Caribbean islands, with 41 of those island endemics. These species are placed within 14 sections of which 7 contain only island endemics. In addition to the 41 Caribbean island endemics, there are two species of *Calosphace* endemic to the Galapagos Islands. The island endemics within

Calosphace are in dire need of further study; the only recent treatment is Torke's (2000) revision of *Ekmania*, a section of eight species endemic to the island of Hispaniola.

Staminal Evolution in Calosphace:

Staminal evolution within *Calosphace* in many ways parallels the evolutionary trajectory found within sister clade, *Audibertia*. The trend is from expression two fertile anther sacs, or thecae, per stamen to the expression of only one (Table 1.2). The pleisiomorphic state is observed in *Calosphace, Audibertia*, and *Dorystaechas*, *i.e.* the expression of two anther sacs per stamen separated by an elongated connective (Walker and Sytsma 2007). (The connective is only minimally elongated in *Dorystaechas.*) In the species of *Calosphace* that do not express a secondary theca, the lower/posterior connective branch is still present and frequently fused. However in the species of *Audibertia* that do not express secondary thecae, the posterior connective branch is completely lacking. The trend within *Calosphace* moves from two fertile thecae per stamen, connectives not fused (stamen type 1); to one thecae per stamen, connectives not fused or only slightly (stamen type 2); and lastly to one thecae per connective, connectives fused by epidermal hairs (stamen types 3 and 4) (Table 1.2). Within this final group, comprised of the majority of *Calosphace*, further trends are observed, namely the trend from an early-developing, anterior-facing (or facing the throat of the corolla tube), veinous or vasculature-containing tooth (assurgent) (stamen type 3) to the absence of such a tooth (stamen type 4). In this latter group or those lacking an assurgent tooth, some species do exhibit a tooth, but it is late-developing, does not contain significant vasculature, and usually points toward the base of the corolla tube or posterior-facing (retrorse). (Figure 1.5, Table 1.2)

Salvia axillaris is the only species within *Calosphace* to express two fertile thecae per stamen (stamen type 1); other pleisiomorphic characters shared between *S. axillaris* and the outgroup are the lack of a fully functional lever mechanism and two fully free stamens, *i.e.* the posterior branches of the connectives are not fused. "*Hastatae* clade" (stamen type 2) shares with *S. axillaris* the lack of strongly fused posterior connective branches; however this group demonstrates a primitive lever mechanism and does not express secondary thecae. The posterior branches of the connective are dilated into widened, disc- or plate-like structures of a greatly reduced length compared with the fertile anterior connective branches. These widened posterior branches restrict access to nectaries at the base of the corolla and serve as functionally active levers.

The "*Uliginosae* clade" and the larger clade sister to it do not express secondary thecae (like the "*Hastatae* clade"), but they do exhibit posterior connective branches strongly fused late in development by epidermal hairs. This character is unique in *Salvia*. These two, fused, lower branches of the connective form a widened, plate-like gubernaculum which serves to block the throat of the corolla and act as lever. All of the species of the "*Uliginosae* clade" and many of the species in the large group sister to it have appendages or teeth-like structures on the abaxial or ventral side of the connectives which can be of various sizes and shapes.

The connectives of the "*Uliginosae* clade", "*Tomentellae* clade II", and "*Tomentellae* clade III" have synapomorphic, ventral teeth. This paraphyletic group (monophyletic in the ITS phylogeny) possesses teeth that are anterior-facing (forward or toward the mouth of the corolla), develop early (present prior to the elongation of the posterior connective), and contain significant vasculature (stamen type 3). Small veins or vascular tissue can be seen bending down into these teeth. Claßen-Bockhoff *et al*. (2004) suggest that this forward-facing or assurgent tooth is actually the aborted, secondary theca. Developmentally, the assurgent teeth of this group are the terminal end of the posterior connective branch; the elongation of the posterior branch is a secondary outgrowth of tissue between the aborted theca and the filament attachment point. The "*Hastatae* clade" exhibits a similar structure but without the posterior outgrowth of the lower connective branch.

Though the "*Uliginosae* clade" and "*Tomentellae* clade" share uniquely assurgent teeth, there are many other species having connectives with ventral appendages. In the many species of the large monophyletic clade sister to "*Tomentellae* clade III", rear- or posterior-facing teeth are frequently but not universally expressed, sometimes both present and absent in different individuals of the same species. They seem to arise late in development, after the posterior connective branch has already elongated considerably. Claßen-Bockhoff *et al*. (2004) suggest that the tissue of the aborted secondary theca is present at the posterior terminus of the connective in these species (and not in the tooth as is found in the *Uliginosae/Tomentellae* group). Structurally and developmentally these retrorse teeth, which may or may not be expressed in the most derived lineages of *Calosphace*, usually appear very different than the assurgent teeth of the "*Uliginosae* clade" and "*Tomentellae* clades II and III". While assurgent teeth appear to be largely present prior to the elongation of the connective, retrorse teeth are formed much later in development.

Certain species such as *S. rypara, S. sphacelaefolia,* and the species of section *Sigmoideae* have teeth which superficially resemble assurgent teeth, however these teeth have more in common with the retrorse teeth just described, albeit somewhat larger and in some cases nearly forward-facing (Figure 1.5). In these species, vascular tissue in the form of veins, while at times present, is not bent or angled strongly into the teeth as is seen in those species with truly assurgent teeth. Developmentally, the teeth of *S. rypara* were shown to have more in common with the smaller, retrorse teeth of species such as *S. leucantha* than with the larger, assurgent teeth of species such as *S. uliginosa* (Claßen-Bockhoff *et al*. 2004).

Furthermore, in regards to pollination syndromes we may conclude briefly that bee-pollinated species (mellitophilous) are paraphyletic with the bird-pollinated (ornithophilous) species deeply imbedded within the mellitophilous taxa. (See Wester (2007) for treatment of these to pollination syndromes, description of characters defining each syndrome, and a list of the taxa containing each syndrome.) *Salvia axillaris* (sister to the rest of the subgenus) is bee-pollinated, and our results indicate that ornithophily had multiple, independent origins within *Calosphace.* As discussed in Wester (2007), some ornithophilous species have lost lever-functionality of staminal connectives; as is the case with ornithophily, so also this loss of functioning levers has arisen multiple times in parallel within *Calosphace, e.g. S. elegans* and *S. haenkei.*

In summary, staminal morphology appears to play a critical role not only in driving speciation within subgenus *Calosphace* as has been suggested elsewhere (Claßen-Bockhoff *et al*., 2004), but is also seen to be crucial to our understanding of the major lineages and phylogeny of the *Calosphace*, as it has been for our understanding of *Salvia* at large (Walker and Sytsma, 2007). As can be seen in the tree-mapping of staminal morphology (Figure 1.5), there is clearly phylogenetic structure to the morphological trends in staminal morphology.

Biogeographic implications:

Our results support previous assertions that the common ancestor of *Calosphace* (and *Audibertia)* probably arrived in what is now Mexico (probably on the western coast prior to the geological shift of Baja and California along the San Andreas fault). The primary center of diversity for subgenus *Calosphace*, Mexico and Central America, contains a greater number of taxa than the Andean and Brazilian centers of diversity. The species of Mexico and Central America form a paraphyletic group with the South American lineages deeply embedded within them. The majority of the South American lineages seem to be the result of long-distance dispersal followed by rapid radiation, however, a few of the Andean species and/or clades appear rather to be the result of a radiation-driven range extension of Central American lineages.

A number of dispersal mechanisms of *Salvia* mericarps have been reported (Bauman and Meeuse, 1992); barochory (dispersal by weight), ombrochory (rain dispersal), and zoochory (animal dispersal) are especially common. Also reported are ballistic dispersal (mericarps are ejected after the movement of the inflorescences by wind, rain or a passing animal) and macrogeochory (dispersal in the form of desert rollers or tumbleweeds). The long-distance dispersal events hypothesized within *Calosphace* are best explained by zoochory. Small birds frequently eat the highly nutritive seeds. In addition, epizoochory has been reported (Standley and Williams, 1973): the calyces of many species are vested with sticky, glandular pubescence and may thereby easily attach to passing animals or perhaps birds thus being carried to some distant place.

The first dispersal event within the phylogeny is represented within the "*Hastatae* clade", *i.e.* the species of section *Hastatae.* This clade is sister to *S. cacaliifolia* of Guatemala and Chiapas indicating a probable dispersion from Central America. Within the "*Uliginosae* clade", two clades of South American species seem to have arisen independently. The first, represented in our analysis by *S. uliginosa, S. procurrens,* and *S. ovalifolia,* appears to be the result of a long-distance dispersal from Central America to Brazil with subsequent radiation into Uruguay and north-eastern Argentina. As discussed previously, *S. ovalifolia* may be of hybrid origin or at least some form of reticulation event. The second South American lineage arising from within the "*Uliginosae* clade" is comprised of the species of section *Corrugatae*. These species appear again to be the results of a long-distance dispersal event followed by rapid radiation albeit in this case in the northern Andes. "*Tomentellae* clade II" represents a third independent origin of *Salvia* in South America.

Without better resolution in the backbone of the *Calosphace* phylogenetic tree, it is difficult to determine whether *S. venulosa* (sister to *S. divinorum*) represents another dispersal or independent origin in South America. This may prove to be the case, but cannot be known with any certainty until other related species are identified and the phylogeny is better resolved. The next major and largest group of South American species is represented by several, unresolved polytomies within the "core *Calosphace"*; here again, it is impossible without better phylogenetic resolution to determine whether this large assemblage of species is the result of one dispersal event followed by radiation or multiple independent origins. However, what seems apparent is a dispersal event from the Andes to the eastern Brazilian center of diversity; these Brazilian species (*S. hilarii, S. articulata, S. cerradicola, S. brevipes, S. tomentella,* and *S. grewiifolia*) are sister to the clade containing the Andean species *S. dombeyi* Epl. and *S. formosa* L'Hárit. These Brazilian species, representing sections *Nobiles, Rudes, Albolanatae* (Epl.)Epl., and *Hoehneana* (Epl.)Epl., collectively form a paraphyletic group with two Andean (*S. rypara* and *S. praeclara* Epl.) species embedded within the otherwise Brazilian clade. This group of Brazilian species appears to be derived from an Andean lineage (unlike the Brazilian clade within "*Uliginosae* clade"), and in this case there appears to have been a back-dispersal to the Andes represented by *S. rypara* and *S. praeclara.*

There are only two other South American lineages that can be commented upon. The first is *S. angulata* Benth. (sect. *Angulatae*) which appears embedded within an otherwise Central American clade. Its placement within the "*Angulatae* clade" may represent an independent dispersal event to the Central Andes. The other Andean lineage evidently of independent origin is represented by the "Andean *Purpurea* clade". Several members of this clade, namely *S. carnea* and *S. tiliifolia* Vahl have extensive ranges from

Central Mexico will into the Sothern Andes. It is therefore probable that this South American lineage is not the result of long-distance dispersal but rather an extended radiation originating in Central America and continuing South into the Andes. This radiation would have to post-date the Panamanian land-bridge that connected North and South America (~12 mya), however, considering the derived phylogenetic nature of this clade, this seems entirely plausible.

In summary, the results of our phylogenetic analyses suggest that the presence of *Calosphace* in South America is the result of multiple, independent introductions, in most cases via long–distance dispersal. In one instance, "Andean *Purpurea* clade" which contains several species with ranges from Mexico to the Andes, it appears more likely the continuation of rapid radiation that began in Central America and continued through Panama and into the Andes. The species of the Brazilian center of diversity seem to represent two distinct dispersal events. The first of these two introductions appears to have been a long-distance dispersal from Central America (represented by the Brazilian *Uliginosae*) and the second, represented by sections *Nobilies* and *Rudes*, appears to be the result of dispersal from the Central Andes, moreover, in this case our results indicate a back-dispersal to the Andes from within this clade. In all, it seems that as few as seven introductions, one directly to what is now eastern Brazil and the other six to the Andes, are required to account for the multiple Brazilian and Andean lineages present within the ancestral Mexican center of diversity.

Disjunct sections:

Numerous of Epling's sections were disjunct — comprised of species from multiple centers of diversity; this was done seemingly without enough consideration of the geographical limitations of such an approach. We were able to sample eleven of these sections from multiple geographic regions, and in most cases these sections were polyphyletic along regional lines. While many appeared polyphyletic, e.g. *Tubiflorae, Longipes, Albolanatae, Purpurea, Maxonia,* and *Angulatae*, there were a few exceptions. Sections *Tomentellae* and *Uliginosae* both appeared paraphyletic in our analysis (with the exception of *S. villosa* (*Uliginosae*) strongly supported in the "*Fulgentes* clade"). Sections *Nobiles* and *Rudes* as originally circumscribed were disjunct containing species from both South and Central America, however in dos Santos' (1996) treatment of section *Rudes,* the Central American taxon, *S. sphacelaefolia* the sole Mexican species of that section, was removed. Our results support that removal and indicate a close relationship with the species of section *Sigmoideae*. The three Central American species of *Nobiles, S. gesneraeflora, S. adenophora,* and *S. disjuncta,* were similarly removed from *Nobiles* (Santos 1991) and reassigned to section *Cardinalis/Holwaya* (see discussion of the "*Fulgentes* clade"); our results also support that reassignment. It is too early to tell, with our broad but rather limited sampling and our somewhat poor resolution which other, non-disjunct sections may or may not be monophyletic, but certainly many of the larger sections appear to be non-monophyletic, e.g. *Farinaceae, Flocculosae,* and *Scorodonia.*

Concluding remarks:

We conclude that Epling's taxonomic hypotheses reflected in his *Calosphace* classification are largely artificial; that is, many of Epling's many infra-subgeneric sections are not supported as monophyletic by the results of our molecular phylogenetic analyses. While certain of the smaller sections consisting of but a few taxa restricted to one geographical region are supported as monophyletic (*e.g. Incarnatae, Blakea, Corrugatae,* and *Hastatae*), the vast majority of the sampled sections are polyphyletic. Specifically, we can reject the monophyly of sections *Albolanatae, Angulatae, Conzattiana , Flocculosae, Longipes, Malacophyllae, Maxonia, Polystachyae, Purpurea, Scorodonia, Tomentellae, Tubiflorae,* and *Uliginosae.* Furthermore, several species from which multiple accessions were sampled are not supported as monophyletic: *S. microphylla, S. mexicana,* and *S. polystachya.*

While our results underscore the need for further work on the subgenus, a few general trends can be commented upon. Several major lineages within the subgenus, specifically the more basal clades, are well supported and clarified. *Salvia axillaris* is well-supported as sister to the rest of *Calosphace*; the "*Hastatae* clade" (sections *Hastatae, Standleyana,* and *Blakea*) is well-supported as sister again to the rest of the subgenus; and finally the "*Uliginosae* clade" is again well-supported sister to the remaining species. The phylogenetic relationships of these major lineages parallel floral evolution, particularly the trends seen in the staminal connectives. The trend is from two fertile thecae per free connective (*S. axillaris*), to one fertile theca per free connective without significant posterior elongation ("*Hastatae* clade"), to one fertile thecae per fused connective with a posterior outgrowth from an assurgent tooth that is developmentally homologous to the secondary thecae ("*Uliginosae* clade" and "*Tomentellae* clade II" and "*Tomentellae* clade III"), to one fertile thecae per fused connective with posterior elongation without an assurgent tooth ("core *Calosphace*").

Our results support a Central American origin of *Calosphace* with subsequent dispersal events to South America; as few as seven long-distance dispersal events could account for the species diversity in the Andes and Eastern Brazil. Long-distance dispersals followed by rapid radiation appears to be the norm. It certainly appears that biogeography deserves greater credit in the phylogeny of *Calosphace* than was implicit in Epling's classification; the disjunct pattern reflected in many of many of Epling's sections is largely artificial. Indeed, much of the sectional polyphyly observed in our results is due to this lack of intra-sectional disjunction. Taxa morphologically similar enough to be grouped together in the same section by Epling are not necessarily closely related phylogenetically; convergent evolution is probably responsible for this pattern. General patterns of morphological change and character evolution are seen to occur in parallel within North and South American lineages. The phylogeny of the subgenus has more geographical structuring than is immediately evidenced by the morphology.

In order to more precisely define and delimit the relationships between the major lineages within *Calosphace*, additional gene regions should be sequenced and further sampling especially within the more derived "core *Calosphace*" lineages will be required. However, it is our hope that the present paper will provide a reference point for additional work on this important group.

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Table 1.1. Numbers of taxa and characters with tree statistics for maximum parsimony analyses of ITS, *psbA-trnH*, and combined datasets.

Data Set	Number of taxa		Maximum Parsimony					
			Sequence Characters					Tree length
			Total	Constant	Total Variable	Parsimony informative		
	Ingroup	Total				number	% of total	
ITS	177	181	654	252	402	276	42.2	1835
psbA	180	184	497	340	157	77	15.4	286
combined	170	174	1151	615	536	432	39.7	2177

Table 1.2. Major staminal characters of the four *Calosphace* stamen types corresponding with the four major phylogenetic lineages.

Stamen Type (corresponding w/ 4 major lineages)	# of fertile thecae	Lever mechanism	Posterior connective	Posterior Connective appendage
1	2	No	Free	(2nd theca)
2	1	primitive	Free	Disc / platelike
3	1	yes	Fused	Assurgent tooth
4	1	yes	Fused	None or retrorse tooth

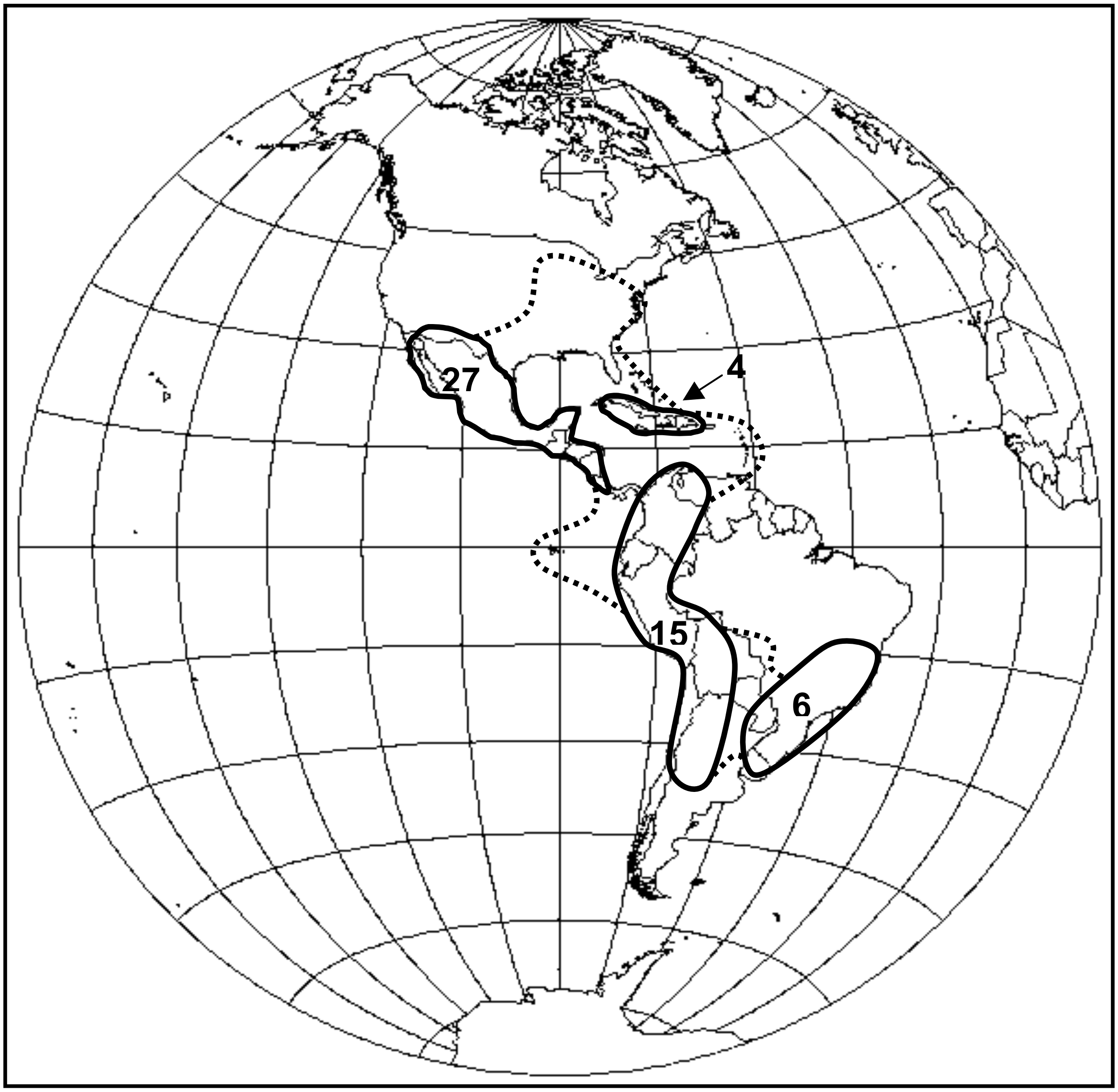

Figure 1.1. Subgenus *Calosphace* distribution. Geographic regions enclosed by solid line represents major centers of diversity and speciation while dotted line represents total distribution. Numbers represent endemic species per center of diversity.

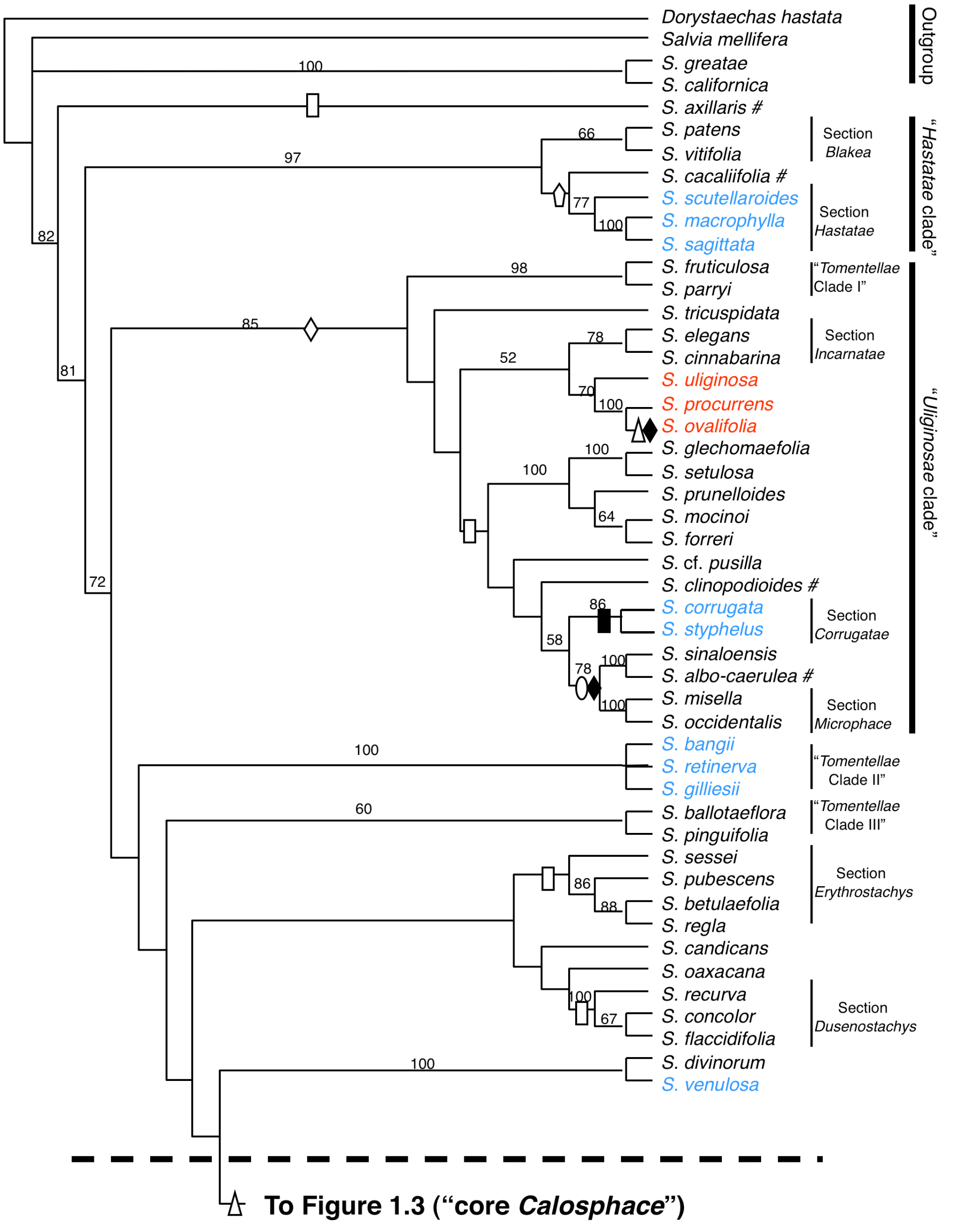

Figure 1.2. Strict consensus of 50,000 based on MP analysis of combined dataset. Bootstrap values above 50% are shown above the branches. Taxa in black are from the Mexico/Central America center of diversity, taxa in blue are Andean, while taxa in red are from the Eastern Brazilian center of diversity. Shapes on branches represent indels: ▯ = ITS1; △ = psbA1; ⬯ = psbA2; ◇ = psbA3; and ⬠ = psbA4. Solid depictions of these symbols represent indel reversals. # following a species name indicates a monotypic section.

