UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

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INSTITUTO DE BIOLOGÍA
SISTEMÁTICA

Filogenia y diversificación de S*alvia* (Lamiaceae) en México: desentrañando una radiación evolutiva

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

ITZI FRAGOSO MARTÍNEZ

TUTOR PRINCIPAL DE TESIS: DR. GERARDO ADOLFO SALAZAR CHÁVEZ
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COMITÉ TUTOR: DRA. MARTHA JUANA MARTÍNEZ GORDILLO
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MÉXICO, CD. MX. ENERO, 2019

UNAM POSGRADO
Ciencias Biológicas

COORDINACIÓN

OFICIO CPCB/1173/2018

Asunto: Oficio de Jurado para Examen de Grado.

M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
Presente

Me permito informar a usted que en la reunión del Subcomité por Campo de Conocimiento de Biología Evolutiva y Sistemática del Posgrado en Ciencias Biológicas, celebrada el día 24 de septiembre de 2018, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la alumna **FRAGOSO MARTÍNEZ ITZI** con número de cuenta **303035899** con la tesis titulada: **"FILOGENIA Y DIVERSIFICACIÓN DE *SALVIA* (LAMIACEAE) EN MÉXICO: DESENTRAÑANDO UNA RADIACIÓN EVOLUTIVA."**, realizada bajo la dirección del **DR. GERARDO ADOLFO SALAZAR CHAVEZ:**

Presidente:	DRA. SONIA VÁZQUEZ SANTANA
Vocal:	DRA. ALEJANDRA CITLALLI MORENO LETELIER
Secretario:	DRA. MARTHA JUANA MARTÍNEZ GORDILLO
Suplente:	DR. GUILLERMO IBARRA MANRÍQUEZ
Suplente	DRA. SUSANA AURORA MAGALLÓN PUEBLA

Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, Cd. Mx., a 4 de diciembre de 2018.

DR. ADOLFO GERARDO NAVARRO SIGÜENZA
COORDINADOR DEL PROGRAMA

c.c.p. Expediente del (la) interesado (a).

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Índice

Resumen

En este trabajo se estudian los posibles factores asociados a la radiación del género más diverso de la flora mexicana: *Salvia* (309 especies divididas en tres subgéneros). El subgénero *Calosphace*, con 600 especies, es endémico de América y representa el linaje con mayor cantidad de especies en *Salvia s.l.* Su mayor diversidad se concentra en los bosques de montaña tropical, siendo México su mayor centro de diversidad (295 spp.), seguido por Los Andes, Brasil y Las Antillas. Se ha planteado que, tanto los procesos orogénicos en Meso- y Sudamérica registrados en el Neógeno, así como el cambio reiterado a una polinización por aves a partir de la melitofilia, pueden estar relacionados con un aumento en las tasas de diversificación de *Calosphace*. Sin embargo, estas hipótesis no han sido puestas a prueba de manera formal.

La pregunta que se aborda en este trabajo es: ¿Por qué hay tantas salvias en el Neotrópico? Para responderla se plantean los siguientes objetivos: 1) generar un marco filogenético robusto, con un muestreo representativo de *Calosphace*. Dicha filogenia es la base para: 2) estimar los tiempos de divergencia de los clados dentro del subgénero y explorar posibles correlaciones con escenarios paleogeográficos o paleoecológicos, y 3) analizar si existen cambios significativos en las tasas de diversificación en *Calosphace* y determinar su asociación con factores intrínsecos y/o extrínsecos de los organismos.

Para generar una filogenia base de *Calosphace* se amplió el muestreo taxonómico y de marcadores, comparado con los incluidos en estudios previos. Se muestrearon 304 especies (ca. 50% de la diversidad del subgénero), para tres marcadores moleculares (ITS, *trn*L-*trn*F y *psb*A-*trn*H), priorizando la inclusión de grupos caribeños, sudamericanos y mexicanos poco representados. Se obtuvo una filogenia más robusta que en estudios previos y permitió la identificación de linajes principales dentro del grupo, así como la reconstrucción de áreas y síndromes de polinización ancestrales. *Calosphace* se originó en Mesoamérica y hubo múltiples colonizaciones hacia Sudamérica; mientras que los linajes caribeños provienen de Los Andes. El síndrome de polinización ancestral de *Calosphace* es la melitofilia y se detectaron al menos 13 transiciones a la ornitofilia y solamente una reversión.