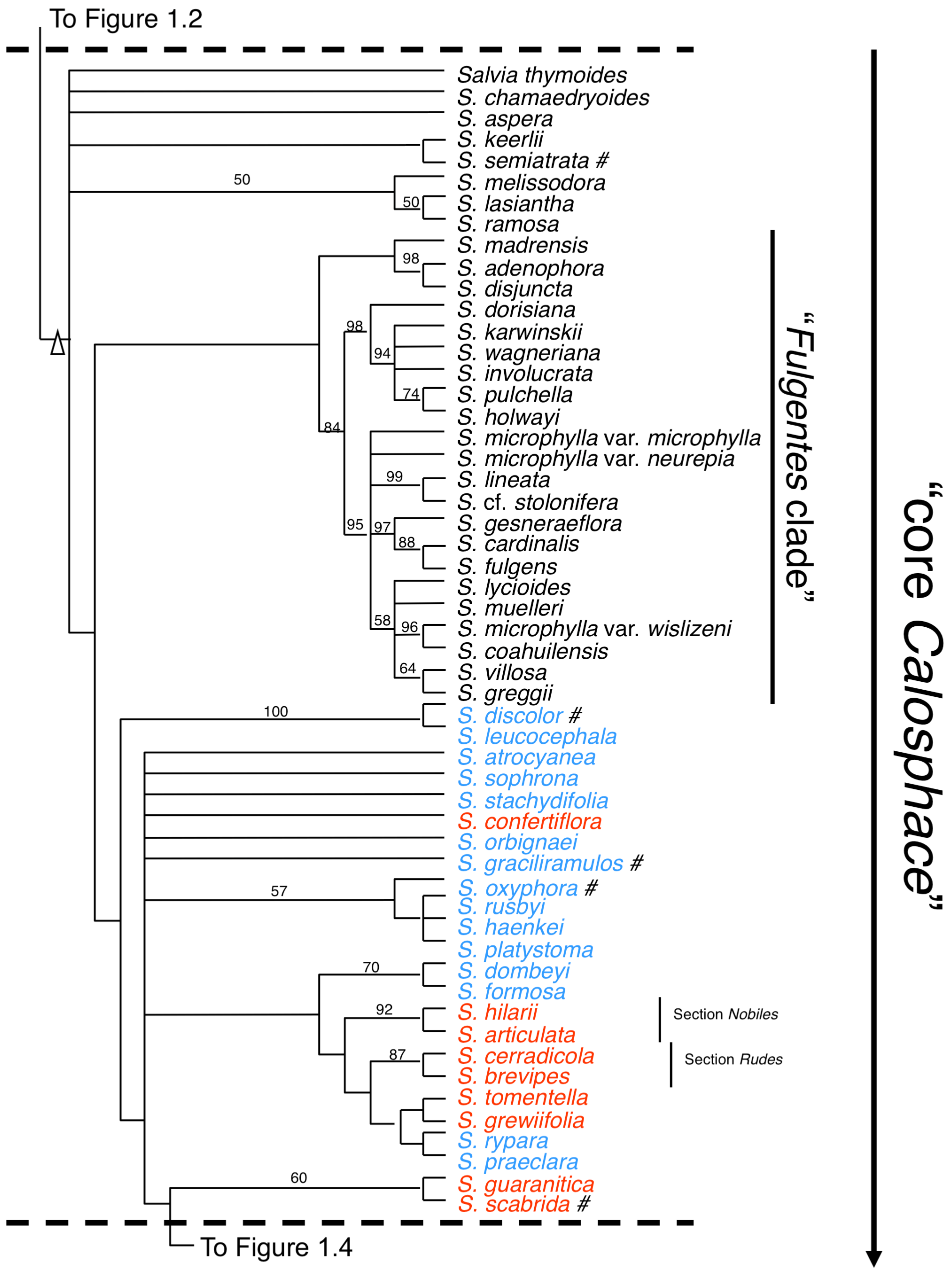

Figure 1.3. Strict consensus of 50,000 based on MP analysis of combined dataset. Bootstrap values above 50% are shown above the branches. Centers of diversity are represented by color: black = Mexico/Central America, blue = Andes, and red = Eastern Brazil. ∆= Indel psbA4. # following a species name indicates a monotypic section.

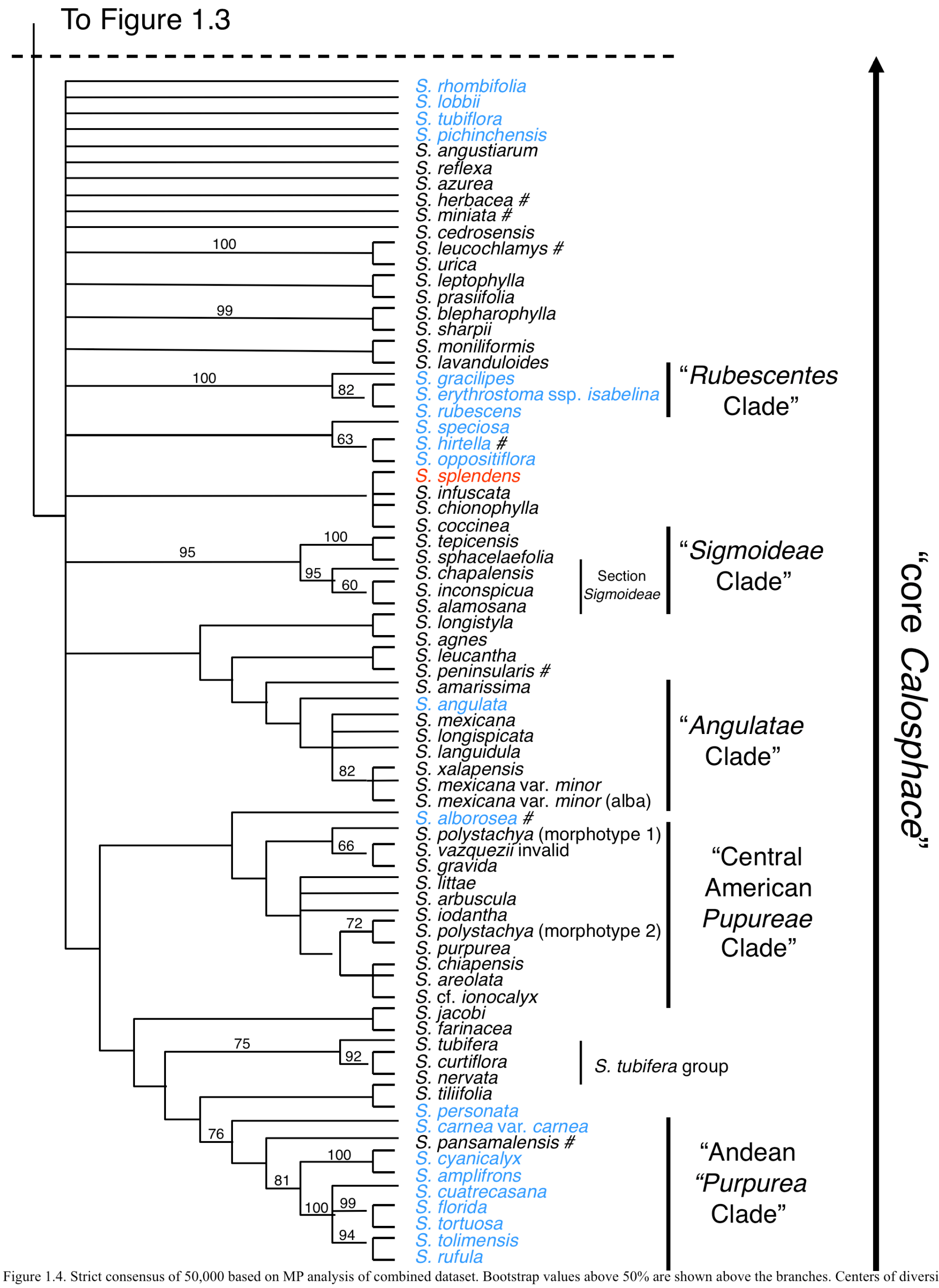

Figure 1.4. Strict consensus of 50,000 based on MP analysis of combined dataset. Bootstrap values above 50% are shown above the branches. Centers of diversity are represented by color: black = Mexico/Central America, blue = Andes, and red = Eastern Brazil. # following a species name indicates a monotypic section.

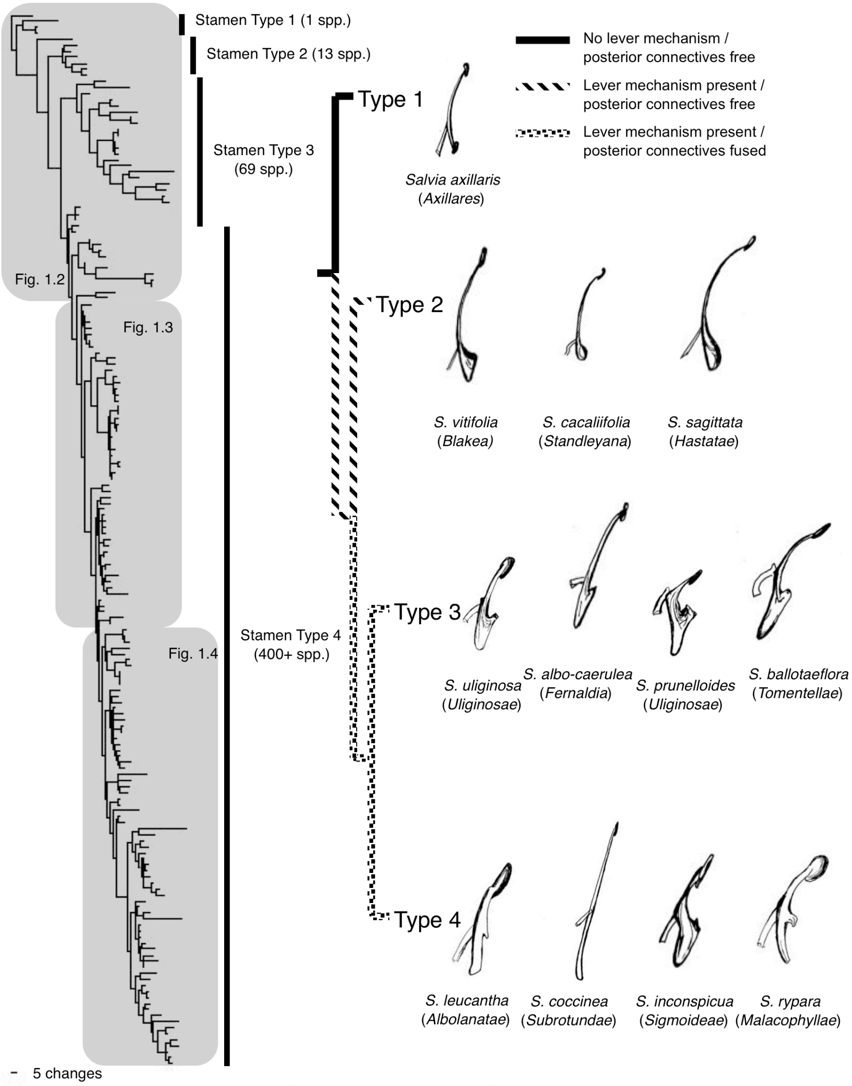

Figure 1.5. The phylogeny and diversity of staminal morphology within *Calosphace*. Phylogram with branch lengths is one randomly chosen from 50,000 equally parsimonious trees from the MP analysis of the combined dataset. Representative species (section in parentheses) of each stamen type are shown.

CHAPTER 2:

Evolution and Origins of the Hallucinogenic Sage, *Salvia divinorum*:

A Molecular Phylogenetic Study.

Aaron A. Jenks, Seung-Chul Kim and Jay Walker

Salvia divinorum **is a hallucinogenic plant of the mint family (*Labiatae*) that is used in traditional healing ceremonies by the Mazatecs of Oaxaca, Mexico, and has recently generated scientific attention due to its unique pharmacology. It has been classified within *Salvia* subgenus *Calosphace* section *Dusenostachys* and hypothesized to be an interspecific hybrid. We sequenced multiple DNA regions (ITS, *trnL-trnF,* and *psbA-trnH*) of 52 species — representing the major lineages of subgenus *Calosphace* — and six accessions of *S. divinorum* to test its phylogenetic position and putative hybridity. Our molecular phylogenetic results suggest that *S. divinorum* should not be classified within *Dusenostachys* and that it is not a hybrid. Additionally, we discover that the closest relative of this psychoactive Mexican sage is *Salvia venulosa*, a rare endemic of Colombia.**

Keywords: *Salvia divinorum*, *Ska Maria Pastora, Salvia,* sage, psychoactive plants, ethnobotany, *Dusenostachys,* entheogen, diviner's sage.

INTRODUCTION

Salvia divinorum Epling & Játiva-M. is powerful psychoactive plant that together with *teonanácatl,* the so-called "magic mushrooms" (*Psilocybe* spp. [*Strophariaceae*]) (Schultes, 1940; Wasson, 1961), and the hallucinogenic morning glory, *ololiuqui* (*Turbina corymbosa* (L.) Raf. [*Convolvulaceae*]) (Schultes, 1941), plays an important role in psycho-spiritual mythology and healing ceremonies of the Mazatecs of northeastern Oaxaca, Mexico (Wasson, 1962). *Salvia divinorum,* known to the Mazatecs as *xka pastora, La Maria,* or *Ska Maria pastora* ("the leaves of Mary the shepherdess"), is a plant steeped in mystery. It is used in quietude and darkness to access a consciousness normally hidden from the rational mind, but unlike other hallucinogenic plants of indigenous Mexico, it appears to have been unobserved by the Spanish chroniclers. Indeed, Western knowledge of this plant is quite recent. The earliest mention of vision-inducing leaves being used by the Mazatecs is by Johnson (1939) who noted a tea prepared from the leaves of *hierba Maria,* although there is no proof that this refers specifically to *S. divinorum*. Reko (1945) and Weitlaner (1952) also mention the use of unidentified vision-inducing leaves, *hoja de las adivinación*, by the Mazatecs and neighboring Cuicatecs. Weitlaner (1952) additionally reported than an herb called *yerba de la Virgen* was ceremonially employed by the Otomí of Hidalgo for the healing visions it engendered. However, the first time *Ska pastora* was identified was 1957 when the plant was first collected and recognized as a species of *Salvia* by the Mexican ethnoecologist, A. Gomez-Pompa (personal communications, 1957). Shortly thereafter while in Mexico investigating Mazatec mushroom cults, Wasson (1962) observed a traditional healing ceremony employing the leaves of *Ska pastora* performed by the *curandera* María Sabina. *Ska pastora* was subsequently identified as a new species, *S. divinorum* (Epling and Játiva-M., 1962).

Research on *Salvia divinorum* by pharmacologists and plant chemists has been extensive due to its unique secondary compounds. The chemical pharmacologically responsible for hallucinogenic activity in *S. divinorum* is salvinorin A (Siebert, 1994), a clerodane diterpenoid. Terpenes and terpenoids (molecules similar in structure to terpenes) are ubiquitous in *Salvia*, clerodane diterpenoids being present in every tested species of subgenus *Calosphace* (Benth.) Benth. (the subgenus to which *S. divinorum* belongs), functioning as anti-feedants and herbivory deterrents (Cole, 1992; Simmonds and Blaney, 1992; Sosa *et al*., 1994; Rodríquez-Hahn *et al*., 1995). Salvinorin A, however, is known only from *S. divinorum,* first isolated by Ortega *et al*. (1982), and independently isolated and verified as having psychoactivity in mice by Valdes III *et al*. (1984). It would be ten more years until salvinorin A was shown conclusively to have hallucinogenic activity in humans (Siebert, 1994), and salvinorin A has recently been shown to function as a highly selective κ-opioid receptor agonist (Roth *et al.,* 2002; Yan and Roth, 2004). It is the first naturally occurring, non-alkaloid (non-nitrogenous) opioid-receptor ligand to be discovered (Roth *et al*., 2002; Chavkin *et al*., 2004). A recent flurry of papers detailing the pharmacology of salvinorin A and *S. divinorum* has been published (*e.g.*, Bigham *et al*., 2003; Mowry *et al*., 2003; Munro and Rizzacasa, 2003;

Munro *et al*., 2003; Butelman *et al*., 2004; Tidgewell *et al*., 2004; Harding *et al*., 2005; Lee *et al*., 2005**;** Wang *et al*., 2005), and medicinal application in a clinical setting has been documented (Hanes, 2001, 2003; Vortherms and Roth, 2006). All of this indicates the therapeutic value of this plant and potential for further research and perhaps pharmaceutical development.

Recently, in addition to all of scientific research on *S. divinorum* and the pharmacologically active compound, salvinorin A, it has also received the attention of the general public, both written about in popular magazines (Parks, 2007) and reported upon by many newspapers (*e.g.*, Jones, 2001; Allday, 2007; Sack and McDonald, 2008) and television (Rohrer, 2007), due primarily to its recreational, hallucinogenic usage (Dennehy *et al*., 2005; González *et al*., 2006). Several countries and multiple states within the U.S.A. have enacted reactionary and largely misguided laws that make even the possession of *S. divinorum* a felony (sagewisdom.org); which has in turn sparked research by the forensic and law enforcement agencies (J. Hurd, personal communication; National Drug Intelligence Center 2003, Bertea *et al*., 2006). However despite all of the attention, surprisingly little is known concerning the species' natural history, specifically its reproductive biology, evolution, and phylogenetic affinities.

The first botanical observations were made by R. Gordon Wasson. While he primarily reported upon Mazatec ethnobotany and culture including the visionary and ceremonial uses of *Ska Pastora,* he also reported that the species only rarely flowers and never sets seed (Wasson, 1962). Wasson additionally proposed that *Salvia divinorum* was *pipilzintzintli,* a previously unidentified sacred Aztec plant reportedly used for visionary purposes, an assertion for which there appears to be supporting evidence in the Dresden Codex (Emboden, 1983). With his tentative identification of this plant as *pipilzintzintli*, Wasson hypothesized that the knowledge and use of the species was known throughout the Aztec empire and that the species' range and distribution may have extended over a much larger area of Mexico than its current, limited range in the remote Sierra Mazateca of northeastern Oaxaca.

Wasson sent flowering specimens of *Ska Pastora* to Carl Epling, an expert of New World *Labiatae,* for identification who described *Salvia divinorum* as a new species (Epling and Jativa-M, 1962). Epling had previously revised *Salvia* L. subgenus *Calosphace* (Benth.) Benth. (Epling, 1939), the largest (~500 spp.) of five *Salvia* subgenera (Bentham, 1876; Walker, 2006). In his comprehensive monograph, Epling erected 91 sections within the subgenus with species ranging from the United States, through Mexico and Central America, and throughout much of South America (Epling, 1939). *Saliva divinorum* was placed in section *Dusenostachys* (Epl.) Epl. and was hypothesized to be closely related to *S. cyanea* Benth. (placed as synonym under *S. concolor* Lamb. ex Benth., sect. *Dusenostachys*) (Epling and Jativa-M,, 1962), a species endemic to a few locations in the transverse ranges of central Mexico.

The botany of *Salvia divinorum* was most thoroughly examined by Reisfield (1987, 1993) who not only conducted extensive fieldwork but also implemented a series of greenhouse breeding experiments. In the Sierra Mazateca, Reisfield located twelve populations, located within a few square miles of each other, however these populations were reported to be clonal stands, most of which were assumed to be the result of human introduction due to their anthropogenic distribution. Hummingbirds are the primary pollinators as indicated by nectar analyses, corolla morphology and field observations. Chromosome studies indicated that the species was diploid ($2n=22$) with normal meiotic pairing. Pollen viability was shown to be poor (56% aborted or inviable) when compared with three control species, though no pollen-tube inhibition was observed indicating normal pollination (not self-incompatible). Hand pollination experiments resulted in low seed set: of 108 flowers selfed only 11 nutlets were produced (2.5% of total possible based upon four possible per flower), and of 190 crossed (the plants crossed may have been genetically identical) only 24 nutlets resulted (3%). These results were attributed to possible post-zygotic embryo abortion or endosperm failure, however, it is uncertain how meaningful these results are since no other species were pollinated as a control group. These cumulative results were accounted for by three hypothetical explanations: self-incompatibility (although the absence of pollen-tube inhibition mitigates against this possibility), inbreeding depression, or interspecific hybridity. Reisfield concludes that interspecific hybridity, *i.e.* hybrid weakness, inviability, or sterility caused by disharmonious gene or chromosome interactions, is the most likely explanation for the low pollen viability and low seed set.

In recent years, seeds have been reported to be abundantly produced in cultivated plants, both in plants hand pollinated and in plants pollinated by Anna’s Hummingbirds (Siebert, personal communications; McCall, 2008). Several germination studies have been summarized (Hanna, 1999). In one study 12-13 of 70 planted seeds germinated (17-18%) while in another 45 of 153 germinated (29.4%).

In this paper we examine the phylogenetic history of *Salvia divinorum* and test its putative hybrid origin. Specifically, is *S. divinorum* correctly classified and most closely related to the species of section *Dusenostachys*? Is *Dusenostachys* monophyletic? What are the evolutionary history and biogeography of *S. divinorum* and its closest relatives; are they of Mexican origin? Is *S. divinorum* of recent hybrid origin, and if so, what are the putative parents? And, is this species monoclonal, with all individuals genetically identical?

MATERIALS AND METHODS

Taxon Sampling and Outgroup Selection:

Sequence data was obtained for 52 species (including 4 outgroups). This sampling includes taxa from 35 of Epling's sections within *Calosphace*, representing all previously identified clades (Jenks, chapter 1). Six accessions of *Salvia divinorum*, representing five populations from the Sierra Mazateca (Appendix 2), were sequenced to determine whether there was any intra-specific sequence variability. However, only one accession (Appendix 1) was included in the Maximum Parsimony analyses. Outgroup taxa were chosen based upon previous molecular studies (Walker *et al*., 2004; Walker and Sytsma, 2006); these species were *Dorystaechas hastata* Boiss. & Heldr. ex Benth*., Salvia greatae* Brand*., S. californica* Brand., and *S. mellifera* Greene. *Dorystaechas hastate,* a monospecific Asian genus, was strongly supported as sister to the clade comprised of subgenus *Audibertia* (Benth.) Epl. ex Walker and subgenus *Calosphace*. *Salvia greatae,* *S. californica, and S. mellifera* are all placed within subgenus *Audibertia*, strongly supported as sister to *Calosphace* (Walker *et al*., 2004).

All samples were either collected by the authors or obtained from herbarium specimens. Accession information and vouchers for the taxa analyzed in this study are given (Appendix 1).

PCR Amplification, Sequencing, and Sequence Analyses:

Total genomic DNA was extracted via DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA). Extractions were made from fresh, dried, or frozen leaf material. Polymerase chain reaction (PCR) amplifications were typically prepared in 25 ul reactions; PCR reactions were performed on a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Watertown, MA). The PCR profile was as follows: 2 minutes of initial denaturation at 95° C, 35 cycles with a 1minute denaturation at 94° C, 2 minutes annealing at 52° C, and 2 minutes extension at 72° C, with a final extension of 10 minutes at 72° C. The entire nuclear ribosomal internal transcribed spacer (ITS) region (including ITS1, the 5.8S gene, and ITS2) was amplified with primers ITS5 and ITS4, or alternately in the case of more highly degraded DNA from herbarium specimens, in two parts with primer combinations ITS5-ITS2 and ITS3-ITS4 (White *et al*., 1990). The noncoding, intergenic spacer region of chloroplast DNA, *psbA-trnH*, was amplified using forward and reverse primers as described previously (Kim *et al*., 1999). In a previous study (Jenks, chapter 1) we were unable to obtain good chloroplast resolution based on this region, so for this study with greatly decreased sample size we sequenced a second chloroplast spacer region, *trnL-trnF*, in order to improve resolution, allowing comparison of *S. divinorum*'s placement between biparentally-inherited ITS region and maternally-inherited chloroplast DNA while assessing putative hybridity. Forward and reverse primers for *trnL-trnF* were used (Taberlet *et al*., 1991). All PCR products were purified with the QIAquick PCR purification kit (Qiagen, Valencia, CA, USA). Sequencing reactions were carried out for the purified PCR products using Big Dye Terminator Cycle Sequencing reagents (Applied Biosystems, Foster City, California, USA). Sequencing primers used were identical to amplification primers. Sequence fragments were assembled and edited using Sequencer version 4.2.2 (Gene Codes, Ann Arbor, Michigan, USA). The corrected consensus sequences were aligned manually in MacClade v4.06 (Maddison and Maddison, 2003).

Initially all data sets were analyzed independently; however the *psbA-trnH* region proved problematic with resolution very poor to none due to extremely low sequence variability (low % of parsimony informative characters) and identical sequences (all loci identical amongst species) shared by a significant number of the taxa. As a result, the two cpDNA datasets were combined for further analysis. The two datasets (ITS and combined cpDNA matrix) were analyzed using an equally weighted, unordered maximum parsimony (MP) approach (Fitch, 1971) implemented in PAUP version 4.0 (Swofford, 2002). A heuristic search performed with 100 replicates of random addition sequences. Branch-swapping was via stepwise addition with 100 trees held at each step; tree-bisection-recombination (TBR) and MulTrees options were in effect. The maximum number of trees held was 100,000. Branches were collapsed if their score was zero, topological constraints were not enforced, gaps were coded as missing data, and characters were unordered. MP bootstrap analyses (Felsenstein, 1985) were performed using 100 replicates, random taxon addition with 10 replicates per replicate and not more than 100 trees held at each step.

RESULTS

Phylogenetic Analyses:

The ITS data matrix had a total aligned sequence length of 642 characters of which 183 were variable and parsimony-informative (28.5% of total) (Table 2). The phylogenetic analysis yielded 547 equally most-parsimonious trees with a length of 909 steps (CI = 0.4653, HI = 0.5347, RI = 0.5525). The combined *psbA-trnH*/*trnL-F* matrix had a aligned sequence length of 1510 characters of which only 84 were variable and parsimony-informative (5.6% of total) (Table 2). Phylogenetic analysis resulted in more than 100,000 equally parsimonious trees, however, the heuristic MP search was unable to run to completion and was truncated after 4 days. These most parsimonious trees had a length of 250 steps (CI = 0.8200, HI = 0.1800, RI = 0.8732). The 50% majority-rule consensus tree of each data set is presented (Figure 2.1,2 — ITS and combined cpDNA, respectively).

In the ITS phylogeny, subgenus *Calosphace* is well-supported as monophyletic (72% MP Bootstrap Probability (BP)), with *S. axillaris* sister to the rest of the subgenus. Section *Dusenostachys* is strongly supported as monophyletic aside from *S. divinorum*

(100%BP). Section *Erythrostachys* is also well-supported as monophyletic (77%BP). Other clades also supported as monophyletic are *Hastatae* clade (98%BP), *Uliginosae* clade (60%BP), and *Fulgentes* clade (93%BP) within the core *Calosphace* lineage (52%BP). These previously identified clades (Jenks, chapter 1) are comprised of species from multiple sections and are named after the section with taxonomic priority or, in case of equal priority the section, with the greatest number of species represented within the clade. *Salvia divinorum* and *S. venulosa* are strongly supported as sister species (98%BP). (Figure 2.1)

Calosphace is strongly supported as monophyletic (100%BP) in the cpDNA phylogeny as well. *Hastatae* clade (100%BP), *Uliginosae* clade (100%BP), and the core *Calosphace* lineage (100%BP) are each strongly-supported as monophyletic as is section *Dusenostachys* excluding *S. divinorum* (100%BP), although the relationships amongst these clades varies slightly between the ITS and cpDNA phylogenies. *Salvia divinorum* and *S. venulosa* are strongly supported as sister to each other (100%BP) in the cpDNA phylogeny.

Sequence Analysis:

The ITS sequence data of all six sampled accessions of *Salvia divinorum* were searched, base-pair by base-pair, for sequence additivity. Since ITS is inherited bi-parentally, a recent interspecific hybrid would exhibit DNA regions with a high percentage of polymorphisms, *i.e.* two bands at each site, one from each parent donor. None of the sampled accessions had a high percentage of polymorphisms; four accessions had no polymorphisms while two had the same, single-locus polymorphism. This indicates that there is some slight genetic variability among the sampled populations of *S. divinorum* since two different ITS sequences were present. The two sequenced accessions of *S. venulosa*, representing two populations, shared an identical ITS sequence containing no polymorphic loci.

DISCUSSION

Phylogenetic position and closely related species of *Salvia divinorum*:

Epling's original placement of *Salvia divinorum* within section *Dusenostachys* and closely related to *S. concolor* (Epling and Játiva-M., 1962) is most certainly in error based on our molecular phylogenetic results; the other sampled *Dusenostachys* species, *S. concolor* and *S. flaccidifolia* Epl., form a strongly supported monophyletic clade which does not include *S. divinorum,* in either the ITS (Figure 2.1) or cpDNA phylogenies (Figure 2.2). In our previous study (Jenks, chapter 1), a third *Dusenostachys* species, *S. recurva* Benth., was also well resolved within this section — strongly supported (100%BP) as sister to *S. concolor* and *S. flaccidifolia* — and again not closely related to *S. divinorum*. Morphologically, *Dusenostachys* is characterized by large, ventricose violet-blue corollas, calyces with upper lips possessed of 5 or 7 major veins, typically hollow fragile stems, and a generally hydrophytic habit as exhibited by a willingness to form large clonal stands in seasonal or perennially-flowing waterways. While *S. divinorum* is certainly hydrophytic and on account of its habit could be, perhaps, associated with *Dusenostachys*, its floral morphology especially is very different. *Salvia divinorum* possesses white corollas of moderate size and slightly sigmoidal rather than ventricose conformation, and it has violet calyces with upper lips distinctly three-veined. Perhaps if Epling had obtained more and better materials, he would have seen fit to remove *S. divinorum* from *Dusenostachys*, as he did with *S. madrensis* Seem., a species also first described within *Dusenostachys* but later removed to another section, *Longipes* Epl. (Epling, 1944). It should be noted that our results do indeed support Epling's removal of *S. madrensis* from *Dusenostachys* (Figures 2.1,2). Aside from a single Brazilian species of *Dusenostachys*, *S. oligantha* Dusen., with doubtful affinities to the remaining species of the section, *Dusenostachys* is comprised of entirely endemics of central and south Mexico. While our results do not support the inclusion of *S. divinorum* within *Dusenostachys, S. divinorum* interestingly is strongly supported as sister to a rare, Colombian endemic, *S. venulosa,* in both the ITS and cpDNA phylogenies (Figures 2.1,2).

There are a number of morphological similarities between *S. divinorum* and *S. venulosa*; they are quite similar in appearance, strikingly resembling each other in habit, vegetatively, and in reproductive structures. Both species have racemes of well-spaced verticillasters of three to six flowers subtended by deciduous violet bracts. The calyces of both species are violet and pedicelate, while the interiorly epapillate corollas are densely villous externally and have upper and lower labia of nearly equal length. The pubescent styles of each are slightly excerted while the anthers are included within the galea. Both species have "Type 4" stamens (Jenks, chapter 1), the most derived of four stamen types;

this stamen type is defined as having an elongated, lever-forming connective, the posterior branches of which are sterile, fused, and frequently possess a small retrorse tooth. The floral differences between the two species are corollas of different colors (*S. divinorum* = white to pink; *S. venulosa* = a light red claret) and calyces frequently with differing numbers of veins on their upper lips (*S. divinorum* = 3; *S. venulosa* = 3 or more often 5). Vegetatively, these two, low-growing species bear a striking resemblance to one another with their narrowly ovate, typically glabrous leaves, acuminate at the apex and attenuate at the base (the blade decurrent onto the petiole and the petiole scarcely differentiated if at al from the blade). The hollow stems of both species are decumbent and when in contact with moist soil or water rooting vigorously at the nodes and (more uniquely) even along the internodes.

Salvia venulosa is a very local endemic, known from only three locations found in deeply shaded, moist ravines in the westernmost cordillera of the Colombian Andes in the district of Risaralda from 1500 to 2000 m elevation. No other Colombian natives appear to be grow in this northwestern most extension of the Colombian Andes (Wood and Harley, 1988) There is no ethnobotanical record of medicinal or other uses of *S. venulosa*. The species is placed in section *Tubiflorae* (Epl.) Epl., a primarily Colombian section (Epling, 1939). Two other species of the section are sampled in our study, *S. tubifera* Cav. of Mexico and *S. tortuosa* Kunth of Colombia, however, *S. venulosa* is not closely related to either species (Figures 2.1,2), *Tubiflorae* in our results proving to be highly polyphyletic. It should be mentioned that, morphologically *S. tortuosa* does not appear to be closely related to the rest of the section (Wood and Harley, 1988), therefore further sampling of Colombian *Tubiflorae* (specifically, *S. camaraefolia* Benth., *S. secundiflora* Rusby and *S. falcata* Wood & Harley) is needed to determine whether any of these species are closely related to *S. divinorum* and *S. venulosa*.

Biogeography of *Salvia divinorum*:

Previous work has conclusively established that Mexico is the center of diversity (Neisess; 1983; Reisfield, 1987; Ramamoorthy *et al*., 1988; Harley and Heywood, 1992; Jenks, Chapter 1) for the monophyletic (Walker *et al*., 2004; Jenks, chapter 1) subgenus *Calosphace* and that the South American species and lineages arose from within the Mexican taxa (Jenks, chapter 1). Our previous results show that there have been multiple long-distance dispersal events (as few as seven) from Mexico to South America (Jenks, chapter 1). Concerning the strongly supported sister relationship between *Salvia divinorum* and *S. venulosa,* the question arises whether this represents and independent South American dispersal event. Further sampling of the Colombian *Tubiflorae* is required to determine whether *S. divinorum* and *S. venulosa* are part of a larger Colombian lineage (in which case the presence of *S. divinorum* in Mexico is perhaps explainable by a back-dispersal). At present, the most parsimonious explanation is that the common ancestor of both species arose in the Mexican center of diversity with subsequent dispersal of the *S. venulosa* ancestor to the northern Andes.

Regardless, the reported lack of reproduction of *S. divinorum,* aside from human-mediated vegetative propagation, in the Sierra Mezateca, the only region from which it is known, is somewhat problematic if that is indeed the native range of the species and introduces again the question of whether *S. divinorum* is the Aztec plant *pipilzintzintli* as has widely been hypothesized (Wasson, 1962; Ott, 1995). *Pipilzintzintli* was drunk by the Aztecs (as *S. divinorum* is by the Mazatecs) to psychoactive effect (Vetancurt, 1698) and is depicted in the Dresden Codex as having bilabiate corollas (Emboden, 1983) providing some evidence for this identification. If *pipilzintzintli* is correctly identified with this species, it would be reasonable to assume *S. divinorum* was once used and perhaps found growing over a much larger geographic area than it is currently known. The reports of other indigenous groups besides the Mazatecs, *e.g.* the Cuicatec, Chinantec, and Otomí, using leaves in the same manner for divination and visions (Reko, 1945; Weitlaner, 1952) only supports the hypothesis of a once-larger range and distribution. Ott (1995) even makes a case for *S. divinorum* being non-native and a post-conquest introduction to the Sierra Mazateca based most strongly upon linguistic evidence; there is no indigenous name for *S. divinorum* in the Mazatec language, *Ska Maria Pastora* ("Leaves of Mary the Shepherdess") being a Spanish modernism referencing two post-Spanish introductions: the Virgin Mary and sheep. Seeming to support this are the lack of any observed wild populations in the Sierra Mazateca and Reisfield's (1993) observations that all of the known populations there are clonal and all were at least potentially the result of human introduction. Nahua trade arteries passed through the Papaloapan drainage basin (Reisfield, 1987), in the eastern Mazatec country, indicating at least a potential anthropogenic vector for presence of *S. divinorum* in the Sierra Mazateca. Regardless, of whether or not *S. divinorum* is native to the Sierra Mazateca, the conditions there do not seem fully adequate for sexual reproduction, suggesting that the origins of this plant are elsewhere.

Hybrid Origin and Reproductive Biology of *Salvia divinorum*:

In the most thorough experimental study of *Salvia divinorum,* Reisfield (1993) concluded that this species was probably of recent interspecific hybrid origin and possibly self-incompatible or suffering from inbreeding depression. However, our results demonstrate unambiguously that *S. divinorum* is not a recent hybrid. By using DNA sequences of paternally- and maternally-inherited regions, we are able to determine hybrid origin of plant species. Hybrids typically show 1) nuclear additivity, showing biparental inheritance, and 2) incongruence between the nuclear and uniparentally (maternally) inherited chloroplast phylogenies. Additivity, as evidenced by a high degree of polymorphisms, was not observed in the ITS sequence of *S. divinorum* (6 accessions sampled) (or in the 2 accessions sampled of *S. venulosa*). Neither do our results show incongruence between the ITS (Figure 2.1) or chloroplast (Figure 2.2) phylogenies, *i.e. S. divinorum* is strongly supported as sister to *S. venulosa* in both analyses and together form a clade that is positioned similarly in both trees. Therefore, if *S. divinorum* is not a hybrid as our results suggest, how can we account for Reisfield's (1993) findings that led him to conclude that it was an interspecific hybrid?

While Reisfield (1993) observed no irregular pairing during meiosis, some stage of gametogenesis was irregular since half the pollen grains aborted (882 of 1587, significantly more than the three other species observed), the haploid stage of the lifecycle being partially sterile. While our results indicate that this is not due to interspecific inbreeding depression (as Reisfield speculated), a lack of genetic diversity within the Sierra Mazateca due to repeated clonal propagation in an already limited gene-pool could account for this depression. In his pollination experiment, Reisfield (1993) reported extremely low percentages of *Salvia divinorum* nutlet maturation: 11 nutlets grew to maturation from 108 selfed flowers (2.5% when calculating 4 possible per flower) and 24 nutlets from 190 crosses (3%) (although the crosses might have been between two genetically identical individuals). These results, however, were not controlled with data sampled from other species. The reported low percentages may not be significant, since no other species were tested; it is uncommon in many species of *Calosphace* for all four nutlets to mature within a pollinated flower, and seed set in some species appears to be naturally low (A. Jenks, unpublished data). Reisfield concluded that the low seed set was most likely the results of hybridity (and accompanying disharmonious genic or chromosomal interactions) or perhaps self-incompatibility or inbreeding depression. Self-incompatibility had been speculated previously to be the cause of low or lack of seed set (Valdéz *et al*., 1987), but Reisfield (1993) observed no pollen-tube inhibitions during pollination as would be expected in a self-incompatible species. Further pollination experiments should be commenced to determine whether *S. divinorum* truly has a lower percentage of nutlet maturation than other species of subgenus *Calosphace*. Of the nutlets that do mature, the reported 30% germination rate (Hanna, 1999) is not lower than expected for *Calosphace* (A. Jenks, unpublished data: germination studies on 20 spp.)

Conclusions:

Salvia divinorum is not closely related to the other sampled species of the Mexican section *Dusenostachys* as previously hypothesized (Epling and Játiva-M., 1962) and should no longer be classified within that section. In both the ITS and cpDNA phylogenetic trees, *S. divinorum* was strongly supported as sister species to *S. venulosa;* in both trees resolved within a basal grade and not the core *Calosphace* lineage. *Salvia divinorum* and *S. venulosa* are not closely related to the other sampled species of the largely Colombian section *Tubiflorae* (in which *S. venulosa* classified) which was highly polyphyletic in our results.

Salvia divinorum is not an interspecific hybrid as previously suspected (Reisfield, 1993) since it shows neither additivity in the biparentally-inherited, nuclear ITS sequence nor incongruence between the ITS and chloroplast DNA phylogenetic trees. *Salvia divinorum* appears to be a reproductively capable species, however because of its extensive anthropogenically-mediated, clonal propagation in the Sierra Mazateca, its ability to reproduce sexually seems to be somewhat limited as evidenced by the decreased pollen viability previously reported (Reisfield, 1993). While it is uncertain whether *Salvia divinorum* prospered over a larger geographic range or whether it is correctly identified as the Aztec *Pipilzintzintli* (although both speculations are certainly plausible based on the scant available evidence), there has doubtless been an ancient alliance between *Salvia divinorum* and humans who have used it to visionary effect. However, this close association does not, *de facto*, mean that this species is a cultigen, *i.e.*

a species requiring of human intervention to thrive and prosper. Further research is needed to determine what barriers to sexual reproduction might be responsible for the seemingly low sexual viability of the species and to determine the genetic diversity within the species, although there appears to be at least some variability in the ITS sequence among the six sequenced accessions. And, while *S. venulosa* does not appear to biosynthesize the psychoactive clerodane diterpenoid, salvinorin A (D. Siebert, personal communication), further studies are underway to determine the chemical constituents of this species.

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Table 2. Numbers of taxa and characters with tree statistics for the maximum parsimony analyses of both ITS and combined cpDNA datasets.

Data Set	Number of taxa		Maximum Parsimony					
			Sequence Characters					Tree length
			Total	Constant	Total Variable	Parsimony informative		
	Ingroup	Total				number	% of total	
ITS	48	52	642	363	279	183	28.5	909
Combined cpDNA	48	52	1510	1321	189	84	5.6	250

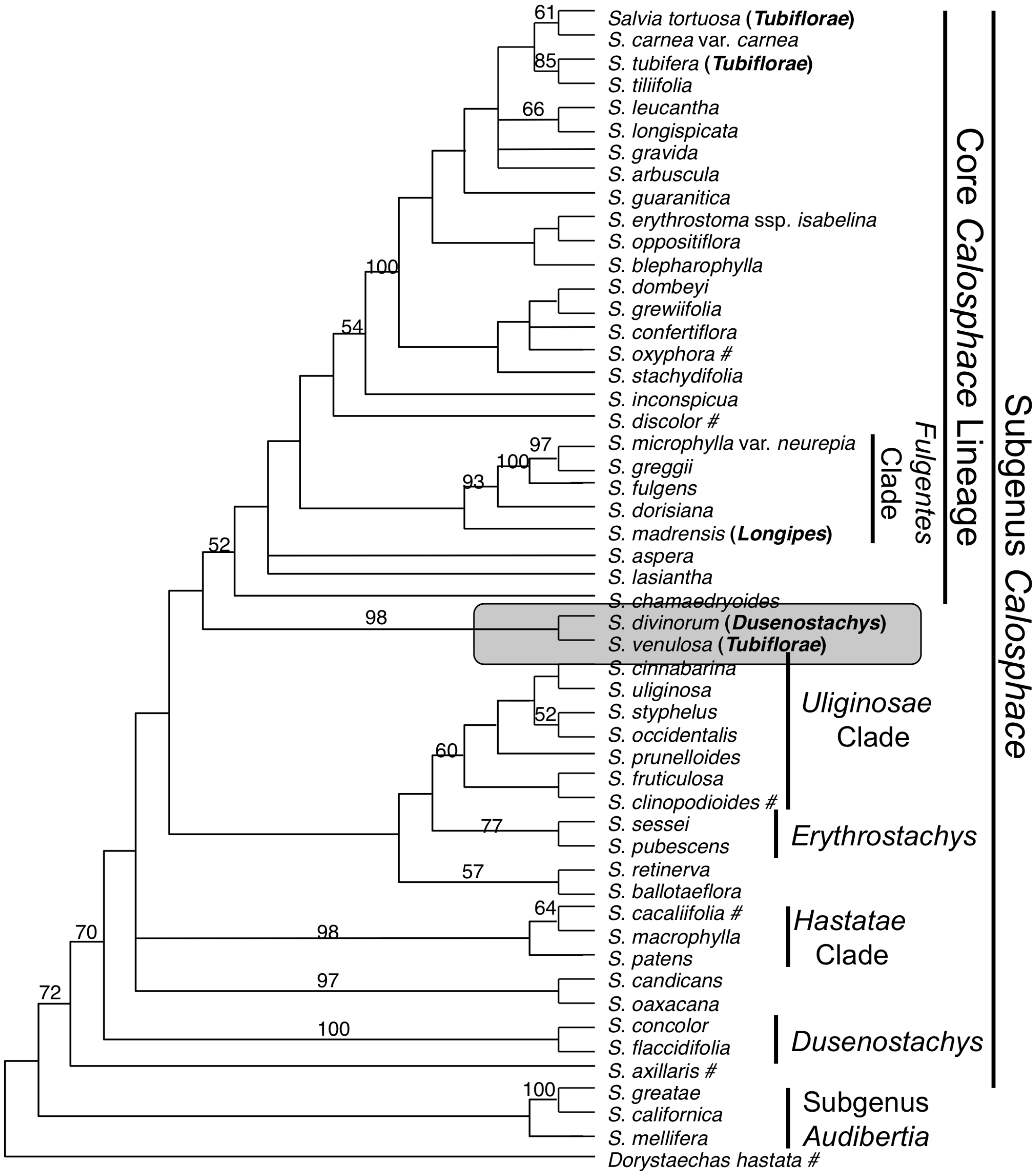

Figure 2.1. Majority-rule (50%) consensus tree of 547 based on MP analysis of ITS dataset. The MP bootstrap support values above 50% are shown above the branches. A # following a species name indicates a monotypic section. Bold names in parentheses following species names are their respective taxonomic sections as discussed in the text.