La estimación de los tiempos de divergencia sugiere que *Calosphace* se originó hace ca. 27.5 (20.6-35.1) ma, entre el límite del Oligoceno y el Mioceno. Se detectó un solo aumento en las tasas de diversificación del subgénero, que coincide con el clado core Calosphace, linaje con ca. 70% de las especies hasta ahora muestreadas y que presenta ramas internas cortas y una espina poco resuelta. El aumento en las tasas de especiación parece estar relacionado principalmente con factores extrínsecos, como un ligero incremento de temperatura global a mediados del Mioceno, el establecimiento de los tipos de vegetación áridos y la disponibilidad de nichos, derivado de los procesos orogénicos del Mioceno. La radiación de los colibríes en Sudamérica, Norteamérica y Las Antillas, pudo haber jugado un papel secundario en la radiación de *Calosphace*, al ser más reciente que core Calosphace. Los análisis de diversificación asociadados al estado de carácter revelaron que, contrario a lo esperado, las especies ornitófilas tienen las mismas tasas de diversificación (r) que las melitófilas. Las especies ambófilas son las que presentan valores de r más elevados, posiblemente por la capacidad de asegurar polinizadores. Todos los centros de diversidad mostraron tasas de diversificación más altas que Mesoamérica, pero menores de dispersión. Esta región exporta linajes a los otros centros de diversidad, donde radian localmente y se dispersan de manera limitada.

La exploración de los métodos filogenómicos (Enriquecimiento Anclado Híbrido, AHE) para la reconstruir las relaciones de *Calosphace* dio resultados promisorios, al recuperar 448 loci. Los árboles filogenéticos tuvieron valores altos de apoyo en la mayoría de las ramas internas, solamente tres de estas ramas tuvieron valores bajos, dos de los cuales mejoraron cuando se eliminó el ruido filogenético de la matriz de datos. La historia evolutiva de cualquier grupo es resultado de la interacción de factores intrínsecos y extrínsecos, que actúan de manera sinérgica, para dar lugar a los patrones de diversidad observados en la actualidad. Los resultados obtenidos señalan que los factores extrínsecos son los responsables principales de la riqueza de especies de *Calosphace*. Cabe mencionar que este trabajo se enfocó en evaluar los síndromes de polinización del subgénero como único factor intrínseco, por lo que no puede descartarse que existan otros caracteres, como la poliploidía o el hábito, que podrían desempeñar un rol primordial en la radiación de las salvias Neotropicales y que merecen ser explorados en estudios futuros. Finalmente, es posible que existan radiaciones anidadas dentro de core Calosphace, pero que las limitaciones de nuestro muestreo o la poca variación de los datos a ciertos niveles filogenéticos hayan impedido detectarlas. Por lo tanto, es necesario ampliar el muestreo taxonómico para incluir especies del este de Sudamérica, así como emplear métodos filogenómicos que permitan generar filogenias más robustas y resueltas.

Abstract

In this study are investigated the possible factors associated to the radiation of the most diverse genus of the Mexica flora: *Salvia* (309 species divided into three subgenera). The subgenus *Calosphace*, with 600 species, is endemic of America and the most species-rich lineage of *Salvia s.l.* Most of its diversity is found in the tropical montane forest, being Mexico its main diversity center (295 spp.), followed by the Andes, Brazil and the Antilles. It has been hypothesized that, the Meso- and South American orogenic processes of the Neogene, as well as the repeated shift to ornithophily from melittophily, could have been related to the increase in diversification rates of *Calosphace*. However, these hypotheses have not been formally tested yet.

This dissertation is aimed at answering one question: Why are there so many sages in the Neotropics? To solve this inquiry, we had the following objectives: 1) to generate a robust phylogenetic framework, including a representative sampling of *Calosphace*. This phylogeny was the base to: 2) estimate divergence times of the clades within the subgenus and explore possible correlations between its ages and palaeogeographic and palaeocologic scenarios or factors, and 3) analyze if there are significative shifts in the diversification rates in *Calosphace* and determine if they are associated with intrinsic and/or extrinsc factors.

To generate a base phylogeny of *Calosphace*, taxonomic and character sampling was expanded, compared to those included in previous studies. We sampled 304 species (ca. 50% of the subgenus diversity), for three molecular markers (ITS, *trn*L-*trn*F y *psb*A-*trn*H), prioritizing the inclusion of poorly represented Caribbean, South American and Mexican taxa. The phylogeny obtained was more robust than previous studies and allowed the recognition of main lineages within the group, as well as the reconstruction of ancestral areas and pollination syndromes. *Calosphace* originated in Mesoamerica and there were multiple colonizations of South America; while the Caribbean lineages came from the Andes. The ancestral pollination syndrome of the subgenus is melittophily and at least 13 transitions to ornithophily were detected and only one reversal.

Divergence time estimation suggests that *Calosphace* originated ca. 27.5 (20.6-35.1) ma ago, during the Oligocene-Miocene limit. Only one shift in the diversification rates was detected in the subgenus, at the onset of the core Calosphace clade, a lineage that includes ca. 70% of the species currently sampled and is characterized by short internal branches and a poorly resolved backbone. The increase in speciation rates in this clade seems to be related mainly to extrinsic factors, such as the raise in global temperature during mid Miocene, the establishment of arid vegetation types and niche availability, derived from the orogenic processes of the Miocene. Hummingbird radiation in South America, followed by North America and the Antilles could have played a secondary role in the radiation of *Calosphace*, being more recent than core Calosphace. State-dependent diversification analyses revealed that, contrary to expected, ornithophilous species have the same diversification rates (r) than the melittophilous species. The intermediate species had higher r values, possibly due to their ability of secure pollinators. All the diversity centers showed higher diversification rates than Mesoamerica, but lower dispersal rates. This region exports lineages to the remaining centers, where they radiate locally and disperse limitedly.