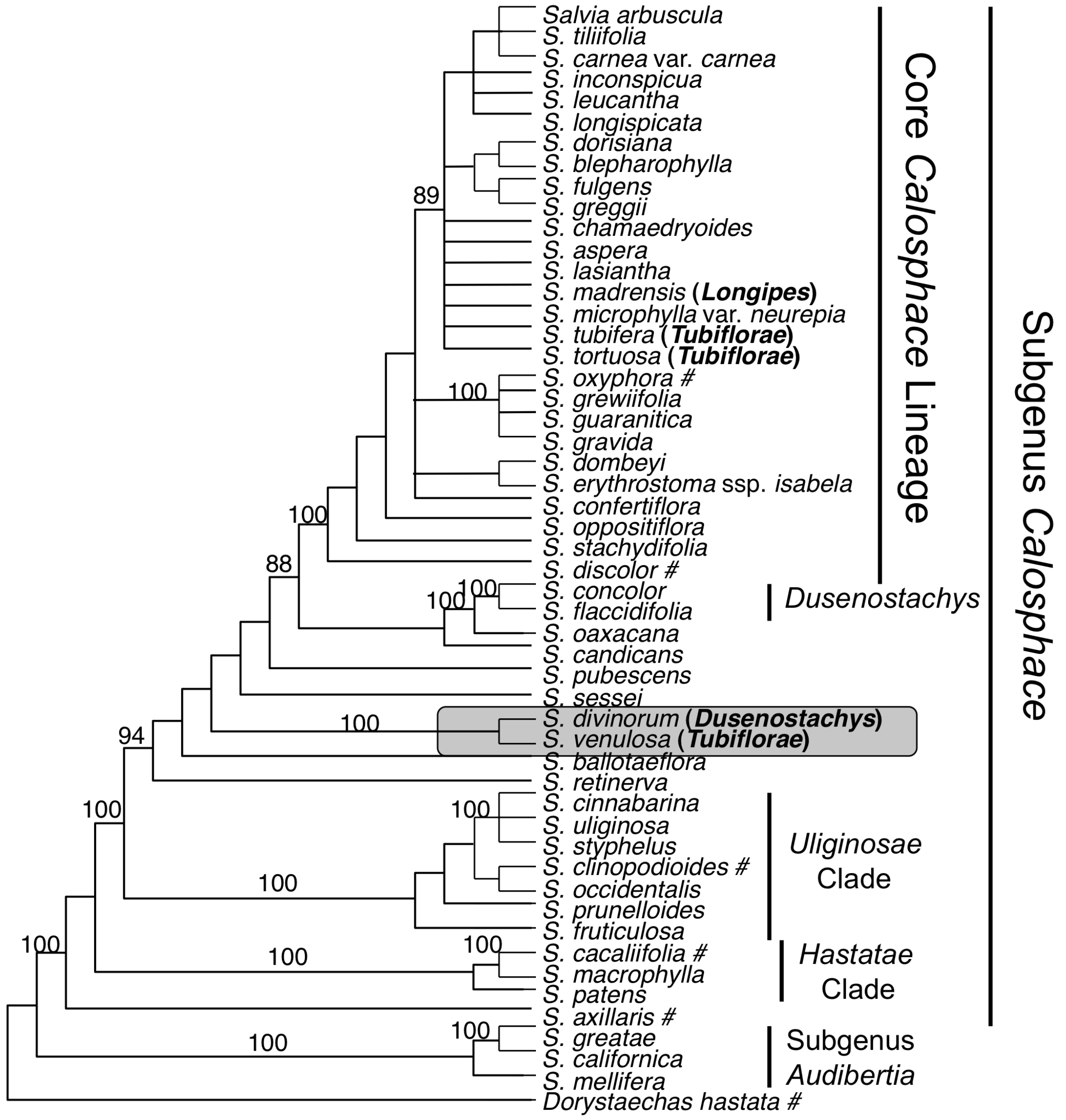

Figure 2.2. Majority-rule (50%) consensus tree of 100,000 based on MP analysis of combined cpDNA dataset *(trnL-F* and *psbA-trnH*). The MP bootstrap support values above 50% are shown above the branches. A # following a species name indicates a monotypic section. Bold names in parentheses following species names are their respective taxonomic sections as discussed in the text.

CHAPTER 3:

Medicinal Plant Complexes of *Salvia* Subgenus *Calosphace*: An Ethnobotanical Study of New World Sages

Aaron A. Jenks

The species of *Salvia* subgenus *Calosphace* are used medicinally and ritually in numerous traditions of folk healing among indigenous cultures of North and South America. These species of sage contain numerous bioactive terpenes, especially clerodane diterpenes, which may contribute to their effectiveness as folk medicines. Ethnobotanical data was gathered for more than 150 species; those sharing the common names, medicinal uses or curative properties, physical appearances, and similar chemical compounds associated into medicinal plant complexes. We identified five new, previously undocumented complexes: *mirto, ñucchu, lí‘l++, cantueso,* and *manga-paqui*. *Mirto* (5 spp., Mexico) is used extensively in the treatment of folk illnesses, especially *susto,* and for nervous system and digestive complaints. *Ñucchu* (7 spp., Peru) is used as a symbolic element in the *semana santa* processions of Cuzco and is used in the treatment of respiratory ailments. *Lí’l++* (3 spp., Mexico) is a Chinantec complex used for food and medicine, *cantueso* (2 spp., Mexico) is used for respiratory ailments in central Mexico, and *manga-paqui* (3 spp., Ecuador) is used in Ecuador for treating problems of the kidneys and liver.

Keywords: Sage, *Salvia, Calosphace,* ethnobotany, *Mirto, Ñucchu, lí’l++, Cantueso, Manga-paqui, susto, taitacha temblores,* folk illness, terpenes, ethnopharmacology

INTRODUCTION

Sage (*Salvia* L. species) is widely appreciated for its great beauty and has charmed its way into gardens around the world, with several hundred species and varieties available in horticultural trade (Clebsch, 2003; Froissart, 2008). There are more than 900 species of sage in the wild, and they are found most abundantly in the Mediterranean Basin, Central Asia, South Africa, the Far East and throughout much of the Western Hemisphere (Walker *et al.*, 2004; Jenks, chapter 1). These plants have proved useful to humans in many ways. As early as 1597, common sage (*S. officinalis* L.) was cultivated as an important culinary spice and medicinal herb (Grieve, 1931). Sages (*S. hispanica* L. and *S. columbariae* Benth.) are nutritious and nourishing staple foods upon which whole cultures have been built (Cahill, 2001, 2003, 2005), while others (*S. sclarea* L. and *S. horminum* L.) have been used to add flavor to and increase the intoxicating properties of ales and wines (Grieve, 1931; Buhner, 1997). A hallucinogenic sage, *S. divinorum* Epling and Játiva-M., is used by Mazatec shamans in Mexico (Wasson, 1962; Jenks, chapter 2) and has potential medical use in clinical settings (Hanes, 2001, 2003; Vortherms and Roth, 2006) due to its novel pharmacology as a highly selective kappa-opioid receptor agonist (*e.g.*, Roth *et al.*, 2002; Yan and Roth, 2004). The most frequent uses of *Salvia* are medicinal; around the globe many species are highly regarded for their usefulness in the treatment of illness and disease and employed in the healing practices and rituals of many indigenous cultures. And nowhere, perhaps, are sages more used medicinally and ritually than in the Americas.

This paper is another in the series (Jenks, chapter 1, 2) on *Salvia* subgenus *Calosphace* (Benth.) Benth. This strictly New World subgenus is the largest of four subgenera originally described (Bentham, 1876) and contains approximately 500 species (Epling, 1939) found throughout North and South America (Figure 3). Aside from a few important species whose ethnobotany has been extensively documented, *i.e. chia* (especially *S. hispanica*) (*e.g.*, Cahill, 2001, 2003; Ayerza and Coates, 2005; Cahill, 2005) and *Salvia divinorum* (*e.g.*, Wasson, 1962; Valdéz *et al.*, 1983, 1987; Reisfield, 1993; Ott, 1995), the extensive medicinal usage of *Calosphace* species has been greatly underrepresented and largely unreported in the literature. However, the chemical constituents of a number of the species have been elucidated. The secondary plant metabolites of *Calosphace* species are primarily terpenes and terpenoids; no alkaloids have to date been isolated. Multiple types of terpenes have been isolated including monoterpenes and sesquiterpenes (volatile oils), diterpenes (typically resins), and triterpenes. The biological activities of terpenes have rendered them valuable in a variety of pharmaceutical and agricultural applications (Hoffmann, 2003). Two well-known examples are paclitaxel (Taxol) from yew trees — an important drug used in the treatment of cancer — and artemisinin from *Artemisia annua* that is active against quinine-resistant strains of malaria. Of all the terpenes isolated from *Calosphace* species, diterpenes are the most significant being both ubiquitous and characteristic, although each individual compound is usually restricted to one or several closely related species (as is reportedly typical of diterpenes (Hoffmann, 2003)). Several classes of diterpenes are present in *Calosphace*. Abiatane and primarane (both tricyclic diterpenes) have been isolated from a few species (*e.g.,* Esquivel *et. al.,* 1987, 2005), while the bicyclic clerodane diterpenes are more common and are present accross *Calosphace* (at least among examined species). A number of these terpenes have been shown to be biologically or pharmacologically active (*e.g.,* Siebert, 1994; Romussi *et al.,* 2001; Capasso *et al.*, 2004a, 2004b; Imanshahidi and Hosseinzadeh, 2006; Topcu, 2006), including compounds active at the benzodiazepine and opioid receptors (Marder *et al.*, 1996; Viola *et al.*, 1997; Roth *et al.*, 2002). This prevalence of bioactive terpenes and the likelihood of others of importance yet to be discovered within *Calosphace* coupled with the frequent usage of these species in traditional folk medicine renders the subgenus an enticing research subject and the focus of this study.

Unlike traditional ethnobotanical research that is centered upon an individual indigenous population and the plants they utilize or is delimited by a discrete geopolitical or biogeographic region, this study is based upon plant relationships, the phylogenetic patterns of evolution. This approach is founded upon our hypothesis that among closely related plants you expect to find trans-cultural ethnomedicinal uses and conserved pharmacological properties based upon similar, active secondary metabolites. *Calosphace* is strongly supported as monophyletic (derived from a common ancestor) (Walker *et al.*, 2004), indicating all of the species within the subgenus are each-other's closest relatives; *Calosphace* is therefore a well-defined, natural group within which our ethnobotanical investigations can confidently be conducted. We hypothesize that *Calosphace* species will assort into medicinal plant complexes (MPC's); MPC's are defined as containing "plant species sharing common names, morphological and/or aromatic characteristics, and curative properties" by Linares and Bye (1987) who identified four complexes from Mexico each comprised of from two to six species. Our research objectives are to (1) identify the major complexes within *Salvia* subgenus *Calosphace*, (2) identify and inventory the species within each complex, and (3) determine the usage of each indigenous plant within each complex. Additionally, we will (4) identify the suite of characteristics (similar appearance, medicinal application, or both) that defines each complex and (5) whether there are any potentially active compounds known from species within each complex or studies on the effects of the said compounds or plants.

METHODS

The scope of this project being *Calosphace*-wide and thereby spanning two continents, it was deemed prohibitive to conduct extensive field research in all locations where *Salvia* species are found and used medicinally, *i.e.* most of the Western Hemisphere. Due to this large-scale scope of our project, our research therefore relies heavy upon previously documented knowledge from local informants by other authors. (Nonetheless, we did conduct a scattering of informal interviews in Mexico over several field expeditions). In lieu of the standard methodology involving the documentation of the complete botanical pharmacopoeia of a given indigenous population or biogeographical locale, this study takes a phylogenetic prerogative of subject delimitation: we are evaluating the ethnobotanical data for a truly trans-cultural taxon, *Salvia* subgenus *Calosphace*, in an attempt to identify trans-cultural trends as observable within a given medicinal plant complex. This novel approach is based on the concept that plant relationships can and should provide a basis for a broader discussion of ethnobotany and ethnopharmacology since closely related plants are not only often used similarly but frequently have secondary compounds and pharmacological effect-profiles in common. By identifying the major medicinal plant complexes in this meta-analysis spanning many cultural groups and most of the Americas, we hope to open the door to future work within each of these complexes that should include further ethnobotanical and anthropological field studies as well as research in plant chemistry and pharmacology.

We obtained ethnobotanical and biochemical documentation on more than 150 species from within subgenus *Calosphace* (with as many as 50 uses per species) from over 200 references, herbarium sheets, and field interviews; a database was assembled which was then queried to access data. We searched for the most frequently used common names; the species sharing common names were grouped into complexes and further analyzed to assess similarity of use, morphology, and secondary compounds. In order to determine the accuracy and validity of this approach, the *chia* complex (also known as *chan* or *chante*) was used as a control case since there is an abundance of information (Cahill, 2001, 2003; Ayerza and Coates, 2005; Cahill, 2005) describing the several uses and multiple species of this complex. We identified eighteen *chia* species of subgenus *Calosphace* (Table 3.1) – this does not include the important *chia* species *Salvia columbariae* Benth. (of subgenus *Audibertia* (Benth.) Epl. ex Walker) and *Hyptis suaveolens* (L.) Poit. — which is more species than have been reported previously by any one source, indicating that this method is useful and accurate in identifying species within a MPC. While *chia* is the largest complex within *Calosphace,* the primary use of these species is a food source; medicinal uses are secondary in importance. Since the *Chia* complex has been well documented elsewhere

(Cahill, 2001, 2003; Ayerza and Coates, 2005; Cahill, 2005) and because this paper deals primarily with medicinal, not culinary, plant complexes, *chia* will not be mentioned in our results or discussion.

Scientific names and common names are listed for each plant. A number of common names were found to have multiple spellings (likely reflecting different pronunciations or transcriptions) that are each included in the results. In addition to the Latin binomial for each species and its taxonomic section within subgenus *Calosphace*, we also list the synonyms it has been cited under in both taxonomic and ethnobotanical references. Common names are derived from the references cited in the text; the reference is only indicated after a name when that reference is not mentioned in the text. These common names are frequently restricted to a particular indigenous group, a specific geographic region, or both. When this information is known, it is presented in parentheses following the common name. If the name is applied to a plant in cultivation outside of it's native range this is also indicated. For example, *Salvia involucrata* is cultivated in but not native to Brazil, there known as *Cardeal do México* and *Segundo Pio Corrêa*; its status is indicated thus: (cult., Brazil).

RESULTS

(1) *Mirto* complex:

The *mirto* ("myrtle", Spanish) complex of central and eastern Mexico (Figure 3) is comprised of five species of *Salvia* (Table 3.2), all of which are red-flowered, herbaceous perennial herbs or subshrubs. The aboveground parts of these species are aromatic and frequently resinous; it is perhaps the smell of these glandular secretions that prompts the name *mirto*. These species play an important role in the treatment of a variety of folk illness throughout Mexico and Central America, especially *susto*. Folk illnesses frequently defy concise categorization by the Western medical tradition, moreover, they are culturally mediated (although they can be trans-cultural) and have a sociologic and/or spiritual basis rather than organic. However, the symptoms are considered serious and potentially life threatening (Rubel *et al.*, 1984; Baer and Bastillo, 1993). The species of the *mirto* complex are also extensively used medicinally for conditions of the nervous system, gastro-intestinal (G.I.) complaints, and to a lesser degree, obstetrics and gynecology (OB/GYN). (Table 3.2)

(1a) *Salvia microphylla* Kunth [section *Fulgentes* (Benth.) Epl.]

(Syn. *S. grahami* Benth., *S. obtusa* Mart. and Gal., *S. gasteracantha* Briq., *S. odoratissima* Sessé and Moc.)

Also part of the *mirto* complex is *S. microphylla* var. *neurepia* (Fern.) Epl. (Syn. *S. neurepia* Fern.) (Martínez, 1979), however no references were located that identify the northernmost variety *S. microphylla* var. *wislizeni* Gray (Syn. *S. microphylla* var. *canescens* Gray, *S. lemmoni* Gray), as part of the *Mirto* complex.

Other common names: *Bandera Mexicana* (Oaxaca and elsewhere in Mexico), *Camotillo* (Ecuador), *diente de acamaya* (Puebla), *hierba de mirto, ix tasalak* (Tepehua and Nahua - Puebla), *kamirto nchia* (popoloca - Puebla). *litropuetei* (Otomí - Puebla), *mastranzo, mirto chico, mirto cultivado, Mirto de Casa, mirto de Castilla, Mirto Dulce*

(Mexico and Guatemala), *mishto* (Tzotzil - Chiapas), *mistro* (est. México), *mistru* (est. México), *mustia* (Purhépecha - Michoacan), *Pabellón Mexicano, Salvia del Monte, Salvia Silvestre* (Mexico and Guatemala), *simbaregne* (Mazahua – est. México), *Toronjil* (Guerrero and elsewhere in Mexico), *tzit bomol* (Tzotzil - Chiapas), and *verbena*.

Salvia microphylla is a somewhat variable, frequently woody, subshrub with corollas ranging from red to dark pink in the wild. Variety *microphylla* is found in the transverse ranges of central Mexico, and while the range of variety *neurepia* overlaps somewhat with that of var. *microphylla*, it is found much further North throughout much of the Sierra Orientale. Recent molecular phylogenetic research (Jenks, chapter 1) does not support the monophyly of all three varieties of *S. microphylla*, specifically variety *wislizeni* is not monophyletic with the other two varieties. No references support the inclusion of variety *wislizeni* as part of the *mirto* complex, indicating an interesting congruence of folk taxonomy and our molecular phylogenetic results.

The most frequent method of *mirto* preparation is to make an infusion or tea of the aboveground plant parts (usually leaves and flowers if available). Unless otherwise described below, this is the method used in the treatment of the following conditions. *Salvia microphylla* is commonly used in the treatment of folk illnesses as indeed are all species of the *mirto* complex. *Salvia microphylla* is employed in the ritual healing of a large number of folk illnesses: *susto* (Argueta V., 1994), *espanto* (Martínez Alfaro *et al.*, 1991; Argueta V., 1994), *mal de aire* (Argueta V., 1994), *nervios* (Aguilar *et al.*, 1996), *mal de ojo* (Martínez Alfaro *et al.*, 1991; Argueta V., 1994), and *aigre de viento* (Argueta V., 1994) (Table 3.5). In addition to its use in the ritual healing (or *limpieza*, "cleansing") of folk illnesses, an infusion of the flowering plant is drunk throughout the day to treat the physical symptoms (Martínez Alfaro *et al.*, 1991; Argueta V., 1994; Aguilar *et al.*, 1996). For *susto* and *mal de aire* one glass of the tea is consumed before breakfast and again throughout the day as needed, for *aigre de viento* two glasses are drunk in the morning, and for *espanto* three glasses are drunk before each meal. Additional instructions apply to children with *espanto*: the tea must be made with *Salvia microphylla* and *Agastache* spp. (*Lamiaceae*) and drunk for nine days while a paste made from *Agastache* and *S. microphylla* must be applied each day to the child's body while invoking the spirits of the plants. (Argueta V., 1994). References to an unspecified ceremonial use of *S. microphylla* (M. Tlapa A. and G. Ubierna J. 289, XAL) may be alluding to its use in the ritual *limpieza* performed by a healer in treatment of folk illnesses.

Aside from folk illnesses, the most frequent application of *Salvia microphylla* is for stomachaches and other G.I. complaints (Morton, 1981; Martínez Alfaro *et al.*, 1991; Argueta V., 1994; Estrada C. *et al.*, 2007). A tea of this species is taken to stop diarrhea (Argueta V., 1994; Cano Asseleih *et al.*, 1997), sometimes boiled/decocted with *Chrysanthemum parthenium* (Morton, 1981), "sweet herb" or *estafiate* (*Artemisia ludovicana*) (Martínez Alfaro *et al.*, 1991). It is also used for stomach cramps and colic (Cano Asseleih *et al.,* 1997), indigestion, dysentery, stomach infection, stomach inflammation (and inflammations of other internal organs) and vomiting (Martínez Alfaro *et al.,* 1991; Argueta V., 1994). An infusion made from the leaves and flowers is also a useful treatment for insomnia (Argueta V., 1994; Aguilar *et al.*, 1996). If a child has trouble falling asleep, they are bathed in an infusion of flowering tops of *Salvia microphylla* while branches of the same are placed under the child's pillow. However if they are also crying, the tea is given to them to drink too (Argueta V., 1994). The infusion is also taken for feelings of suffocation (Cano Asseleih *et al.*, 1997) and as an antispasmodic (Martínez Alfaro *et al.*, 1991). In Puebla it is used for premenstrual cramps, excessive vaginal bleeding, childbirth and infertility (Argueta V., 1994). Externally, *S. microphylla* is applied for skin problems such as granulomas (Argueta V., 1994), and it is tinctured in alcohol that is then rubbed on inflamed hands and feet as a treatment for inflammation and rheumatism (Bussmann and Sharon, 2006).

The chemical constituents of aerial parts of *Salvia microphylla* include the triterpenoids erythrodiol-3-acetate, lupeol, and oleanolic acid. Oleanolic acid exhibits antimicrobial, antiulcer, and anti-inflammatory activities (Aydogmus *et al.*, 2006; Topcu, 2006). Diterpenoids present in *S. microphylla* include 7alpha-hydroxysandaracopimaric acid (the most abundant), 7-oxo-sandaracopimaric acid, 7,15-isopimaradien-14α,18-diol, 1α-hydroxy-neoclerodane-3,13-diene-18,19: 15,16-diolide (Esquivel *et al.*, 1987), and 12-methoxycarnosic acid (Aydogmus *et al.*, 2006). Carnosic acid 12-ethyl ether (12-methoxycarnosic acid) is moderately active against *Staphylococcus aureus* (Aydogmus *et al.*, 2006). Two phenolic esters, 2-(*p*-hydroxyphenyl)ethyl eicosaheptanoic acid ester and hexacosylferulate, and two sesquiterpenes, β-eudesmol and 8α-hydroxy-β-eudesmol, were isolated from an acetone extract of *S. microphylla*. β-eudesmol is active against *Alternalia* fungi (Aydogmus *et al.*, 2006). Additionally, *S. microphylla* var. *neurepia* is reported to also contain ursolic acid and β-sitosterol (Argueta V., 1994), and unique pimarane-type diterpenoids (Esquivel *et al.*, 1989).

(1b) *Salvia coccinea* Juss. ex Murr. [sect. *Subrotundae* (Epl.) Epl.]

(Syn. *S. pseudococcinea* Jacq., *S. rosea* Vahl, *S. glaucescens* Pohl, *S. ciliata* Benth., *S. galeottii* Mart., *S. mollissima* Mart. and Gal., *S. coccinea* var. *pseudococcinea* Gray, *S. coccinea* var. *minima* Fern.)