The exploration of the phylogenomic methods (Anchored Hybrid Enrichment, AHE) to reconstruct the relationships of *Calosphace* gave promising results, recovering 448 loci. The phylogenetic trees had high support values in most of the internal branches, only three of such branches had low support values, two of which increased when the phylogenetic noise was eliminated from the data matrix.The evolutionary history of any lineage is the result of the interaction of intrinsic and extrinsic factors that act in synergy, to produce the diversity patterns currently observed. Our results point to the extrinsic factors as the responsible of the species-richness of *Calosphace*. However, this study only focused on the evaluation of the pollination syndrome as intrinsic factor, which is why other traits, such as polyploidy or habit could be playing an important role in the radiation of the Neotropical sages and should be explored in further studies. Finally, it is possible that nested radiations within core Calosphace exist, but due to the limitations of our sampling or the lack of variation of the data at certain phylogenetic levels we were unable to detect them. Thus, it is necessary to expand our taxonomic sampling to include more taxa from eastern South America, as well as to employ phylogenomic methods that will generate more resolved and robust phylogenies.

Introducción general

Un marco conceptual sobre las radiaciones

I.I. ¿Qué es una radiación?

Los términos “radiación” y “radiación adaptativa” han sido utilizados de manera extensiva e indistinta para describir los patrones observados en la distribución heterogénea de la biodiversidad a nivel filogenético (i.e., clados muy diversos hermanos de linajes con pocas especies). Comúnmente, “radiación adaptativa” se ha utilizado para referirse a los linajes con gran riqueza de especies, cuyo contexto filogenético (con frecuencia poco resuelto) sugiere un evento acelerado de diversificación. Todos los conceptos de radiación coinciden en tres componentes comunes: diversificación de linajes, de caracteres y ecológica (Sanderson, 1998). No obstante, existe controversia entre el peso que debe darse a cada uno de estos componentes para considerar a una radiación como “adaptativa”. Givnish (1997a, 2015) considera que la diversificación ecológica es primordial en una radiación, mientras que la de linajes no lo es tanto. Por otro lado, numerosos autores dan más peso a este último componente (Sanderson, 1998; Bateman, 1999; Schulter, 2000; Donoghue & Sanderson, 2015).

El uso de una definición amplia de radiación, eliminando el calificativo “adaptativa”, permite englobar a linajes donde la diversificación ecológica ha tenido mayor importancia (e.g. *Brocchinia*, Givnish *et al*., 1997b; “alianza de compuestas hawaiianas”, Baldwin, 1997); así como grupos que son resultado de procesos donde la diversificación de linajes tuvo mayor peso y no existió una notable diferenciación de nicho entre especies cercanas. En este trabajo utilizaremos la definición de radiación de Bateman (1999): “*Un gran excedente de la tasa natalidad sobre la tasa mortalidad de especies y/o estados de carácter, dentro de un clado específico durante un intervalo de tiempo*”, ya que esta definición permite identificar, de manera práctica y metodológica a los clados que han radiado. Más aún, permite distinguir entre las radiaciones que están dadas por el aumento en las tasas de especiación, la disminución en las tasas de extinción o una combinación de ambas (Donoghue & Sanderson, 2015).

I.II. Factores involucrados en las radiaciones

Diversos estudios han sugerido la idea de innovaciones clave como los factores responsables de causar la radiación de un grupo (Walker & Sytsma, 2007; Claßen-Bockhoff *et al*., 2004; Drummond *et al*., 2012; Matuszak *et al*., 2016). No obstante, cada vez surgen más trabajos donde se discute la influencia de las interacciones entre distintos factores (intrínsecos y extrínsecos), sobre los cambios en las tasas de diversificación en distintos grupos de plantas (Drummond *et al*., 2012; Givnish *et al*., 2014; Silvestro *et al.*, 2014; Schwery *et al*., 2015; Spriggs *et al.*, 2015; Lagomarsino *et al*., 2016; Roalson & Roberts, 2016). Por ello han surgido nuevos términos que permiten referirse a dichos atributos que interactúan y clasificaciones que ayudan a catalogarlos por su origen o por su tiempo de aparición en la historia de un linaje (Bouchenak-Khelladi *et al.*, 2015; Donoghue & Sanderson, 2015).

Al considerar el origen de dichos atributos, éstos pueden clasificarse en intrínsecos y extrínsecos. Los primeros se refieren a características propias de los organismos, (morfológicas o fisiológicas). Cuando un conjunto de caracteres actúa de forma sinérgica, por ejemplo, para dar lugar a una estructura o ruta metabólica; Donoghue & Sanderson (2015) emplean el término sinnovación ("*synnovation*") y cuando ésta tiene efecto positivo sobre las tasas de diversificación, constituye una "sinnovación clave". Por otro lado, los factores extrínsecos son externos al organismo y se refieren a características ambientales, de distribución (e.g., la migración a nuevos territorios) o resultado de las interacciones bióticas (Donoghue & Sanderson, 2015; O'Meara *et al.*, 2016). Una radiación puede ser desencadenada por uno u otro tipo de factores, o una combinación de ambos, es decir, una confluencia clave ("*key confluence*"; Donoghue & Sanderson, 2015).

Dependiendo del momento de aparición de estos atributos con respecto a la escala temporal de una radiación, pueden catalogarse en tres grupos según la clasificación de Bouchenak-Khelladi *et al.* (2015). Los precursores ("*background*") se establecen antes de la radiación y crean las condiciones adecuadas para que más adelante esta pueda darse. Los detonantes (*"trigger"*) suceden al mismo tiempo que la radiación, considerando que existen confluencias y sinnovaciones, un detonante puede ser el último factor en surgir, desencadenando la radiación. Los moduladores (*"modulator"*) se establecen una vez que la radiación ha empezado y pueden ser detonadores de radiaciones anidadas (i.e., radiaciones dentro de otras más inclusivas).

Para estudiar a las radiaciones y los factores que están involucrados en ellas, es necesario un sistema de estudio que presente una gran diversidad de especies, morfológica y/o ecológica; con un marco filogenético robusto, que permita el conocimiento de los tiempos de divergencia de los linajes que lo conforman. Esto con el fin de llevar a cabo estimaciones de tasas de diversificación y determinar su relación con factores intrínsecos o extrínsecos.

Las salvias neotropicales como modelo para estudiar las radiaciones en los bosques de montaña

II.I. *Salvia s.l.*

Dentro de la familia Lamiaceae, *Salvia* L. es el género con mayor número de especies (ca. 1,000 spp.), las cuales se distribuyen principalmente en tres centros de diversidad: el Neotrópico (600), Asia central y el Mediterráneo (250) y el Este asiático (90) (Walker *et al*., 2004). Tradicionalmente, *Salvia* se distinguía del resto de los géneros de la familia por la presencia de dos estambres con anteras monotecas, separadas por un conectivo alargado, que al fusionarse y en conjunto con las tecas posteriores (en ocasiones fértiles), forman un apéndice, generalmente estéril, con forma de palanca (Harley *et al*., 2004; **Fig. 1a**). Dicha estructura se conoce como palanca estaminal ("*staminal lever*"; Walker & Sytsma, 2007), balancín o gubernáculo (Bentham, 1876; Espejo y Ramamoorthy, 1993) y restringe el acceso al néctar, que se deposita en la base de la corola. Para poder obtener la recompensa, los polinizadores accionan el mecanismo de palanca y al empujarla, la parte dorsal del polinizador entra en contacto con las tecas, cargándose de polen (**Figs. 1b-c**; Claßen-Bockhoff *et al*., 2004; Walker & Sytsma, 2007).

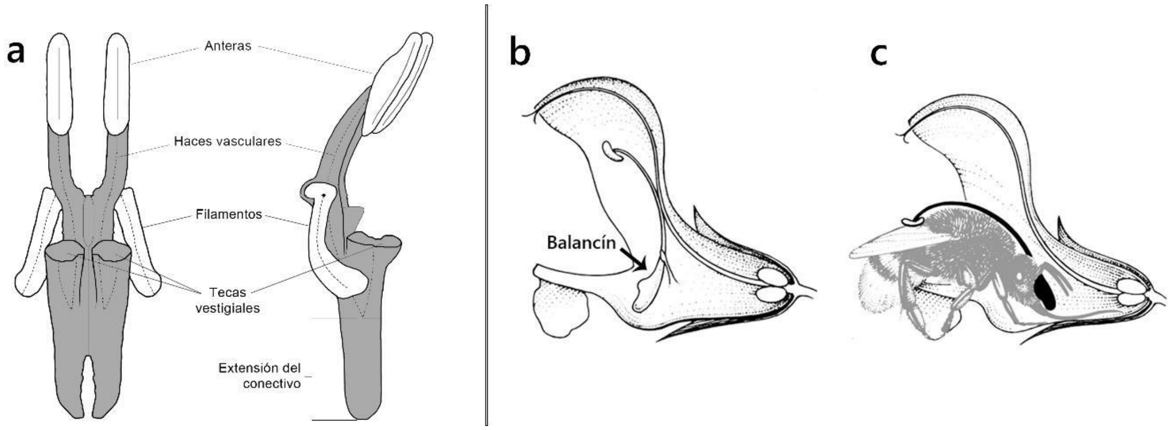


Figura 1. a) Morfología del androceo de *Salvia* subgénero *Calosphace* (clado Uliginosae), en gris se muestra el conectivo (Modificado de Barrera-Aveleida, 2017); b) corte longitudinal de una flor de *Salvia pratensis*, que muestra la ubicación del androceo cuando el mecanismo de palanca está desactivado y el acceso al néctar está bloqueado; c) mecanismo de palanca accionado por el polinizador haciendo contacto con el polen al intentar acceder al néctar (Modificado de Walker *et al*., 2004).