Other common names: *Betónica, Cardeal do Brasil* (Brazil), *chacajxiú, chactapek, chäkajxiw, chak lool* (Yucatán), *Chak-tsits* (Maya – Yucatán and Quintana Roo), *chaktsitsil* (Quintana Roo), *Chichinguaste rojo* (El Salvador), *clavel* (Belize), *cornetilla* (V. Vázquez T. 1003*), flor de chupamirto, flor de sol, hierba de espanto, hierba tinta, hoja de viento, ix chäkta'pek', ju'che', jut'ut' ts'ojol* (San Luis Potosí), *k'aax. macancachauat* (Totonaca - Veracruz) (Martínez, 1979), *makaukachagua* (Totonaco), *mirto cimarro* (C. Vargas R. and P. Sima 459), *Mirto cimarrón, mirto rojo, myrto, segundo Pio Corrêa* (Brazil), *Tabaquillo* (Petén, Guatemala), *tasitsxnico* (Quintana Roo), *tila, toronjil, tsaab sets* (Yucatán), *Tsab-tsits* (Maya – Yucatán and Quintana Roo) (Martínez, 1979), *Tsab-xiu* (Maya - Yucatán) (Martínez, 1979), *tsa kil xiw* (Yucatán), *tsitsil xiw* (Yucatán), *Ts'unum-pak* (Maya - Yucatán) (Martínez, 1979), *tsunu 'un baak* (Maya – Yucatán), *tsunuum bak* (Maya – Quintana Roo), *tzajal sikil nich wamal* (Tzeltal - Chiapas), *uxum ts'ojol* (Tenek – San Luis Potosí), and *x-p'oklampiix* (Maya – Yucatán)

Salvia coccinea is a short-lived, herbaceous perennial with corollas that are red or occasionally pink. It is widely cultivated as an ornamental (Clebsch, 2005; Froissart, 2008). It was thought to be a native of Brazil (Epling, 1939; Epling and Toledo, 1943) — later authors (Wood and Harley, 1988) have considered it native as far north as Colombia — but has since become naturalized and indeed is a cultivated ornamental in many tropical and subtropical areas around the globe. There is extensive documentation of its medicinal use by many indigenous peoples of Mexico and a paucity of such documentation from Brazil where it is seemingly cultivated merely as an ornamental (Epling and Toledo, 1943). The ethnology and frequent medicinal use in Mexico, at least suggest a possible Central American origin of this species. Alas, molecular phylogenetic analyses have so far been inconclusive concerning its putative Mexican endemism (Jenks, chapter 1).

Salvia coccinea is typically prepared via infusion of the plants' aerial parts (Lidia Ambriz C. 10, John Brett 675, Feliciano Gómez Santiz 238, C. Vargas R. and P. Sima 459). *Salvia coccinea* is used in the treatment of the folk illnesses, *aire* (Atran *et al.*, 2004), *espanto* (M.M. Juárez S. 81), and *mal de viento* (Argueta V., 1994) (Table 3.5). When children are afflicted with *aire*, not only are they made to drink a tea of *S. coccinea*, but they are also bathed in it. This is said to reverse the loss of appetite and wasting sickness that is associated with *aire* (Atran *et al.*, 2004). In addition to the use of *S. coccinea* in the ritualized treatment of folk illnesses, the Yucatán Maya use it as a protective against *Ik* (Roys, 1931), the capricious and feared deities of *cenotes* (Hlebakos, 2006).

Salvia coccinea affects the nervous system primarily as an antispasmodic and sedative, being used as a tranquilizer and muscle relaxant (Argueta V., 1994; Atran *et al.*, 2004); an oil made from this species is used to massage sore muscles (Atran *et al.*, 2004). An infusion of the stems is taken for headaches (Estrada C. *et al.*, 2007). The infusion is taken internally and used as eyewash for ocular pain (John Brett 675 and 471; Roys, 1931), and the seeds are placed in the eye to remove debris (Estrada C. *et al.*, 2007) (the seeds swell up when wet and become coated with mucilage to which foreign objects adhere). It is also taken for the pain of toothaches (Roys, 1931).

Salvia coccinea is used in the treatment of various G.I. complaints including stomachache, colic, dysentery, and diarrhea (Lidia Ambriz C. 10; Alcorn, 1984; Argueta V., 1994). In obstetrics and gynecology, it is used to regulate menstruation and to control vaginal bleeding and hemorrhaging (Feliciano Gómez Santiz 238; Roys, 1931; Argueta V., 1994). Externally, the red corollas are rubbed on women's cheeks as a rouge to redden them (von Reis and Lipp Jr., 1982), and the foliage is used to staunch nosebleeds (Cano Asseleih *et al.*, 1997). Lastly, it is called *tabaquillo* in the Petén region of Guatemala (Standley and Williams, 1973) indicating that it may be smoked there for recreational or ceremonial purposes.

The plant has beta-sitosterol (Argueta V., 1994), four triterpenes (methyl-1-3β-hydroxy-1-oxo-olean-12-en-28-methylcarboxylate (Mukherjee *et al.*, 2004), 3β-hydroxy-1-oxo-olean-12-en-28-methylcarboxylate, 3β,28-dihyroxyolean-12,20(30)-diene, and uvaol (Topcu, 2006)), and salviacoccin (a clerodane diterpenoid) (Savona *et al.*, 1982). The flowers have anthocyanin pigments: salvianin-, cyanidine-, and pelargondidin-glucosides (Tomas-Barberan *et al.*, 1987).

(1c) *Salvia elegans* Vahl [sect. *Incarnatae* Epl.]

(Syn. *S. incarnata* Cav., *S. microculis* Poir., *S. punicea* Mart. and Gal., *S. microcalyx* Scheele*, S. longiflora* Sessé and Moc., *S. camertoni* Regel, and *S. rutilans* Carr.)

Other common names: *flor del cerro, hierba del burro, jetcho deni* (Otomí), *k'anrrejna* (Mazahua - Michoacan), *limoncillo, mirto de flor roja, mirto inglés, mirto mocho, perritos rojos,* pineapple-scented sage, *salvia, and toronjil de monte.*

As are all of the members of the *mirto* complex, *Salvia elegans* is red-flowered and typically bird-pollinated. The leaves exude a very sweet, often pineapple-like scent when lightly rubbed. This perennial herb is quite widespread, ranging from the Sonora desert (*S. elegans* var. *sonorensis* Fern.) throughout central Mexico and as far East as the Sierra Orientale.

A whole plant infusion of *Salvia elegans* is given to individuals suffering from the folk illnesses *espanto* and *aire*, who drink the tea and bath in it (L. Zamora and P. Barquín 619; Argueta V., 1994) (Table 3.5). Whether due to one of these folk illnesses or some other cause, insomnia with accompanying sighs and crying in adults and particularly children is treated by drinking an infusion of *S. elegans* and placing a flowering branch of the plant near one’s head or under the pillow while falling asleep (R. Monroy 4146; Argueta V., 1994). In central Mexico (D.F. and est. México), a tea is made from the leaves of this species for stomachaches while wounds and edemas are plastered with cataplasms made from crushed stems and roots. In Puebla, and infusion of aerial parts is drunk by new mothers for post-partum pain, and the newborns are bathed in it. (Argueta V., 1994)

There are a number of aromatic and volatile compounds found in *Salvia elegans* including the monoterpene, linalool, which is reported to have sedative effects and is used frequently in cosmetics and perfumery (Makino *et al.*, 1996). The closest relative of *S. elegans* (Epling, 1939; Jenks, chapter 1)*, S. cinnabarina* Mart. and Gal., contains a secoisopimarane diterpenoid (3,4-secoisopimar-4(18),7,15-triene-3-oic acid) that exerts antispasmodic activity on the intestines (Romussi *et al.*, 2001) and inhibits both rat bladder contractility (Capasso *et al.*, 2004a) and mouse intestinal motility (Capasso *et al.*, 2004b). It is probable that *S. elegans* also contains this or another closely related diterpenoid. In two mammalian studies that employed both positive and negative controls, hydroalcoholic (60% ethanol) extracts were shown to be significantly active. A leaf extract had significant motor depressant, sedative, and antidepressant effects and slight anti-anxiety effects. The extract induced a drastic reduction in the rats' ability to acquire learned responses, and the observed patterns indicated serotonergic brain systems were involved (Mora *et al.*, 2006). In the second study, an extract of leaves and flowers had significant anti-anxiety and anti-depressant effects (Herrera-Ruiz *et al.*, 2006).

(1d). *Salvia fulgens* Cav. [sect. *Fulgentes* (Benth.) Epl.]

(Syn. *S. boucheana* Kunthm *S. cardinalis* Kunth, *S. incana* Mart.andGal., *S. grandiflora* Sessé and Moc., *S. orizabensis* Fern., *S. pendula* Sessé and Moc., *S. schaffneri* Fern.)

There is some question concerning the synonymy of several of these taxa, however for the present they are best treated as synonyms of *S. fulgens*. References to their medical application and inclusion in the complex are available for both *S. cardinalis,* as *mirto* (F. Rodríguez Z. s/n, C. Garcia A. s/n, M. Tlapa A. and G. Ubierna J. 375), and *S. schaffneri* Fern., as *mirto grande* (Martínez, 1979).

Other common names: *pinyesi* (Mazahua – *est*. México) *Cardeal do México* (cult., Brazil) (Epling and Toledo, 1943), *segundo Pio Corrêa* (cult., Brazil) (Epling and Toledo, 1943), and *ts'imbarenzé* (Mazahua - Michoacan).

Salvia fulgens, *sensu latu,* ranges across Mexico from the Sierra Madre Occidentale of Michoacan and Jalisco across the transverse ranges east to Puebla and the western slopes of the Sierra Orientale. This species has brilliant scarlet corollas and like many of the large-flowered species of *Calosphace* is cultivated in many gardens and arboreta outside of its native range.

The uses of *S. fulgens* parallel those of *S. microphylla*; it is used in the treatment of folk illnesses both in the ritual cleansing ceremony and given as a tea (the ærial parts are decocted) to induce sleep. This soporific tea is an especially useful treatment for sick or restless children, and as with *S. microphylla,* a branch of the plant is placed near the child's head to give them peaceful sleep (Argueta V., 1994). In Puebla, the decoction is used to bathe newborn babies, and the branches are seemingly used in *limpiezas*, the ritual "cleansing" performed to heal certain folk illnesses (Argueta V., 1994) (Table 3.5). Additionally, the tea is used for stomachache (C. García A. s/n; Esquivel *et al.*, 1987). In Michoacan, this species is collected as fodder for livestock such as sheep and horses

(Farfán *et al.*, 2007). The aerial parts of the plant contain the clarodane diterpenoid, salvigenolide, the pimarane-type diterpenoid, sandaracopimaric acid, and β-sitosterol (Esquivel *et al.*, 1985, 1987).

(1e). *Salvia involucrata* Cav. [sect. *Holwaya* Ramamoorthy]

(Syn. *S. puberula* Fern., *S. laevigata* Kunth, S. *palafoxiana* Sessé and Moc., *S. ventricosa* Sessé and Moc.)

Other common names: *Cardeal do México* (cult., Brazil) (Epling and Toledo, 1943), *mirto real* (Puebla, Mex.), and *segundo Pio Corrêa* (cult., Brazil) (Epling and Toledo, 1943)

The inflorescences of *Salvia involucrata* are among the most beautiful of sage flowers, the deep magenta to bright pink corollas and calyces are enclosed within similarly colored involucral bracts before they mature and emerge into flower. This species is widespread although not abundant throughout central and eastern Mexico, and as with many of the larger, showy species, this too is frequently cultivated.

Salvia involucrata is used in the treatment of the folk illness, *aire* (Argueta V., 1994) (Table 3.5). This species is related to both *S. fulgens* and *S. microphylla*; all three species are well-supported within the monophyletic *Fulgentes* clade (Jenks, chapter 1). It is therefore not surprising that all three have similar ethnomedicinal uses. The water-soluble fraction of a whole leaf extract had dose-dependant receptor activity typical of materials with cholinergic activity, *i.e.* it screened as an acetylcholine receptor agonist, indicating its potential for use in the treatment of neurodegenerative diseases such as Alzheimer's disease (Wake *et al.*, 2000).

Lastly, while these five species of *Salvia* are the most significant members of the *mirto* complex and most sources refer exclusively to these sages, occasionally other species are known in various parts of Mexico as *mirto*, i.e. *Bouvardia glaberrima* Engelm. (Rubiaceae) in Sonora; *Bouvardia ternifolia* (Cav.) Schl. (Rubiaceae) in Coahuila and Durango; *Lippia geminata* Kunth (Verbenaceae) in Tamaulipas; *Loeselia mexicana* (Lam.) Brand. (Polemoniaceae) in Querétaro and México; and *Stachys coccinea* Jacq. (Lamiaceae) (Martínez, 1979).

(2) ***Ñucchu*** complex:

The *ñucchu* complex is comprised of seven species of *Salvia* from the Peruvian Andes (Figure 3). These species are characterized by their red corollas, and are generally perennial herbs. These species are notable for their prominence in religious celebrations and for the use in indigenous herbal medicine, specifically employed in the treatment of respiratory and nervous system illness (Table 3.3). The name *ñucchu* appears to be a Quechua word reserved for species of *Salvia* and perhaps other mints (Lamiaceae); it is spelled variously in the literature, i.e. *ñujch'u, ñuqchu, nucchu, nujchu,* and *nukch'u*.

(2a). *Salvia oppositiflora* Ruiz and Pávon [sect. *Biflorae* (Benth.) Epl.]
(Syn. *S. grata* Vahl, *S. strictiflora* Hook., and *S. cupheifolia* Kunth)

Other common names include: *chucchu* (Ferreyra, 1984), *salvia ñuqchu, puka ñuqchu* ("red" *ñuqchu*), and *saqraq ñuqchu* ("devil's" *ñuqchu*) (all Quechua).

Salvia oppositiflora is a low-growing perennial herb with red flowers from Peru. Within the inflorescence there are only two flowers per verticillaster as with all members of section *Biflorae*, including *S. tubiflora* below.

The most important contemporary use of *S. oppositiflora* is its use in the Holy Week and Corpus Christi celebrations in Cuzco (Roersch and van der Hoogte, 1988; Franquemont *et al.*, 1990; Roersch, 1994). Franquemont *et al.* (1990) report that the red flowers are gathered by children and then thrown on the figure of Christ during processions. The streets are red from the large number of flowers thrown from the balconies, and the flowers are representative of the blood of Christ. Roersch and van der Hoogte (1988) associated *S. oppositiflora* with the Lord of the Earthquakes, while Roersch (1994) later states that the flowers are strung into necklaces which are placed around the necks of the Saints brought out for the Corpus Christi parades.

Medicinally, the flowering tops of this species are infused and used for respiratory illnesses such as pneumonia and asthma; it also acts as a sudorific (Roersch and van der Hoogte, 1988; Roersch, 1994; Moscoso Castilla, 1997) and expectorant (Jaramillo and Ahumada-Barona, 1983). It is also used for and eliminating phlegm from the esophagus, for colds and flu (Moscoso Castilla, 1997), who states, "Todos estos males ceden ante el poder curativo de esta hermosa y bendita yerba de los Incas." ["Everywhere, all evil flees in the presence of the curative power of this beautiful and blessed herb of the Inca."]

An essential oil isolated from leaves and stems of *S. oppositiflora* was analyzed and found to be composed primarily of oxygenated terpenoids (59.5%). The primary constituents were guaiol (34.2%), a compound tentatively identified as eudesmol (10.3%), and bulnesol (9.4%). (Fernández-Zuniga *et al.*, 1996)

(2b). *Salvia tubiflora* Smith [sect. *Biflorae* (Benth.) Epl.]

(Syn. *S. biflora* Ruiz and Pavón, *S. excisa* Ruiz and Pav., *S. scrobiculata* Meyen, *S. biflora* var. *glabrata* Benth.)

Other common names include: *Añasqentu* (Quechua - Paria), *Pampa salwiya* (Aymara), *Ruda* (Paria - Oruro)

Another Peruvian species of section *Biflorae*, *Salvia tubiflora* also has red flowers in pairs and is a perennial herb with more pubescent leaves than *S. oppositiflora*. *Salvia tubiflora*, like *S. oppositiflora,* was sacred to the Inca and used in religious "cults" (Perez de Barradas, 1957). It was also used to treat respiratory illnesses such as pleurisy (Herrera, 1940; Perez de Barradas, 1957). Perez de Barradas (1957) also states this plant was used as a soporific (to put one to sleep), but he may have simply been misquoting earlier reports (Herrera, 1940) that stated it was used as a sudorific. De Lucca D. and Zalles A. (1992) state that this species is used similarly to *S. bullulata* Benth. An infusion of the leaves of *S. bullulata* is drunk as a stomach tonic and for headaches while externally it is used as a wash for wounds and hemorrhoids (De Lucca D. and Zalles A., 1992).

(2c). *Salvia dombeyi* Epl. [sect. *Longiflorae* (Benth.) Epl.]

(Syn. *S. longiflora* Ruiz and Pav.)

Other common names include: *Llagas Nujchu* (Quechua) (Moscoso Castilla, 1997), *salwiya* (Aymara and Quechua), and *Yawarkuma* (Quechua)

Salvia dombeyi is known for its beautiful flowers that are up to 12 cm long, the largest *Salvia* flowers. They are red and pendulous; the plant itself is perennial and can become woody with age and quite tall if supported (6 feet or more). The plant is native to Peru and Bolivia, where like the *ñucchu* species of section *Biflorae* it is considered sacred by the Inca (Epling, unpublished; Perez de Barradas, 1957; Franquemont *et al.*, 1990; Moscoso Castilla, 1997).

This plant is cultivated for use in the religious festivals of Holy Week where it is wrapped around the arms of the figure of Christ (Franquemont *et al.*, 1990), similarly to the use of the red flowers of *S. oppositiflora* and *S. tubiflora*. Like those species, a tea of *S. dombeyi* is also used for respiratory ailments (Roersch, 1994). Furthermore, the infusion is good for infirmities of the liver, for urine retention, for epilepsy and muscular debility, spasms, colds and chills. For rheumatism and neuralgia, a liniment of the herb macerated in alcohol is massaged into the affected area, and leaves are applied externally to wounds to sop bleeding. The leaves are also chewed raw to clean teeth and to freshen the breath (De Lucca D. and Zallas A., 1992).

(2d). *Salvia revoluta* Ruiz and Pav. [sect. *Pavonia* (Epl.) Epl.]

(Syn. *S. macbridei* Epl.)

Other common names include: *Churin, Ch'urin* (Callawaya), and *kuma* (Aymara),

Salvia revoluta is another perennial species from Peru; it is shrub in habit and has purple-red flowers. Medicinally, it is considered similar in action to *S. dombeyi* (De Lucca D. and Zallas A., 1992). An infusion of the leaves is drunk to energize and stimulate the circulation and as a blood tonic. It is also used for cases of vomiting, nervousness, diarrhea, headaches and bloody urine or vaginal discharge. It is unknown if but likely that the flowers of this species are used in the Holy Week celebrations like *S. oppositiflora, S. tubiflora,* and *S. dombeyi*.

(2e). *Salvia sarmentosa* Epl. [sect. *Flocculosae* Epl.]

Common name: *ásul ñuqchu* (Quechua) (Franquemont *et al.*, 1990)

Salvia sarmentosa is a small shrub from Peru with blue flowers as the common name indicates. The members of the *ñucchu* complex with small blue flowers (2e,f,g) are not ignorant culturally for religious use as are the species of the complex with large red flowers. Medicinal uses of the small, blue-flowered species are also minor to none. The appellation *ñucchu* and hence their inclusion within this MPC, is due to the relationship recognized by the Quechua based on morphological similarity. In the case of *S. sarmentosa*, it seems to be only known as *ásul ñuqchu*, indicating that it is a different kind or species of *ñucchu* in their system of folk taxonomy. It is probable that other species of *Salvia* or species that have a similar appearance are also classified as *ñucchu* by the Quechua.

(2f), *Salvia rhombifolia* Ruiz and Pav. [sect. *Rhombifoliae* (Epl.) Epl.]

(Syn.: *S. foliosa* Benth., *S. pilosa* Vahl, *S. tafallae* Benth.)

Other common name: *yerba de cancer*

Primarily known from Peru, but occasionally Bolivia and Chile, *Salvia rhombifolia* is an annual species with small, blue flowers. No medicinal applications are reported, however the Spanish name, *yerba de cancer* (Franquemont *et al.*, 1990) implies its use in the treatment of cancer.

(2g). *Salvia occidentalis* Swartz [sect. *Microsphace* (Benth.) Epl.]

(Syn.: *S. procumbens* Ruiz and Pav., *S. martinicensis* Sessé and Moc.)

Other common names: American Field Balsam, American Field Basil, *Banderilla*, *Cansaperro* (Colombia), *Cansa-Perro* (Central America), Catnip, *codillo* (Oaxaca, Mex.), *Corrimiento*, Field Basil, *Gonce de Gallina* (El Salvador), *Gopweek*, Hap-Weed, *hierba del cancer* (Morelos, Mex.), *Hierba de Cangro* (El Salvador), *hmo-tôn* (Chinatec – Oaxaca, Mex.), *K'ak-nap* (Maya – Yucatán, Mex.) (Martínez, 1979), *Moradilla, Moradilla Azul, Mozote de gallina* (El Salvador), *Mozote de Pollo* (El Salvador), *mozotillo* (Honduras) (Standley and Williams, 1973), *Pegapega* (Central America) (Standley and Williams, 1973), *Sacha hierbabuena* (Peru) (von Reis and Lipp Jr., 1982), *trencilla negra* (El Salvador) (Standley and Williams, 1973), *verbena, Vulnerê* (Haiti), and *yaxal nich wamal* (Chiapas, Mex.).

Salvia occidentalis, while it probably originated in Mexico (see Jenks, chapter 1, for support of the Mexican origin of section *Microsphace* to which *S. occidentalis* belongs), is naturalized throughout the Western Hemisphere as is reflected in the common names listed above; it grows from Mexico and Florida, through Central America and the Caribbean into the Andean South America, as far south as Bolivia. This widespread species is a small, annual plant with small blue flowers. It is used extensively as a medicine throughout its range. While it is an important medicine for many indigenous groups, as a member of the *ñucchu* complex it is seemingly insignificant; there is only one reference to *S. occidentalis* as a species of *ñucchu* (Ferreyra, 1984).

Throughout the range of *Salvia occidentalis,* it is most frequently used for G.I. illnesses. In his Ph.D. dissertation, Schultes (1941) reported that the leaves of this species were decocted and drunk in the Tuxtepec region of Oaxaca for dysentery and as a stomach tonic, sometimes blended with *Petrea volubilis* L. In Amazonian Peru it is used to treat colic, nausea, and flatulence (Vásquez, 1989). In Trinidad and the Bahamas, a decoction is taken to relieve colds and influenza and to expel intestinal worms, while an infusion is taken to stop diarrhea, dysentery and vomiting and as a remedy for indigestion (Morton, 1981). It is also used for G.I. complaints such as dysentery in Colombia, El Salvador (von Reis Altschul, 1973; Garcia Barriga, 1975), and Haiti where in addition to its use in treating stomach pain and indigestion (Weniger *et al.*, 1986) it is taken as an emmenagogue and for fevers (Morton, 1981). It is reportedly used as an analgesic in Panama (Duke, 1975), and in Cuba a decoction is taken as a stimulant (Morton, 1981).

Externally, the ground-up leaves are plastered on ulcers of the skin (John Brett 822) as well as wounds, both on humans and livestock (Rubén Bautista Cruz 36). In Colombia, Haiti, and Jamaica it is used as eye drops (the juice is expressed from the leaves and stems) and to treat granulomas, boils and skin problems (Garcia Barriga, 1975; Morton, 1981; Garcia Barriga, 1992).

(3) *Lí'l++* complex:

The name of this MPC is Chinantec; there are species used for food, medicine, and magic by the Chinantec people of Oaxaca, Mexico, and others. (Figure 3; Table 3.4)

(3a). *Salvia purpurea* Cav. [sect. *Purpurea* (Epl.) Epl.]

(Syn. *S. affinis* Cham. and Schlecht., *S. farinosa* Mart. and Gal., *S. graciliflora* Mart. and Gal., *S. oxyphylla* Brandegee, *S. purpurea* var. *pubens* Gray ex Wats.)

Other common names include: *Chan, chaptzuún* (Huehuetenango, Guat.), *chichinguaste, chichinguaste morado, Clarín de monte, cuatro-filos morada, Flor morada* (Honduras), *mintsingi tzitziki* (Michoacan, Mex.), *ponpón lila*, and *tutzunún* (Cobán, Quecchí).

Salvia purpurea is a fairly variable species that ranges from central Mexico south through Guatemala, Honduras, and El Salvador. This erect, perennial shrub can grow up to 8 feet and has beautiful pink to lavender to deep lilac flowers. It is part of the *chia/chan* complex (Standley and Williams, 1973), and as such the seeds are eaten as a nutritive food. The Chinantec cultivate this species for medicinal and ornamental use (Martin, 1996; Caballero *et al.*, 2000). It is employed in magic (Ma. E. Ventura 233) in Veracruz and taken for stomach pain in Michoacan (Argueta V., 1994).

(3b). *Salvia inconspicua* Benth. [sect. *Sigmoideae* Epl.]

(Syn. *S. elongata* Mart. and Gal., *S. multiramea* Fern., *S. protracta* Benth., *S. querceto-pinorum* Epl.)

Other common names include: *chia, jehuite blandito*, and *tlachpahuastle* (Nahua - Puebla)

This is a small shrub from southwestern Mexico with small blue flowers. It is also part of the *chia* complex, and as such its seeds are occasionally consumed. It is a medicinal plant of the Chinantec (Martin, 1996; Caballero *et al.*, 2004). It is used in Puebla for bile (mixed with *Sida rhombifolia*) while an infusion of the plant is used externally for washing wounds and bathing newborns. It is also used in cleansing the body of *mal de ojo* (Martínez Alfaro *et al.*, 1991) (Table 3.5).

(3c). *Salvia sapinea* Epl. [sect. *Scorodonia* Epl.]

Salvia sapinea, a blue-flowered perennial subshrub from Guerrero, is apparently used for ornamental, medicinal, and “other” uses by the Chinantec of Oaxaca (Martin, 1996; Caballero *et al.*, 2004). This species has several interesting flavones: 5,6,4'-trihydroxyflavone-7,4'-dimethyl ether (also called apigenin-7,4'-dimethyl ether) and 5,7,3',4'-tetrahydroxyflavone-6,7-dimethyl ether (also called luteolin-6,7-dimethyl ether or cirsimaritin). Cismaritin was reported to have a high anti-microbial activity against several bacteria strains (Miski *et al.*, 1983; Pereda-Miranda *et al.*, 1986). *Salvia sapinea* also has several triterpenes: 2α,3α-dihydroxyolean-12-en-28 oic acid, oleanolic acid, and ursolic acid; oleanolic and ursolic acids have antimicrobial, anti-inflammatory, antihyperlipidemic, antiulcer, hepatoprotective, hypoglycemic, antifertility, anticarciogenic, and antiangiogenic properties (Topcu, 2006).

(4) *Cantueso* complex:

Cantueso is Spanish name for lavender and thyme. It also applies in Mexican folk medicine, especially in the states of México and Morelos (Figure 3), to two species of *Salvia,* both perennial herbs with small, blue-flowers. (Table 3.4)

(4a). *Salvia lavanduloides* [sect. *Lavanduloideae* Epl.]

(Syn. *S. fratrum* Standley, *S. humboldtiana* Schultes, *S. lavandulaefolia* Spreng.)

Other common names: *Bacal nich* (Tzeltal)*, k'anrrejna* (Mazahua - Michoacan), *Mazorquita, salvia del monte* (Standley and Williams, 1973), and *verbena* (Guatemala).

Salvia lavanduloides is a small, herbaceous perennial with blue flowers that is somewhat variable across its range from central Mexico south through Guatemala. It has been cultivated as a bedding plant in gardens well outside of its natural range. In Nayarit it is apparently used as a honey crop (A. Flores M. and R. López M. 2166).

Medicinally, it is used for relieving cough; the flowers and leaves prepared as a decoction (boiled for 10-15 minutes) which is drunk three times a day for seven to ten days (L. Soto P., L. Hernández L. and A. López 1576; Produssep, no date). The flowers are boiled and the vapours are inhaled to clear stuffy sinuses; the seeds are used for diaper rash and for cleaning diapers (Produssep, no date). In Guatemala it is used to treat malaria (von Reis Altschul, 1973). It is reported to have expectorant, antipyretic, haemostatic and antidysenteric actions (Ortega *et al.*, 1991). It is commonly sold as an item of commerce in markets in Michoacan and elsewhere in central Mexico; the primary use of the purchased product is as a hair rinse, applied the head to prevent hair loss (Martínez, 1967; Farfán *et al.*, 2007). It is also a food source (Farfán *et al.*, 2007). The aerial portions of this species contain the secoclerodane diterpenoids salvianduline A, B (Ortega *et al.*, 1991), C (Moldonaldo *et al.*, 1991), and D (Moldonaldo *et al.*, 1994), the flavone 6-hydroxyluteolin-6,7,3'',4'-tetramethyl ether (Rodriguez *et al.*, 1974), and the triterpenes 3-oxo-ursolic acid methyl ester, oleanolic acid, and ursolic acid (Ortega *et al.*, 1991; Topcu, 2006). The roots contain the abiatane diterpenoids horminone and 7alpha-acetoxyroyleanone and the triterpenes β-sitosterol and ursolic acid (Moldonaldo *et al.*, 1994).

(4b). *Salvia longispicata* Mart. and Gal. [sect. *Angulatae* (Epl.) Epl., subsect. *Rudes* Epl.]

(Syn.: *S. jaliscana* Briq., *S. molina* Fern.)

Other common names include: *la Linda Señora, verbena*

Salvia longispicata is another perennial herb native to central Mexico that has pretty, small, blue flowers. This species is reportedly cultivated and sold in markets for medicinal use in Venezuela (Morton, 1981). It is excitant and sudorific (Martínez, 1967; Morton, 1981)

(5). *Manga-paqui* complex:

This complex requires further study; three species of *Salvia* are currently identified within this Ecuadorian complex (Figure 3; Table 3.4). These species are known to be used for liver and kidney problems (von Reis Altschul, 1973; von Reis and Lipp Jr., 1982; Joyal, 19870, however other medicinal applications probably exist. The three species are *Salvia scutellaroides* Kunth [sect. *Hastatae* (Benth.) Epl.] (Syn. *S. petiolaris* Kunth), *S. macrophylla* Benth. [sect. *Hastatae*], and *S. pichinchensis* Benth. [sect. *Siphonantha* (Epl.) Epl.] (Syn. *S. siphonantha* Briquet). *S. pichinchensis* is known as *Quinde-sungana mangapaque*; the leaves of this species are stuck on one's forehead for headache (von Reis Altschul, 1973). The two species of section *Hastatae* are closely related, phylogenetically (Jenks, chapter 1). All three species of this complex are perennial shrubs with deep blue flowers that are large and showy. A close relative of the two *manga-paqui* species from section *Hastatae*, the Colombian medicinal, *S. palaefolia* Kunth, contains the following triterpenes: β-amyrin, lupeol, olean-12-en-3-one, oleanolic acid, squalene, taraxerol, taraxerone, ursolic acid (Topcu, 2006).

(6-9) Tzeltal complexes:

In their coverage of Tzeltal folk taxonomy in Chiapas, Berlin *et al*. (1974) describe several *Salvia* complexes, some of which have medicinal uses. Many of the species that comprise these complexes have multiple uses among other indigenous groups, but only their Tzeltal uses are listed here. Since the Berlin *et al*. (1974) treatment of these complexes is complete and since regurgitating their work here would be unoriginal and tedious, the complexes and species that comprise them are only briefly mentioned in this paper for the sake of completeness.

(6) *sabal c'unun wamal* complex

Primary species: *Salvia cinnabarina, S. purpurea, S. karwinskii, S. holwayi,* Also included: *S. excelsa, S. disjuncta, S. polystachya, S. rubiginosa, S. longispicata, S. chiapensis, S. incarnata,* and *S. leptophylla.* Also as *sak'al wamal*, *S. coccinea.* Uses: the leaves are the preferred toilet paper since they are large and soft (Berlin *et al*., 1974). In addition to these species *S. pansamalensis, Zijk'al sabal tz'anan wamal*, is included in the complex (Méndez Ton 7353).

(7) *ˆc'aal bakal* complex

Primary species: *Salvia lavanduloides.* Also included: *S. lasiocephala* (listed as syn. *S. hyptoides*)*, S. myriantha, S. tiliifolia,* and *S. recurva.* Uses: the stems and leaves are made into a tea and take to treat diarrhea and whooping cough. (Berlin *et al*., 1974)

(8) *ihk'al niˆc wamal* complex

Primary species: *Salvia urica, S. recurva,* and *Scutellaria* spp. Also included: *S. cacaliifolia* and *S. rubiginosa* (Berlin *et al*., 1974). Additional herbarium data warrant the inclusion of two more species in this complex: *S. lasiocephala* (identified as syn. *S. hyptoides*, *yaxal nich* on González González 463) and *S. occidentalis* (*yaxal nich wamal* on John Brett 822).

(9). *wohĉ'ol 'ok* complex

Primary species: *Salvia iodantha, S. myriantha, S. polystachya,* and *S. purpurea.* Also included: *S. chiapensis, S. karwinskii, S. rubiginosa,* and other, non-*Salvia* taxa (Berlin *et al*., 1974). Interestingly, all of the primary species in this complex except *S. myriantha* are closely related phylogenetically (Walker, 2006; Jenks, chapter 1).

DISCUSSION

Our results indicate that a number of medicinal plant complexes are present within *Calosphace* and these complexes are well represented in two of the major centers of diversity, *i.e.* Mexico and the Andes (Figure 3). While there are a number of medicinal *Salvia* species from the Caribbean and Brazil, it is interesting to note that there appear to be no major medicinal plant complexes of *Salvia* from these centers of *Calosphace* diversity. To determine whether this is due to the smaller numbers of *Calosphace* taxa endemic to these regions (see Figure 3 for the numbers of species present in each center of diversity) or whether sages are simply employed less frequently in folk medicine in the Caribbean and Brazil will require further research. There is however a positive correlation between species diversity and complex diversity (Figure 3), as might be expected. Our results identify seven complexes from Mexico and Central America (although four of them are restricted to the Tzeltal of Chiapas) and two from the Andean cordillera of South America, supporting our hypothesis that species will assort into complexes identified by shared common names, appearances and medicinal uses.

***Mirto* complex:**

The most significant medicinal plant complex in the Mexico/Central America center of *Salvia* diversity is the *mirto* complex, the species of which share the morphological features of red flowers and aromatic, perennial vegetative growth habit. The species of this complex are all used in healing several folk illnesses and in treating conditions of the nervous and G.I. systems; to a lesser degree they are used in gynecology and obstetrics.

Mirto is frequently employed in the treatment of the folk illness *susto*; a more severe form of the illness is also known as *espanto*. *Susto* is usually precipitated by the occurrence of a frightening event or series of unsettling situations. The shock or fright that induces *susto* results in the statistically significant symptoms of loss of appetite, debility, lethargy, lack of motivation, and weightloss (Rubel, 1960; Rubel *et al.*, 1984), although other symptoms could include indigestion, insomnia, headache, heart pain, vomiting, sadness, tuberculosis, and “feeling that one’s hair is being pulled” (Tousignant, 1979). However, *susto* is not truly caused by the frightening experience; this illness derives from the supernatural loss of some vitality. The human spirit initially becomes detached from the body as a result of a frightening or unsettling experience, however his detachment, or soul loss, is attributed to the person failing to show deference or respect to the guardian of the locale (where the fright occurred) or a spirit being of a species of animal, fish or bird. Subsequent sickness is attributed to these supernatural forces that capture of the person's vital force. Other physical symptoms are attributed to spirits or their representations in the form of *aires* (Gillin, 1948; Rubel, 1960; Rubel *et al.*, 1984; Baer and Bustillo, 1993; Lincoln, 2001 — for the related illness *nervios,* see Low, 1981). It is also important that many of the species of *mirto* are used in the treatment of *aire,* which may both accompany *susto* (and may be responsible for some of the physical symptoms) and occur independently (Table 3.5).

A *limpieza* ("cleansing") is a ritualized magical healing ceremony performed by an indigenous healer (*curendera* or *curendero*) to restores psychological balance and spiritual wholeness to the individual suffering from *susto* of other folk illness. In the most common form of the *limpieza* the patient lays down and recounts the frightening or traumatic circumstances that led to them having *susto*. The healer fashions a broom from branches of *mirto* (sometimes other herbs may be used) and ritually sweeps the ritual broom over the patient while uttering prayers, symbolically cleansing the body (Rubel, 1960; Baer and Bustillo, 1993; Argueta V.. 1994). Not only are these aromatic *Salvia* species used to spiritually or psychically heal the patient through the *limpieza*, but the patient also drinks an infusion to treat the symptoms of the illness. Individuals suffering from *susto* and other folk illnesses, particularly children and newborns, may also bathe in the *mirto* infusion. The symptoms of *susto* and other folk illness frequently overlap with the physiological complains that *mirto* is used to treat, e.g. nervous system and G.I. problems. And, while species of the *mirto* complex are the most frequently employed in the treatment of folk illness, there are a number of other species that are used in the *limpieza* and in treatment of the physical symptoms of folk illnesses (Table 3.5).

Mirto species contain many interesting compounds that may be partially responsible for their effectiveness as medicine. Here are several examples. *Salvia microphylla* contains the triterpene oleanolic acid which is antimicrobial, anti-ulcer, and anti-inflammatory (among other properties); these properties may account for some of its effective treatment of stomach aches, diarrhea, and other G.I. illnesses, as well as the indigestion and upset stomach associated with *susto*. The demonstrated anti-bacterial and anti-fungal properties of two *S. microphylla* terpenoids, 12-methoxycarnosic acid and β-eudesmol, suggest its use as an external wash is probably useful. The whole plant extract of *mirto* species *S. elegans* was shown in mammalian studies to be have significant anti-anxiety, anti-depressant, and sedative effects, rendering it extremely useful in treating insomnia, depression, and other symptoms of *susto*.

Ñucchu **complex:**

Our results identify the Peruvian *ñucchu* as the most significant medicinal plant complex from the Andean center of *Calosphace* diversity. The species of this complex appear to form two tiers both morphologically and medicinally/culturally. The most important *ñucchu* species from a cultural and medicinal standpoint are perennial herbs or small shrubs with large red flowers, *e.g. Salvia oppositiflora, S. tubiflora, S.dombeyi,* and *S. revoluta*. These species are used extensively as symbolic decorations in the religious ceremonies and parades in Cuzco, and are important medicinally in the treatment of respiratory illnesses. The second tier of species is comprised of blue-flowered, typically annual herbs whose medicinal and cultural value appears to be insignificant in Peru. That these species, *e.g. S. rhombifolia, S. sarmentosa,* and *S. occidentalis,* share the name *ñucchu* is simply indicative of the perceptivity of the Quechua who recognize some similarity of appearance between these seldom-used *Salvia* species and the culturally and medicinally important, red-flowered species.

It does not appear, however, that *ñucchu* is or was sacred to the Inca simply because of its medicinal attributes. The contemporary use of *ñucchu* in Holy Week processions appears to have had its modern origins in 1650. Cuzco legend states that in the great earthquake of 1650 a statue of Christ in the city's cathedral saved many people from death during the devastating quake. The image was donated by Charles V of Spain, which henceforth became venerated as the patron saint of Cuzco, the city's protector known as *Taitacha Temblores,* the "Lord of the Tremors". Santo Domingo cathedral houses *Taitacha Temblores* was built atop the ancient temple, Coricancha, to the Inca god, Viracocha. Every year on Easter Monday of Holy Week, the image of the *Taitacha* is carried out of the cathedral and through the streets of Cuzco in a grand celebration, bedecked with *ñucchu* and festooned in wreathes of red *Salvia* corollas strung together. As this spectacle proceeds through the city, the crowds of people, some above in balconies, throw yet more *ñucchu* on the *Taitacha* and on the street before he passes; the mass of red *ñucchu* is symbolic of the blood of Christ (Roersch and van der Hoogte, 1988; Franquemont *et al.*, 1990; Roersch, 1994). But when and of what accord did the custom of using *ñucchu* as symbolic decoration for *Taitacha Temblores* arise? Is its sacredness and cultural importance post-Colombian, or was *ñucchu* used to some sacred purpose prior to Pizarro and the Spanish arrival in 1526?

The earliest published reference to *ñucchu* is in the Inca drama, Apu Ollantay (Valdez, 1770), dated to c. 1470 A.D.; however, it wasn't put into writing until 1770, by Dr. Antonio Valdez, the Cuba of Sicuani. Inca dramas were apparently common, but this is the oldest (and perhaps only) one surviving. This drama is pre-Spanish in origin and provides clear evidence that *ñucchu* was known and important to the pre-Christian Inca. The basic plot summary is that of a noble commoner, Ollantay, falling in love with the royal princess, Cusi Coyllor, in blatant defiance of the Inca ruler. Ollantay is banished because of his love for Princess Cusi Coyllor and leads a revolt that ultimately plunges the empire into war and unrest. The mention of *ñucchu* is in act 3, scene 3. These words are spoken about the Inca ruler to Ollantay by a servant.

> A new ruler now reigns in Cuzco
> Tupac Yupanqui [the ruler] is in power
> Against the wish of the people,
> He rose upon a wave of blood;
> Safety he sees in headless bodies,
> The *sunchu* and the *nucchu* red
> Are sent to all he would destroy.

The translator's commentary states that *sunchu* is a large, yellow sunflower (*Asteraceae*) that grows around Cuzco, while *nucchu* is a species of *Salvia* that is considered sacred (Valdez, 1770). While this drama provides clear evidence that *ñucchu* was used by the Inca in pre-Spanish times, however it is less clear from the drama what the uses of *ñucchu* were. Documented pre-Hispanic uses of *ñucchu* are few. The Inca nobility wore headdresses, the beauty of which indicated their social status. Included in their headdresses were several species of flowers both red and yellow (Guaman Pema de Ayala, 1615; Cobo, 1653), and *ñucchu* has been identified in the headdresses of the Inca nobility of the vice regal period (Pardo, 1953; Dean, 1999). There were several species of flowers, *ñucchu* and *kantuta*, that were sacred to the Inca and revered; *ñucchu* flowers appear to have been specifically associated with nobility and authority (Pardo, 1953; Dean, 1999). When an Inca ruler returned to Cuzco after having been away from his city, the people scattered coca leaf, flowers (which probably included *ñucchu*) and colorful feathers on the ground before him as he approached. Cloth, llamas, coca, and flowers (again probably including *ñucchu*) were presented to deities as offerings (MacCormack, 1991). Popular legend states that *ñucchu* was sacred and used as an offering to Viracocha, specifically. Since *ñucchu* was one of the sacred flowers of the Inca, it is likely that *ñucchu* was offered to Viracocha and other deities, but I was not able to find published validation specifically linking *ñucchu* to Viracocha.

While references to specific pre-Spanish *ñucchu* use are few, the contemporary *Taitacha Temblores* procession in which *ñucchu* is abundantly used has a strong historical precedence. It is recorded (Cobo, 1653; MacCormack, 1991) that deceased Inca nobility wielded great power and were venerated and honored in the form of their mummified remains, their physical bodies preserved long after their death. These sacred mummies *(huacas*) were consulted for advice, had dedicated attendant servants, and were held in high esteem, venerated and even worshiped by their descendants. The most powerful ancestor/gods were wrought in gold and offered sacrifices of cloth, llamas, coca, flowers (*ñucchu* and others), and as circumstances dictated, blood sacrifices of guinea pig, llama, and humans. The Inca calendar was “an interconnected fabric of sacred time” (MacCormack, 1991) with many festivals and associated parades. During the numerous religious holidays, the mummies were extravagantly decorated in robes and gold jewelry and paraded through the streets of Cuzco. During the Inca festivals the *huacas* and idols were anointed with sacrificial blood. Blood of sacrificial animals and even children flowed freely in the Cuzco streets during the various holidays and processions.

In the pantheon of Inca gods, Viracocha is invariably considered the ancient, celestial high god and the Creator of all, the Maker of mysterious or divine origin. Viracocha is also associated with the deified Sun and the summer solstice, although Viracocha and the Sun are sometimes considered independent deities (Cobo, 1653; Demarest, 1981). The gilded image of Viracocha was housed in the magnificent golden temple of the Sun, Coricancha (as were many of the *huacas*), and Viracocha was brought out and paraded through the streets of Cuzco on a litter during the major festivals, especially the festival Inti Raymi. When Viracocha was brought out, sacrificial blood was spread over the entire statue as well as the ground around (Cobo, 1653). The festival Inti Raymi occurred at the same time of year as the Catholic Corpus Christi (Dean, 1999), indeed in post-conquest Cuzco, Catholicism absorbed many of the Inca festivals. Both religions are marked by a calendar full of holy days and festivals and a proclivity for showy ceremonies and processions. The Catholics had parades of saints instead of *huacas* and golden gods. The Catholics even co-opted the nobility's use of *ñucchu*; the standard bearers in processions wore ornate tunics with emblems of *ñucchu*. (Dean, 1999). However, the Inca secretly continued to worship their own gods and celebrate Inti Raymi while pretending to observe Corpus Christi (Dean, 1999). *Ñucchu* eventually became a blood surrogate, taking the place of the animal and human sacrifices of the Inca. Instead of Viracocha covered with sacrificial blood as the statue was carried through the bloody streets of Cuzco during Inti Raymi, *Taitacha Temblores* and the streets before him are covered in red *ñucchu* flowers during *Semana Santa*. Perhaps the *Taitacha Temblores* is a contemporary incarnation of Viracocha. Regardless, the current *ñucchu*-saturated Holy Week parade is syncretistic, combining elements of ancient Inca religion and Catholicism.

Other complexes:

The *Lí'l++* complex is associated with the Chinantec and is comprised of three perennial species found in Oaxaca and Guerrero, Mexico. The species of this complex, *Salvia purpurea, S. inconspicua,* and *S. sapinea*, are of special significance to the Chinantec who use them for medicine and food, and as ornamentals. The *Cantueso* complex is comprised of two species of *Calosphace, S. lavanduloides* and *S. longispicata,* which are known as *cantueso* primarily in the Valle de México. These species are cultivated for the commercial market and are sold in markets well outside of their natural range. Medicinally, they are primarily utilized for respiratory conditions, but they are also employed in external applications. The *cantueso* species are perennial herbs with small blue flowers. The *manga-paqui* complex consists of three Ecuadorian species, *S. scutellaroides, S. macrophylla,* and *S. pichenchensis*, which are each perennial plants with large, deep blue, and rather showy flowers. The *manga-paqui* species are primarily used of the treatment of liver and kidney problems. It is likely that these three complexes, *Lí'l++, cantueso,* and *manga-paqui* may prove to each contain more species when studied further in the field. The species of these complexes have been largely unexplored chemically, but since their medicinal uses are well-documented further work into their phytochemistry is warranted. *Lí'l++* and *manga-paqui* appear to have a more restricted geographical and cultural basis, while *cantueso* from the central valley of Mexico is reportedly sold for medicinal purposes as far away as Venezuela (Heinrich, 1992). Lastly, while some of the species of the Tzeltal complexes (6-9) appear to be used for medicinal purposes, these complexes are more important as an example of a well-developed and thoroughly recorded folk taxonomic system.