Aunque existe una gran variación morfológica en las palancas estaminales de los miembros de *Salvia*, la presencia de esta estructura se consideraba una sinapomorfía y el género se asumía como un grupo monofilético. No obstante, las primeras reconstrucciones filogenéticas del grupo revelaron que es un género parafilético, donde las especies, tradicionalmente consideradas como *Salvia,* se encontraban en tres linajes distintos, con otros cinco géneros intercalados entre ellos (Walker *et al*., 2004; **Fig. 2**). Los géneros *Dorystaechas* Boiss. & Heldr. ex Benth., *Meriandra* Benth., *Perovskia* Kar., *Rosmarinus* L. y *Zhumeria* Rech.f. & Wendelbo son poco diversos (ca. 34 spp. en total), carecen de conectivos alargados y por lo tanto de palanca estaminal (Walker & Sytsma, 2007; Walker *et al*., 2015; **Fig. 2**).

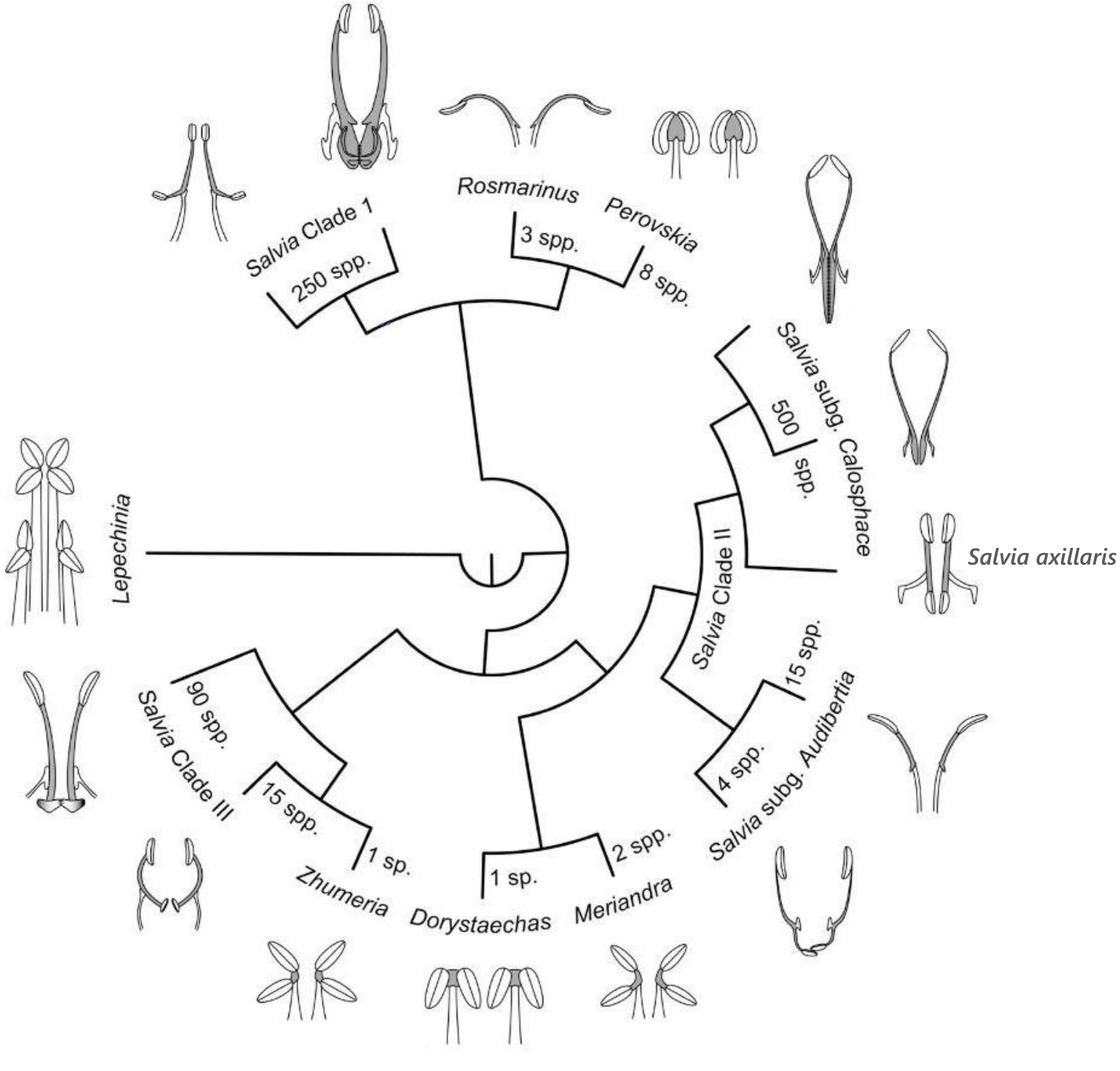


Figura 2. Filogenia resumida de *Salvia s.l.*, donde se muestran los linajes principales, con datos de diversidad y la morfología representativa del androceo de cada uno, en gris se muestra el tejido conectivo (Tomado de Drew *et al*., 2017).