Conclusions:

The results of our study spanning the largest subgenus of *Salvia, Calosphace* (with some 500 species spanning the Western Hemisphere), has supported our hypothesis that species will assort into complexes of medicinal plants that share common names, appearances, and medicinal uses. We have identified five previously unidentified complexes and report on four more. These medicinal plant complexes not surprisingly are associated with the highest levels of *Calosphace* diversity, i.e. the Mexico/Central America and Andean centers of diversity. Of the five complexes, three were identified from Mexico/Central America (*Mirto, Lí 'l++,* and *Cantueso*) and two from the Andes (*Ñucchu* and *Manga-paqui*). *Mirto* (5 spp.) is used extensively in the treatment of *susto* and other illnesses in Mexico; *Ñucchu* (7 spp.) is used as a symbolic element in religious processions and in the treatment of respiratory ailments in Peru; *Lí'l++* (3 spp.) is used for food and medicine by the Chianantec; *Cantueso* (2 spp.) used for respiratory ailments in Mexico*;* and *Manga-paqui* (3 spp.) is used for kidney and liver problems in Ecuador. Each of these complexes contains important medicinal species, many if not all of which contain novel secondary compounds, especially terpenoids, which may prove pharmacologically important. Among these complexes, some appear to have exceptional levels of cultural significance, *i.e. Mirto* and *Ñucchu. Mirto* is used extensively in the treatment of folk illnesses, both ritually in a cleansing ceremony (*limpieza*) and as a tea to treat the physical symptoms of the folk illnesses. *Ñucchu* is sacred to the Inca, a emblem of nobility and an offering to the deities in pre-Hispanic times and used symbolically today as a blood-surrogate in the religious festivals honoring the patron saint of Cuzco, *Taitacha Temblores*.

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Table 3.1. The species of the *Chia* complex within *Salvia* subgenus *Calosphace*, with references indicating their inclusion within the *Chia* complex.

Species	**References**
Salvia misella Kunth	Ayerza Jr. and Coates, 2005
S. subincisa Benth.	von Reis Altschul, 1973 (E. Palmer 305)
S. inconspicua Benth.	Martínez Alfaro, M. *et al.*, 1991
S. thyrsiflora Benth.	Espejo-S. and Ramamoorthy, 1993
S. reflexa Hornem.	Fernald, 1900
	von Reis Altschul, 1973 (E. Palmer 446)
	von Reis Altschul, 1973 (E. Palmer 327)
	Argueta V., 1994
	Curtin, 1997
	Ayerza Jr. and Coates, 2005
S. potus Epl.	Fernald, 1900
	von Reis Altschul, 1973 (E. Palmer 422)
	Williams, 1981
S. hispanica L	Fernald, 1900
	Sahagun, 1950-1982
	Martínez, 1967
	Standley and Williams, 1973
	von Reis Altschul, 1973
	Williams, 1981
	von Reis and Lipp Jr., 1982 (College of Pharmacy s.n. and Palmer 659)
	Argueta V., 1994
	Dweck, 2000
	Fernández-Alonso, 2003
	Ayerza Jr. and Coates, 2005
	Cahill 2001, 2003, 2005
S. lasiocephala Hook.& Arn.	von Reis Altschul, 1973 (R.S. Ferris 5850)
S. melissodora Lag.	Ayerza Jr. and Coates, 2005
S. amarissima Ort.	von Reis Altschul, 1973 (A. Dugés s.n.)
S. leptophylla Benth.	Argueta V., 1994
S. concolor Lamb ex Benth.	Ayerza Jr. and Coates, 2005
S. polystachya Ort.	von Reis Altschul 1973, (Y. Mexia 8841)
	Heinrich, 1992 (Vibrans 445)
	Argueta V., 1994
	Cahill, 2003
	Ayerza Jr. and Coates, 2005
S. leptostachys Benth.	Argueta V., 1994

S. uruapana Fern.	Cahill, 2003
S. tiliaefolia Vahl	Fernald, 1900
	Standley and Williams, 1973
	von Reis Altschul, 1973 (J. Gregg 542)
	Cahill, 2003
	Ayerza Jr. and Coates, 2005
S. iodantha Fern	Argueta V., 1994
S. purpurea Cav	Standley and Williams, 1973

Table 3.2. Species of the *mirto* complex and their uses. See results for references.

Species	Ritual Uses: Folk illnesses, etc.	Internal Uses			External Uses
		Gastro-intestinal	Nervous system	OB/GYN	
Salvia microphylla Kunth	*Susto, espanto, mal de aire, nervios, mal de ojo, aigre de viento*	Stomachache, diarrhea, stomach cramps and colic, indigestion, dysentery, stomach infection, stomach inflammation, vomiting	Insomnia, feelings of suffocation	premenstrual cramps, excessive vaginal bleeding, infertility, childbirth	rheumatism, skin problems such as granulomas
S. *coccinea* Juss. ex Murr.	*Aire, espanto, mal de viento,* protection against *Ik*	Loss of appetite, stomachache, colic, dysentery, diarrhea	Pain, sedative, muscle relaxant, headaches	regulate menstruation, vaginal bleeding and hemorrhaging	red rouge on women's cheeks, nosebleeds, eyewash, *tabaquillo*
S. elegans Vahl	*Espanto, aire*	Stomachache	Insomnia, crying and sighing, post-partum pain		wounds and edemas, post-partum pain and bathing newborns
S. fulgens Cav.	unspecified, probably *sustu*	stomachaches	Insomnia and restlessness		Bathing newborns
S. involucrata Cav.	*aire*				

Table 3.3. *Ñucchu* complex with species and uses. See results for references.

Species	Flowers used in *Semana Santa* procession	Medicinal uses
Salvia oppositiflora Ruiz and Pav.	Yes	Primary use: respiratory system, e.g pneumonia and asthma. It is an expectorant, sudorific, and blood tonic.
S. tubiflora Smith	Yes	Primary use: respiratory system, e.g. pleurisy. Also: as a stomach tonic and for headaches; externally a wash for wounds and hemorrhoids. Possibly soporific.
S. dombeyi Epl.	Yes	Primary use: respiratory system. Also: infirmities of the liver, urine retention, epilepsy and muscular debility, spasms, colds and chills. Externally: as a liniment for rheumatism and neuralgia, leaves as a poultice for wounds and to stop bleeding. Leaves are chewed to clean teeth and to freshen the breath
S. revoluta Ruiz and Pav.	Probably	Primary uses: respiratory system and circulatory stimulant / blood tonic. Also: vomiting, nervousness, diarrhea, headaches, and bloody urine or vaginal discharge
S. sarmentosa Epl.	No	None known
S. rhombifolia Ruiz and Pav.	No	Epidermal sores, boils, and cancers
S. occidentalis Swartz	No	None specifically reported as *ñucchu* or from the Peruvian highlands. However, it is used to treat colic, nausea, and flatulence in the Amazon basin, for treating skin problems in Colombia, and is used extensively throughout its range.

Table 3.4. The medicinal plant complexes *Lí 'l++, Cantueso,* and *Maga-paqui*, the species that comprise each complex, and the medicinal and other applications of each species. See results for references.

Complex	Species	Uses
Lí 'l++	*S. purpurea* Cav.	Medicine, food, ornamental, stomachache, "magic"
	S. inconspicua Benth.	Food, stomach bile, washing newborns, "mal de ojo"
	S. sapinea Epl.	Medicinal, ornamental, "other"
Cantueso	*S. lavanduloides* Kunth	Respiratory conditions, malaria, dysentery, ddiaper rash, food, and as hair rinse.
	S. longispicata Mart. and Gal.	Respiratory conditions
Manga-paqui	*S. scutellaroides* Kunth	Liver and kidney problems
	S. macrophylla Benth.	Liver and kidney problems
	S. pichinchensis Benth.	headache

Table 3.5. Folk illnesses and the species used in the treatment of each. An asterix, *, indicates that species is part of the *mirto* complex. The folk illness which *Salvia fulgens* is used to treat is unspecified in the literature, however the usage implies *susto* or *espanto*. References for *mirto* species are found in results. Other references are as follows: *Salvia filipes* (C. Weimann 58); *S. helianthemifolia* (Argueta V., 1994); *S. inconspicua* (Martínez Alfaro *et al.*, 1995, in reference to synonym, *S. multiramea* Fern.); *S. leucantha* (Argueta V., 1994); and *S. macrostachya* (Fernández-Alonso, 2003).

Species	*Aire/Mal de aire*	*Aigre de viento/Mal de viento*	*Mal de ojo*	*Nervios*	*Susto* and *Espanto*
**S. coccinea* Juss. ex Murr.	X	X			X
**S. elegans* Vahl	X				X
S. filipes Benth.	X				
**S. fulgens* Cav.					(X)
S. helianthemifolia Benth.	X				
S. inconspicua Benth.			X		
**S. involucrata* Cav.	X				
S. leucantha Cav.	X				
S. macrostachya Kunth	X		X		
**S. microphylla* Kunth	X	X	X	X	X

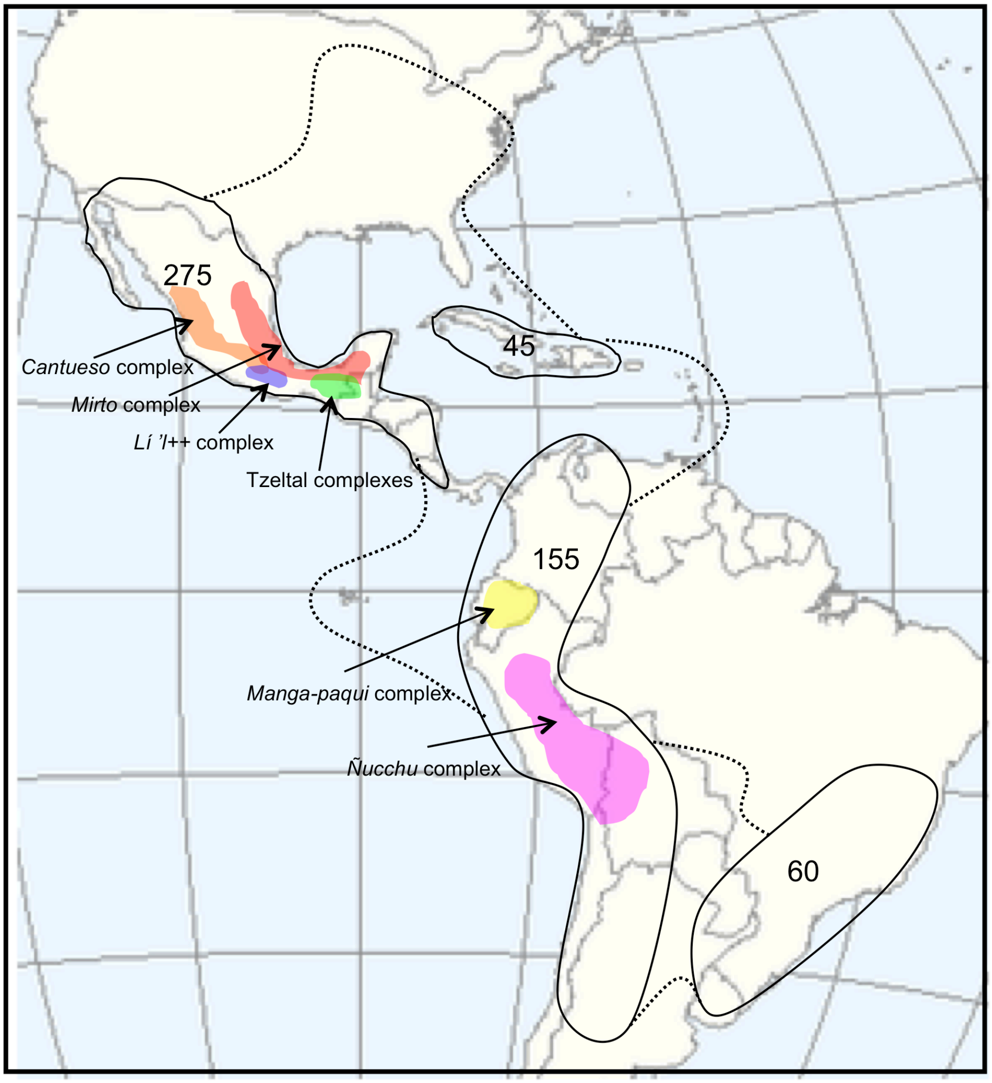

Figure 3. Range and distribution of *Salvia* subgenus *Calosphace* represented by the dotted line. The solid lines represent major centers of species diversity; numbers represent number of species present in each. Colors represent the distribution of the medicinal plant complexes discussed in this article: Red = *Mirto* (1), Pink = *Ñucchu* (2), Blue = *Lí 'l++* (3)*,* Orange = *Cantueso* (4), Yellow = *Manga-paqui* (5), and Green = Tzeltal complexes (6-9),

CONCLUSION

This conclusion is presented in order to summarize the most important findings of my Ph.D. research and perhaps more importantly to proffer areas of further investigation and topics for continued research. Indeed it seems that the greatest achievement of this project is its potential to serve as an outline or framework upon which subsequent work can build, and while the conclusions of each chapter are significant, there is much yet to learn about *Salvia* subgenus *Calosphace.*

In the *Calosphace*-wide molecular phylogenetic analyses we discovered that the previous classification was largely artificial; many of Epling's (1939) taxonomic sections are not natural, especially those comprised of numerous species from multiple centers of diversity. While certain of the smaller sections consisting of only a few taxa restricted to one geographical region are supported as monophyletic (*e.g. Incarnatae, Blakea, Corrugatae, Erythrostachys, Hastatae, Sigmoideae, Nobiles* (*sensu* Santos, 1991), the vast majority of the sampled sections are polyphyletic. Specifically, we can conclusively reject the monophyly of sections *Albolanatae, Angulatae, Conzattiana, Flocculosae, Longipes, Malacophyllae, Maxonia, Polystachyae, Purpurea, Scorodonia, Tomenteallae, Tubiflorae,* and *Uliginosae.* Furthermore, three species from which multiple populations or described varieties were sampled are not supported as monophyletic: *Salvia microphylla, S. mexicana,* and *S. polystachya.*

The most important discovery however, was the elucidation of four, well-supported major lineages that correspond closely with four major stamen types

(Figure 1.2,3,4,and 5; Table 1.2). The first major lineage is represented by a single species, *Salvia axillaris* and strongly supported sister to the rest of *Calosphace*. The second lineage, "*Hastatae* clade" (sections *Hastatae, Standleyana,* and *Blakea*) with 13 species by extension (6 sampled), is strongly supported as sister again to the rest of the subgenus. The third lineage, "*Uliginosae* clade" with a projected 69 spp., is again strongly supported as sister to the remaining species. The fourth lineage, "core *Calosphace*" comprises the largest number of species estimated at close to 400. The phylogenetic relationships of these major lineages parallel floral evolution, particularly the trends seen in the staminal connectives. The trend (Figure 1.5, Table 1.2) is from two fertile thecae per free connective (*S. axillaris*), to one fertile theca per free connective without significant posterior elongation ("*Hastatae* clade"), to one fertile thecae per fused connective with a posterior outgrowth from an assurgent tooth that is developmentally homologous to the secondary thecae ("*Uliginosae* clade" and "*Tomentellae* clade II" and "*Tomentellae* clade III"), to one fertile thecae per fused connective with posterior elongation without an assurgent tooth ("core *Calosphace*").

Our results support a Central American origin of *Calosphace* with subsequent dispersal events to South America; as few as seven long-distance dispersal events could account for the species diversity in the Andes and Eastern Brazil. Long-distance dispersals followed by rapid radition appears to be typical. It certainly appears that biogeography deserves greater credit in the phylogeny of *Calosphace* than was implicit in Epling's classification; the disjunct pattern reflected in many of many of Epling's sections is largely artificial. Indeed, much of the sectional polyphyly observered in our results is due to this lack of intra-sectional disjunction. Taxa morphologically similar enough to be grouped together in the same section by Epling are not necessarily closely related phylogenetically; convergent evolution is probably responsible for this pattern. General patterns of morphological change and character evolution are seen to occur in parallel within North and South American lineages. The phylogeny of the subgenus has more geographical structuring than is immediately evidenced by the morphology.

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In order to more precisely define and delimit the relationships between the major lineages within *Calosphace*, additional gene regions should be sequenced and further sampling especially within the more derived "core *Calosphace*" lineages will be required.

Additionally, morphological scrutiny and analysis will be required to tease apart the species-level relationships.

Perhaps the most fascinating of all the *Calosphace* species is the hallucinogenic sage, *Salvia divinorum.* Our results determined that this species is not closely related to the other members of the Mexican section *Dusenostachys* as it was previously classified (Epling and Játiva-M., 1962) and should no longer be considered part of that section. In both the ITS and cpDNA phylogenetic trees (Figure 2.1,2), *S. divinorum* was strongly supported as sister species to *S. venulosa;* in both trees resolved within a basal grade and not the core *Calosphace* lineage. *Salvia divinorum* and *S. venulosa* are not closely related to the other sampled species of the primarily Colombian section *Tubiflorae* (in which *S. venulosa* is classified) which was highly polyphyletic in our results. Furthermore, *S. divinorum* is not an interspecific hybrid as previously hypothesized (Reisfield, 1993) since it shows neither additivity in the biparentally-inherited, nuclear ITS sequence nor incongruence between the ITS and chloroplast DNA phylogenetic trees. *Salvia divinorum* appears to be a reproductively capable species, however because of its extensive anthropogenicly-mediated, clonal propagation in the Sierra Mazateca, its ability to reproduce sexually seems to be somewhat limited as evidenced by the decreased pollen viability previously reported (Reisfield, 1993). While it is uncertain whether *Salvia divinorum* prospered over a larger geographic range or whether it is correctly identified as the Aztec *Pipilzintzintli* (although both hypotheses are certainly plausible based on the scant available evidence), there has doubtless been an ancient alliance between *Salvia divinorum* and humans who have used it to visionary effect. However, this close association does not, *de facto*, mean that this species is a cultigen, *i.e.* a species requiring of human intervention to thrive and prosper.

Further research is needed to determine what barriers to sexual reproduction might be responsible for the seemingly low sexual viability of *Salvia divinorum* and to determine the genetic diversity within the species. Additionally, it is currently unknown what other species are closely related to *S. divinorum* and *S. venulosa*, whether their closest relatives are to be found in Central America or in Colombia awaits further study. However in further analyses Colombia will receive especial attention, particularly those species of *Tubiflorae* yet unsampled. Pharmacologically, this is a ripe field as well; considerable research dollars have been invested into the discovery, characterization, pharmacology, and analogue development of the terpenes found in *S. divinorum*. The pharmacology of closely related taxa should prove similarly rewarding. And while *S. venulosa* does not appear to biosynthesize the psychoactive clerodane diterpenoid found in *S. divinorum*, salvinorin A, further studies are underway to determine the chemical constituents of *S. venulosa.*

However, there are many other ethnobotanically significant *Calosphace* species as the results of chapter three clearly indicate. While ethnobotanical data was collected for over 150 of the 500 species of *Calosphace*, the purpose of our methodology was to identify medicinal complexes comprised of multiple species. The results support this hypothesis that species will assort into complexes of medicinal plants that share common names, appearances, and medicinal uses. I have identified five previously unidentified complexes and report on four more. These medicinal plant complexes not surprisingly are associated biogeographically with the highest levels of *Calosphace* diversity, *i.e.,* the Mexico/Central America and Andean centers of diversity (Figure 3). Of the five complexes, three were identified from Mexico/Central America (*Mirto, Lí ’l++,* and *Cantueso*) and two from the Andes (*Ñucchu* and *Manga-paqui*). *Mirto* (5 spp.) is used extensively in the treatment of *susto* and other illnesses in Mexico (Table 3.2); *Ñucchu* (7 spp.) is used as a symbolic element in religious prossessions and in the treatment of respiratory ailments in Peru (Table 3.3); *Lí ’l++* (3 spp.) is used for food and medicine by the Chianantec (Table 3.4); *Cantueso* (2 spp.) used for respiratory ailments in Mexico (Table 3.4); and *Manga-paqui* (3 spp.) is used for kidney and liver problems in Ecuador (Table 3.4). Each of these complexes contains important medicinal species, many if not all of which contain novel secondary compounds, especially terpenoids, which may prove pharmacologically important. Among these complexes, some appear to have exceptional levels of cultural significance, *i.e. Mirto* and *Ñucchu. Mirto* is used extensively in the treatment of folk illnesses especially *susto*, both ritually in a healing ceremony (*limpieza*) and as a tea to treat the physical symptoms of the folk illnesses. *Ñucchu* is sacred to the Inca, a emblem of nobility and an offering to the deities in pre-Hispanic times and used symbolically today as a blood-surrogate in the religious festivals honoring the patron saint of Cuzco, *Taitacha Temblores*. Further research, especially ethnobotanical field work on-site where each of these complexes is utilized medicinally, is needed to expand our knowledge of the medicinal uses and cultural significance of these *Calosphace* species.

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APPENDIX 1

List of taxa sampled in this study with voucher information (collector, collection number, and herbarium where specimen is deposited) or literature citation for previous published sequences. Sequences are listed alphabetically by genus and species. Herbarium acronyms, UCR = University of California, Riverside Herbarium, WIS = University of Wisconsin, Madison Herbarium, MO = Missouri Botanic Garden, FIELD = Field Museum of Natural History. Other abbreviations: cult. = from cultivated material; UCRBG = University of California, Riverside, Botanic Garden, Riverside, CA, USA; WSN = Richard DuFresne's World of Salvias Nursery, Star, NC, USA

Dorystaechas hastata Boiss. & Heldr. ex Benth., *1972-0177D* (MJG); ***Salvia adenophora*** Fern., *Crone 7/8/00* (MJG); ***S. agnes*** Epl., *B. Carlson s/n* (UCR); ***S. alamosana*** Rose, *J. Walker 3040* (WIS); ***S. albo-caerulea*** Lind., Walker (2006); ***S. alborsea*** Epl. & Játiva-M., *Sagastegui et al 15910* (FIELD); ***S. amarissima*** Ort., *Lott 3404* (UCR); ***S. amplifrons*** Briq., *P. Wester 141* (MJG); ***S. angulata*** Benth., Walker (2006); ***S. angustiarum*** Epl., *Peterson 629* (WIS); ***S. arbuscula*** Fern., *Tenorio 16911* (WIS); ***S. areolata*** Epl., *Cahill 2966* (UCR); ***S. articulata*** Epl., Walker (2006); ***S. aspera*** Mart. & Gal., *J. Walker 3021* (WIS); ***S. atrocyanea*** Epl., *P. Wester 3* (MJG); ***S. axillaris*** Moc. & Sessé ex Benth., *Breedlove 59702* (WIS); ***S. azurea*** Michx. ex Lam., *J. Walker 3222* (WIS); ***S. ballotaeflora*** Benth., *J.B. Walker 2547* (WIS); ***S. bangii*** Rusby, *P. Wester 10* (MJG); ***S. betulaefolia*** Epl., *Van Devender s/n* (UCR); ***S. blepharophylla*** Brandegee, cult., UCRBG; ***S. brevipes*** Benth., Walker (2006); ***S. cacaliifolia*** Benth., *J.B. Walker 1203* (WIS); ***S. californica*** Brandegee, *J.B. Walker 2520* (WIS); ***S. candicans*** Mart. & Gal., *J. Walker 3001* (WIS); ***S. cardinalis*** Kunth, cult., WSN; ***S. carnea* var. *carnea*** Kunth, *Boeke 2366* (MO); ***S. cedrosensis*** Greene, cult., Rancho Santa Ana Bot. Gard., CA, USA; ***S. cerradicola*** Santos, Walker (2006); ***S.* cf. *ionocalyx*** Epl., cult. (labeled incorrectly as "*S. chiapensis*"), UCRBG; ***S.* cf. *pusilla*** Fern., *Crone 29/7/00* (MJG); ***S.* cf. *stolonifera*** Benth., *J. Walker 3002* (WIS); ***S. chamaedryoides*** Cav., *J. Walker 2009* (WIS); ***S. chapalensis*** Briq., *J. Walker 3029* (WIS); ***S. chiapensis*** Fern., cult., UCRBG; ***S. chionophylla*** Fern., *Jenks 267* (UCR); ***S. cinnabarina*** Mart. & Gal., *J. Walker 3030* (WIS); ***S. clinopodioides*** Kunth, *Iltis 28770* (WIS); ***S. coahuilensis*** Fern., cult., UCRBG; ***S. coccinea*** Juss. ex Murr.,, cult., UCRBG; ***S. concolor*** Lamb. ex Benth., *Reisfield 1286* (WIS); ***S. confertiflora*** Pohl, *1959-9166A* (WIS); ***S. corrugata*** Vahl., *Jenks 256* (UCR); ***S. cuatrecasana*** Epl., Walker (2006); ***S. curtiflora*** Epl., *P. Wester 294* (MJG); ***S. cyanicalyx*** Epl., *Schmidt-Lebuhn 578* (MJG); ***S. discolor*** Kunth, *J.B. Walker 2546* (WIS); ***S. disjuncta*** Fern., *J. Walker 3018* (WIS); ***S. divinorum*** Epl. & Játiva-M., *Jenks 237* (UCR); ***S. dombeyi*** Epl., *1959-9269A* (WIS); ***S. dorisiana*** Standley, *2000-1632A* (WIS); ***S. elegans*** Vahl, *J.B. Walker 1204* (WIS); ***S. erythrostoma* ssp. *isabelina*** Fernández-A., *Fernandez & Castillo 12308* (MO); ***S. faccidifolia*** Fern., *Reisfield & Sundberg 1212* (MO); ***S. farinacea*** Benth., *J.B. Walker 2525* (WIS); ***S. florida*** Benth., *Schmidt-Lebuhn 450* (MJG); ***S. formosa*** L'Hérit., *Gentry et al 19240* (FIELD); ***S. formosa*** L'Hérit., *Gentry et al 19240* (FIELD); ***S. forreri*** Greene, *J.B. Walker 2098* (WIS); ***S. fruticulosa*** Benth., *Crone 1/8/00* (MJG); ***S. fulgens*** Cav., *1967-1496A* (WIS); ***S. gesneraeflora*** Lindl. & Paxton, cult., UCRBG; ***S. gilliesii*** Benth., *P. Wester 13* (MJG); ***S. glechomaefolia*** Kunth, *Jenks 248* (UCR); ***S. gracilipes*** Epl., *Steyermark 55997* (FIELD); ***S. graciliramulos*** Epl.&Játiva-M., *P. Wester 14* (MJG); ***S. gravida*** Epl., *Jenks 254* (UCR); ***S. greatae*** Brandegee, *J.B. Walker 2511* (WIS); ***S. greggii*** Gray, cult., UCRBG; ***S. grewiifolia*** S. Moore, *P. Wester 15* (MJG); ***S. guaranitica*** St.Hil. ex Benth., cult., UCRBG; ***S. haenkei*** Benth., *P. Wester 71* (MJG); ***S. herbacea*** Benth., *Torres 448* (WIS); ***S. hilarii*** Benth., Walker (2006); ***S. hirtella*** Vahl, *Schmidt-Lebuhn 395* (WIS); ***S. holwayi*** Blake, cult., UCRBG; ***S. inconspicua*** Benth., *J. Walker 3045* (WIS); ***S. infuscata*** Epl., *Cowan 5011* (UCR); ***S. involucrata*** Cav., cult., UCRBG; ***S. iodantha*** Fern., cult., UCRBG; ***S. jacobi*** Epl., cult. (as *S.* sp. "Blue Chiquita"), WSN; ***S. karwinskii*** Benth., cult., UCRBG; ***S. keerlii*** Benth., cult., WSN; ***S. languidula*** Epl., *Sanders 10409* (UCR); ***S. lasiantha*** Benth., *J. Walker 3009* (WIS); ***S. lavanduloides*** Kunth, *J. Walker 3044* (WIS); ***S. leptophylla*** Benth., *1978-1159A* (WIS); ***S. leucantha*** Cav., *J.B. Walker 1205* (WIS); ***S. leucocephala*** Kunth, Walker (2006); ***S. leucochlamys*** Epl., *Cahill 2963* (UCR); ***S. lineata*** Benth., *Crone 12/8/00* (MJG); ***S. littae*** Vis., cult., WSN; ***S. lobbii*** Epl., *Schmidt-Lebuhn 475* (MJG); ***S. longispicata*** Mart. & Gal., *J. Walker 3034* (WIS); ***S. longistyla*** Benth., *J. Walker 3036* (WIS); ***S. lycioides*** Gray, *J.B. Walker 2047* (WIS); ***S. macrophylla*** Benth., *Riina 1472* (WIS); ***S. madrensis*** Seem., *Peterson 738* (WIS); ***S. melissodora*** Lag., *Jenks 258* (UCR); ***S. mellifera*** Greene, *J.B. Walker 2550* (WIS); ***S. mexicana*** L., *Jenks & Tempchin 224* (UCR); ***S. mexicana* var. *minor*** Benth. (forma *alba*), *Jenks 238* (UCR); ***S. mexicana* var. *minor*** Benth., cult., UCRBG; ***S. microphylla*** Kunth, *Jenks & Tempchin 207* (UCR); ***S. microphylla* var. *neurepia*** (Fern.) Epl., *1959-9360* (WIS); ***S. microphylla* var. *wislizeni*** Gray, (UCR); ***S. miniata*** Fern., *Holst 5895* (MO); ***S. misella*** Kunth, *J. Walker 3025* (WIS); ***S. mocinoi*** Benth., *Crone 15/9/00* (MJG); ***S. moniliformis*** Fern., *Crone 15/9/00* (MJG); ***S. muelleri*** Epl., *Jenks 251* (UCR); ***S. nervata*** Mart. & Gal., *P. Wester 292* (MJG); ***S. oaxacana*** Fern., *Salinas-T. F-2705* (UCR); ***S. occidentalis*** Swartz, *J.B. Walker 612* (WIS); ***S. oppositiflora*** Ruiz & Pav., *Riina 1477* (WIS); ***S. orbignii*** Benth., *P. Wester 43* (MJG); ***S. ovalifolia*** St. Hil. ex Benth., *Sytsma 7226* (WIS); ***S. oxyphora*** Briq., *P. Wester 16* (MJG); ***S. pansamalensis*** J.D. Smith, *A. Mendez Ton 7353* (WIS); ***S. parryii*** Gray, cult., UCRBG; ***S. patens*** Cav., *1973-9197* (RBG); ***S. peninsularis*** Brandegee, *Moran 18786* (MO); ***S. personata*** Epl., *P. Wester 17* (MJG); ***S. pichinchensis*** Benth., *Ollgaard & Balslev 9508* (FIELD); ***S. pinguifolia*** (Fern.) Woot. & Standl., *P. Wester 372* (MJG); ***S. platystoma*** Epl., *P. Wester*

18 (MJG); ***S. polystachya*** Ort. (morphotype 1), *Jenks & Tempchin 205* (UCR); ***S. polystachya*** Ort. (morphotype 2), *Jenks & Tempchin 216* (UCR); ***S. praeclara*** Epl., *P. Wester 32* (MJG); ***S. prasiifolia*** Benth., cult. (as *S. caudata* "El Cielo Blue"), WSN; **S.** ***procurrens*** Benth., *Bonif 941* (WIS); ***S. prunelloides*** Benth., *Crone 15/9/00* (MJG); ***S. pubescens*** Benth., *J. Walker 3043* (WIS); ***S. pulchella*** D.C., cult., WSN; ***S. purpurea*** Cav., cult., UCRBG; ***S. ramosa*** Brandegee, *J. Walker 3008* (WIS); ***S. recurva*** Benth., *Jenks & Tempchin 212* (UCR); ***S. reflexa*** Hornem., *J. Walker 3020* (WIS); ***S. regla*** Cav., *Jenks 260* (UCR); ***S. retinerva*** Briq., *Riina 1508a* (WIS); ***S. rubescens*** Kunth, *P. Wester 442* (MJG); ***S. rufula*** Kunth., Walker (2006); ***S. rusbyi*** Britton ex Rusby, *P. Wester 31* (MJG); ***S. rypara*** Briq., *P. Wester 32* (MJG); ***S. sagittata*** Ruiz & Pav., cult., UCRBG; ***S. scabrida*** Pohl, *Pirani 5225* (WIS); ***S. scutellaroides*** Kunth, *Schmidt-Lebuhn 496* (MJG); ***S. semiatrata*** Zucc., *Jenks 269* (UCR); ***S. sessei*** Benth., *Crone 15/9/00* (MJG); ***S. setulosa*** Fern., *Crone 30/7/00* (MJG); ***S. sharpii*** Epl. & Math., *J.B. Walker s/n* (WIS); ***S. sinaloensis*** Fern., *Jenks 250* (UCR); ***S. sohrona*** Briq., *P. Wester 34* (MJG); ***S. speciosa*** Presl. ex Benth., *Soriano 465* (FIELD); ***S. sphacelaefolia*** Epl., *Lubinsky 264* (UCR); ***S. splendens*** Sellow ex Roem. & Schultes, cult., UCRBG; ***S. stachydifolia*** Benth., *P. Wester 35* (MJG); ***S. styphelus*** Epl., *Schmidt-Lebuhn 495* (MJG); ***S. tepicensis*** Fern., *Van Devender 95-470* (UCR); ***S. thymoides*** Benth., cult., WSN; ***S. tiliifolia*** Vahl, *P. Wester 32* (MJG); ***S. tolimensis*** Kunth., Walker (2006); ***S. tomentella*** Pohl, Walker (2006); ***S. tortuosa*** Kunth, Walker (2006); ***S. tricuspidata*** Mart. & Gal., *J. Walker 3037* (WIS); ***S. tubifera*** Cav., *P. Wester 231* (MJG); ***S. tubiflora*** Smith, *Jenks 242* (UCR); ***S. uliginosa*** Benth., *1984-9105B* (WIS); ***S. urica*** Epl., cult., UCRBG; ***S. vazquezii*** Iltis & Ram. (invalid), *P. Wester 196* (MJG); ***S. venulosa*** Epl., *Wood 5381* (MO); ***S. villosa*** Fern., *Jenks 263* (UCR); ***S. vitifolia*** Benth., *J. Walker 3032* (WIS); ***S. wagneriana*** Polak., cult., UCRBG; ***S. xalapensis*** Benth., *Jenks & Tempchin 214* (UCR).

APPENDIX 2

Salvia divinorum Epl. & Játiva-M. has been collected numerous times in the Sierra Mazateca, Oaxaca, Mexico. Most of these accessions are available as herbarium sheets (the most extensive and complete field collections were by A.S. Reisfield in 1983-1984), however a number of live collections have also been introduced into cultivation. This appendix lists only two types of accessions, the known cultivated accessions with all known information regarding each and Reisfield's complete collection data. While there are other herbarium materials available collected by other botanists, these are frequently made from cultivated material, and in the case of the rare, field-collected accessions, they either have very vague or general location data (as is the case of the Type specimen) or appear to have been collected from one of the populations precisely described by Reisfield. DNA was isolated from each of the accessions listed below, however only those with * preceding the name were sequenced from this study (specifically, as described in chapter 2). All live materials were generously made available by Daniel Siebert, Sept. 2004.

Live accessions from the Sierra Mazateca, Oaxaca, Mexico:

**Salvia divinorum*, "Owens". Collected by Jack Owens on Cerro Rabon.

**S. divinorum*, "La Fuerza" ("The Force"). Collected by Kathleen Harrison in January 2001 near Llano de Arnica, a small village near San Jose Tenango, (Her informant said, however, that it was from another location originally.)

**S. divinorum*, "Cerro Quemado". Collected by L.J.Valdez III near Cerro Quemado, next to a wet coffee plantation, alt. 800-1000m, March 2, 1980.

**S. divinorum*, “Juletta” (also “Julieta”). Collected by Daniel Siebert (DS9902) February 14, 1999, in Huantla de Jimenez.

*S. divinorum, “*Bunnell clone”. This also known as the “Wasson and Hofmann” clone; however, it was been documented that Wasson did not bring live material back to the U.S., rather this collection was introduced by Sterling Bunnell, the first clone of *S. divinorum* brought into cultivation (Siebert, 2003).

S. divinorum, “Blosser clone” (also sold as the “Palatable clone”). The anthropologist Bret Blosser brough two clones back from his work in the Sierra Mazateca. It is unclear which of those two clones is widely available as the “Blosser clone”.

Live materials from other sources:

S. divinorum, “Luna”. This is a sport of the Bunnell strain arising in Terence McKenna’s collection, Botanical Dimension in Hawaii

S. divinorum, “DSO3” (“Paradox”). This seed-raised strain, started in 1994, from “Bunnell clone” parents.

S. divinorum, “Resilience” Seed raised strain started in 2002 from “Blosser clones” (#1 or #2?).

Reisfield’s *S. divinorum* collections by collection number; citations are lifted verbatim from his herbarium sheet notations except my notations set inside brackets. It should also be noted that there are discrepancies between Reisfield’s herbarium labels and the brief information concerning each collection in his “specimens examined” at the end of his 1993 publication.

Reisfield & Solheim 1077:

“06 Jan 1984, 18 08’N 96 52’W, 1400m, 2km NNW of Huautla de Jimenez.

Perennial herb, mostly 0.5-1.5 m tall, spreading vegetatively, some stems trailing along rock and/or in water, stems in water rooting especially copiously at nodes and sometimes along internodes, locally abundant, growing within 1.5 m of water. Broken, trailing and dropping stems usually resume erect growth at stem apex or by axillary branches, new vigorous shoots often arising from axils of old senescent stems. Living stems apparently all ultimately arise from now dead stems lying on or in the ground, these old stems sometimes appearing asa woody caudex. Many stems are cut by people who collect leaves for medicinal use. Several old dried inflorescence branches present lacking flowers and bracts (fallen), fruit absent, a younger rachis still with color (light purple)."

"Very wet, somewhat disturbed, shaded ravine at edge of coffee plantation. Rocky clay soil. Growing with *Calceolaria, Liquidambar macrophylla, Yucca, Ilex, Sambucus, Clethra.* The coffee plantation apparently replaces the cloud forest (dominated by *Hedyosmum mexicanum* with *Liquidambar macrophylla*) of which there are remnants nearby."

Reisfield & Solheim 1090 [I was unable to observe this collection number and the following, 1092; this information is from Reisfield (1993).]:
15 Jan 1984, 1 km SW of Huautla market.

Reisfield & Solheim 1092 [Information from Reisfield (1993)]:
15 Jan. 1984, ca. 2 km SW of Huautla market

Reisfield & Solheim 1093:

"16 Jan 1984, 18 14'N 96 50'W, 1200m, Chauhtemoc, ~4km NE of Santa Maria Chilchotla, near Clemencia and Santa Rosa., Mnpio. Saqnta Maria Chilchotla.

"Perennial herb, locally abundant, mostly 0.5-1.5m tall, rarely to 3m tall, he taller plants decumbent for part of their length. Broken, trailing and drooping stems usually resume erect growth at their apex or by axillary branches, new vigorous shoots often arising from axils of old senescent stems. Living shoots apparently all ultimately arise from now dead stems lying on or in the ground, these old stems often appearing as a woody caudex. Plants spreading vegetatively, rooting at nodes and sometimes along internodes, growing in shade and filtered sun. Many dried inflorescence branches with bracts and/or calyces adhering but not mature fruit present. Several stems in full flower including 1 inflorescence with 8 open flowers and many buds, all flowering stems growing in at least some sun, mostly 1-2m tall. Corolla pubescent, pure white, stamens, style and stigma white, bracts, calyx, and rachis light purple.

"A broad, undisturbed, humid shaded ravine with only several pools of standing water ubt almost certainly flooded during wetter periods. The channel of the ravine mostly without vegetation, *S. divinorum* and associates colonizing the banks of the channel. *S. divinorum* grows no farther than 3 to 4m from the channel. Wet, rocky clay soil. Ravine is in cloud forest grading into perennial-leavedforest, with *Hedyosmum mexicanum, Liquidambar macrophylla, Siparunaandina, Posoqueria latifolia, Heliconia, Deppea, Chamaedorea.*"

Reisfield and Solheim 1102 [potentially the same location as the "Cerro Quemado" strain collected by Valdéz III, collected on the other side of town from 1103a/b and 1106/1242]

"8 Feb.1984, 18 09'N 96 35'W, 230m, Cerro Quemado, 0.5km W of Centro. Mpio. Sand Pedro Ixcatlan.

"Somewhat open patch in narrow, moist rocky ravine, Black soil with humus and much rotted wood, indisturbed perennial-leaved forest with *Begonia, pepromia, Syngonium, Chamaedorea*."

Reisfield and Solheim 1103a "=1103 plant A"
Reisfield and Solheim 1103b [plant b handwritten; multiple sheets of each were made]:

"09 Feb 1984, 18 09'N, 93 35'W, 360m, Cerro Camaron, ~0.75km E of the town Cerro Quemado, Mnpio. San Pedro Ixcatlan.

"Perennial herb mostly 0.5-1.5m tall, spreading vegetatively, rooting copiously at nodes and sometimes along internodes. Plants scattered, forming dense stands in some areas. Broken and dropping stems usually resume erect growth at stem apex or by axillary branches, new vigorous shoots often arising from axils of old senescent stems.Living stems apparently all ultimately arise from now dead stems lying onor in the ground, these old stems often appearing as a woody acudex. Plants mostly sterile but several flowering stems present, 1-2m tall, in filtered sun. Corolla pubescent, pure whilte calyx, pedicel, bracts and rachislight purple.

"Mostly sunny, east-facing , rocky (limestone) slope with clay soil, near and in slash and burn cornfield with scattered *Coffea arabica, Musa, Theobroma cacao, Citrus, Vanillaplanifolia, Bixa orellana, Persea, Rivina humulus, Xanthosoma, Salvia occidentalis, Piper, Commelina, Cordia alliodora, Acalypha, Cecropia, Pilea microphylla,* surrounded byvery disturbed perennial-leaved forest."

Reisfield & Solheim 1106 [the same collection location as 1242 and seemingly near 1103a/b]:

"9 Feb. 1984, 18 09'N 96 35'W, 400m, Trailside moist clay soil in shade to full sun, mostly filtered sunlight, with *Jatropha, Cecropia, Siparune, Piper, Urera caracasana, Cordia alliodora, Bixa orellana, Heliconia, Rivina humulus, Dioscorea, Iresine, Chamaedorea, Acalypha*, in disturbed perennial-leaved forest, Cerro Camaron, 1km E of village of Cerro Quemado, Mpio. San Pedro Ixcatlan."

Reisfield & Solheim 1107 [The sheets of this collection number and the following, 1108, were unavailable; location information from Reisfield (1993).]:

9 Feb 1984, 1.5 km W of Cerro Quemado

Reisfield & Solheim 1108 [Locaion information from Reisfield (1993).]:

10 Feb 1984, ca. 1 km NNW of Cerro Quemado centro.

**Reisfield & Solheim 1109*:

"13 Feb 1984, 18 03'N 96 40'W, 1050m, Cerro Alto, 2km NE of Ayautla. Mnpio. San Bartolome Ayautla.

" Perennial herb, mostly 0.5-1.5m tall, flowering stems 1-2(-3)m tall, plants rooting copiously at nodes and sometimes along internodes, spreading vegetatively, scattered plants forming dense stands in some areas. Broken and dropping stems usually resume erect growth at stem apex or by acxillary branches, new vigorous shoots often arising from axils of old senescent stems. Living stems apparently all ultimately arise from now dead sems lying on or in the ground, these old stems often appearing as a woody caudex. Plants partially shaded, flowering stems receiving at least some sun. Corolla pubescent, pure white, stamens, style and stigma white, calyx, pedicel, bracts, and rachis light purple.

"Northeast facing slope, moist clay soil with humus, mostly shaded trailside and near trail in disturbed perennial-leaved forest of *Hedyosmum mexicanum, Oreopanax xalapensis, Siparuna, Xanthosoma, Heliocarpus, Heliconia, Inga sapindoides, Costus, Chamaedorea.*"

Reisfield & Solheim 1111:

"13 Feb 1984, 18 03'N 96 42'W, 1100m, La Soledad, 3.5km WNW of Ayautla, just off road to San Juan. Mnpiol San Bartolome Ayautla.

"Perennial herb, mostly 0.5-1.5m tall, locally abundant, plants [Multiple it would seem]growing within 3m of water, mostly shaded, spreading vegetatively, some stems trail along rock and/or water, stems in water rooting especially copiously at nodes and sometimes along internodes. Broken, trailing and dropping stems usually resume erect growth at stem apex or by axillary branches, new vigorous shoots arising from axils of old senescent stems. Living stems apparently all ultimately arise from now dead stems lying on or in the ground, these old stems often appearing as a woody caudex. Some stems cut, population apparently visited by people who collect leaves for medicinal use. Several inflorescence branches present and still with color (light purple), flowers and bracts absent (apparently fallen), fruit absent.

"Very wet, somewhat disturbed ravine with running water, rocky clay soil, plant litter. With *Sambucus, Piper* spp., *Pothomorphe, Siparuna andina.*"

Reisfield & Solheim 1112 [Location information from Reisfield (1993).]:

"13 Feb. 1984, 2.5 km W of Ayautla on road to San Juan."

Reisfield 1117:

"1985, grown at University of Wisconsin from cuttings from Valdez's collections from Ayautla, Cerro Rabon, Mar. 7, 1980."

Reisfield 1242:

"27 Oct. 1985, 18 09'N 96 35'W, 400m, Cerro Camaron, 1 km E of Cerro Quemado on an Eastern face of the Sierra Mazateca, Mpio. San Pedro Ixcatlan. [Same location as 1106 and very close to the collection location of 1103a/b, apparently Reisfield revisited this population to observe potential pollinators]

"Perennial herb, mostly .5-1.5 (2.0)m tall; stem square, hollow, succulent-translucent and breaking easily; pedicel, bract, calyx, and rachis light purple; corolla pure white; plants forming dense stands in some areas, spreading vegetatively, rooting at nodes and sometimes along internodes; living stems apparently all ultimately arise from now dead stems lying on or in the ground, these old stems often appearing as a woody caudex; flowers open during the night, most calyces pierced by nectar robbers by morning; no mature fruit observed but some ovules enlarged; **p**opulation visited by hummingbirds at dawn, nectar depleted and pollen transferred from visited flowers.

"Trailside, in shade and sun, with *Cecropia, Jatropha, Iresine, Chamaedorea,* in disturbed perennial leaved forest."