Para abordar la parafilia de *Salvia s.l.* existen dos propuestas opuestas, ambas filogenética y taxonómicamente válidas: ampliar la circunscripción de *Salvia*, para incluir a los cinco géneros intercalados y así tener un género monofilético (Drew *et al*., 2017), o segregar en seis géneros distintos a las especies tradicionalmente incluidas en *Salvia* (Will *et al*., 2015; Will &Claßen-Bockhoff, 2017). Considerando a la propuesta de Drew *et al*. (2017) más práctica, la única característica diagnóstica de género ampliado es la presencia de conectivos engrosados (i.e., *Dorystaechas*, *Meriandra*, *Perovskia*, y *Zhumeria*) o alargados, y el mecanismo de palanca estaminal habría surgido tres veces de forma independiente dentro del género (Walker & Sytsma, 2007). Debido a que esta estructura se encuentra en los tres linajes más diversos de *Salvia*, se ha propuesto como una innovación clave para el género (Claßen-Bockhoff *et al*., 2004), sugiriendo que su aparición pudo desencadenar tres radiaciones paralelas, situadas en los centros de mayor diversidad de *Salvia*: la región de la Cuenca Mediterránea, el Este de Asia y el Neotrópico (Walker & Sytsma, 2007; Drew *et al*., 2017).

La mayoría de las especies de *Salvia s.l.* son polinizadas por abejas, solamente en dos clados ha surgido la polinización por aves. En el clado I (clado de *Salvia officinalis sensu* Drew *et al*., 2017; **Fig. 2**), cinco especies africanas son polinizadas por aves paseriformes y una especie del Nuevo Mundo por colibríes (Wester & Claßen-Bockhoff, 2006; Will & Claßen-Bockhoff, 2014). En el clado II (**Fig. 2**), que incluye a las especies del Neotrópico (*Calosphace*) y su grupo hermano, de la Provincia Florística de California (*Audibertia*), ca. 190 especies son polinizadas por colibríes (Wester & Claßen-Bockhoff, 2011).

II.II. *Salvia* subgénero *Calosphace*

Comparado con otras áreas tropicales de África y Australasia, el Neotrópico contiene el mayor número de especies de plantas con semilla (90-110 mil spp.), albergando al 37% de su diversidad a nivel mundial (Antonelli & Sanmartín, 2011). Entre otros factores, Antonelli *et al*. (2015) determinaron que una de las principales diferencias entre las áreas tropicales es la dinámica evolutiva, pues en el caso de las angiospermas Neotropicales, la alta diversidad de especies está dada por altas tasas de recambio de especies (tasas altas de extinción y especiación), es decir, nuevas especies se generan y son reemplazadas rápidamente. Comparado con los otros dos centros de diversidad de *Salvia s.l.*, el linaje Neotropical, que corresponde al subgénero *Calosphace* (**Fig. 2**; de aquí en adelante sólo se referirá al grupo por el nombre del subgénero), es el más diverso. Las más de 600 especies de *Calosphace* se encuentran distribuidas en cuatro centros de diversidad (Jenks *et al*., 2013): México y Centroamérica (ca. 300 spp.), Los Andes (200 spp.), el este de Brasil (60 spp.) y las Antillas

(45 spp). Se considera que México es el centro de origen de *Calosphace* (Jenks *et al*., 2013). Fragoso-Martínez *et al*. (2018) han sugerido que existieron numerosas colonizaciones de Sudamérica por parte de linajes mesoamericanos, y al menos dos colonizaciones de Las Antillas por parte de linajes andinos.

Salvia es el género más diverso de angiospermas en México (Villaseñor, 2016) y de sus 309 especies, 295 pertenecen a *Calosphace* (Martínez-Gordillo *et al.*, 2013; Martínez-Gordillo *et al.*, 2017). México, no solamente alberga la mayor cantidad de especies de este subgénero en el Neotrópico, su nivel de endemismo es notable (77%; Martínez-Gordillo *et al.*, 2017), por lo que se ha propuesto, al país, como su centro primario de especiación (Ramamoorthy 1984). Las salvias Neotropicales se distribuyen principalmente en los bosques de montaña, en el caso de las especies mexicanas, la mayoría se encuentran en bosques de pino, encino, mixtos y bosques mesófilos de montaña, siendo uno de los elementos más conspicuos del estrato herbáceo o arbustivo (Domínguez-Vázquez *et al*., 2002; Ramamoorthy & Elliott, 1998).

Calosphace se distingue morfológicamente de los otros subgéneros por presentar una palanca estaminal con el conectivo alargado y la ausencia de tecas posteriores fértiles (Walker & Sytsma, 2007; Will & Claßen-Bockhoff, 2017). Con excepción de *Salvia axillaris*, que es la única especie del subgénero que expresa tecas posteriores y es el grupo hermano del resto (**Fig. 2**), los demás taxa presentan una morfología estaminal muy diversa, que se encuentra relacionada, en parte, con los polinizadores (Barrera-Aveleida, 2017). *Calosphace* es el único linaje de *Salvia s.l.* donde la polinización por aves ha surgido al menos en 13 ocasiones distintas; además de registrase una reversión a melitofilia en un linaje ornitófilo (Fragoso-Martínez *et al*., 2018). Por otra parte, las transiciones hacia la ornitofilia o la reversión a la melitofilia requieren del paso por un estado intermedio o ambófilo (Fragoso-Martínez *et al.*, 2018), el cual está presente en ca. 10% de las especies, que son polinizadas tanto por aves como por abejas (Wester & Claßen-Bockhoff, 2011).

Wester & Claßen-Bockhoff (2011) destacan algunas de las características que distinguen a las especies melitófilas como son: la presencia de un labio inferior alargado formando una plataforma de aterrizaje, estambres insertos en el labio superior de la corola, colores azules (**Figs. 3a-d**), blancos o violetas y guías de néctar (**Figs. 3c-d**). Las especies ambófilas tienen una morfología intermedia entre ambos síndromes (**Figs. 3e-h**), mientras que las especies ornitófilas tienen tubos de la corola rectos y alargados (**Figs. 3i-n**), estambres generalmente exsertos y labios de la corola de tamaño reducido (**Figs. 3k-n**).

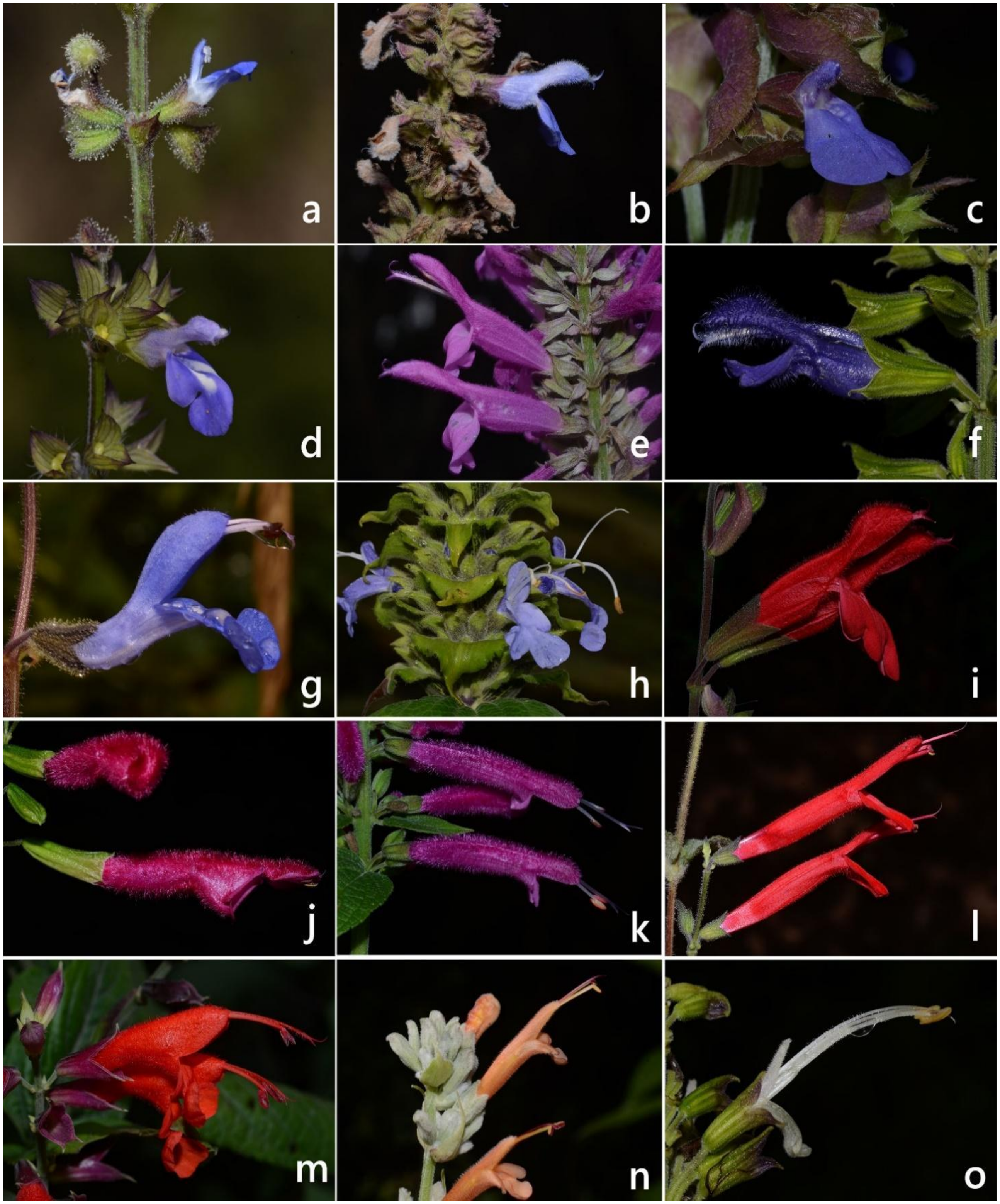


Figura 3. Ejemplo de la diversidad morfológica floral de *Calosphace*. a-d. Especies melitófilas con androceo inserto en el labio superior de la corola, corola azul o violeta: a) *Salvia misella*, b) *S. polystachya*, c) *S. mocinoi* con brácteas coloridas persistentes, d) *S. setulosa* con guías de néctar blancas en el labio inferior de la corola. e-g. Especies de amplia distribución, polinizadas por aves y abejas; dos con estambres insertos: e) *S. purpurea*, f) *S. mexicana*. g-h, con estambres exsertos: g) *S. scutellarioides* y h) *S. macrostachya*. i-j. Especies ornitófilas con estambres insertos: i) *S. gesneriflora*, j) *S. nervata*. k-n. Especies ornitófilas con estambres exsertos: k) *S. iodantha*, l) *S. elegans*, m) *S. pauciserrata*, n) *S. bahorucona*. Especie falenófila con estambres exsertos, o) *S. arborescens*.

La clasificación infragenérica de *Calosphace*, propuesta por Epling (1939), está basada principalmente en morfología floral (**Fig. 3**) y distribución geográfica. Es así, como en dicha obra, en conjunto con una serie de publicaciones posteriores (Apéndice 1), Epling propuso 99 secciones, 35 de ellas monotípicas. Los estudios filogenéticos en *Calosphace* concuerdan en que la mayoría de estas agrupaciones no son monofiléticas y sus circunscripciones están basadas en caracteres homoplásicos (Jenks *et al*., 2013; Fragoso-Martínez *et al*., 2018). Algunos de estos caracteres convergentes pueden ser los estambres exsertos (**Fig. 3**), que se encuentran presentes en especies de varios clados (e.g., Angulatae, Biflorae, Hastatae, etc.), o la presencia de papilas al interior del tubo de la corola (comunes en Fulgentes, y algunas especies de Angulatae y Membranaceae). Encontrar caracteres que eran considerados de valor taxonómico, sin un patrón aparente en la filogenia de *Calosphace*, indica que son resultado de presiones impuestas por el mismo grupo de polinizadores (en este caso colibríes).

Aunque es claro que *Calosphace* ha tenido una gran diversificación y buena parte de las especies se encuentran en México, se desconoce qué factores están involucrados en esta radiación. Por lo tanto, este grupo puede servir como modelo para entender los procesos que han formado la gran diversidad florística de las montañas neotropicales. Este proyecto está enfocado en generar una filogenia robusta de los principales linajes del subgénero *Calosphace*, con el fin de poder llevar a cabo análisis sobre tiempos de divergencia, tasas de diversificación, así como evaluar posibles correlaciones entre la radiación de algunos clados con posibles eventos paleogeográficos.

Existen dos principales factores que se consideran directamente relacionados con la diversificación de las salvias neotropicales: *1) Polinizadores*. La presencia de la palanca estaminal se ha considerado como un evento que pudo haber desencadenado tres radiaciones en *Salvia s.l.*, una de ellas en *Calosphace* (Claßen-Bockhoff *et al*., 2004; Walker & Sytsma, 2007). No obstante, el éxito y subsecuente modificación de esta estructura involucra la interacción con los polinizadores y con el resto de los órganos florales. Dado que la polinización por vertebrados, particularmente por colibríes, ha sido asociada con el aumento en las tasas de diversificación en distintos grupos andinos de angiospermas (Givnish *et al*., 2014; Lagomarsino *et al*., 2016; Roalson & Roberts, 2016), *Calosphace* podría tratarse de un caso similar, especialmente porque ha experimentado múltiples transiciones a la ornitofilia a partir de la melitofilia y solamente una reversión (Fragoso-Martínez *et al*., 2018).

2) Procesos orogénicos (Jenks *et al*., 2013; Ramamoorthy & Elliot, 1998). El Mediterráneo es el área ancestral que se ha reconstruido para el linaje de *Salvia s.l.*, pero se considera que *Calosphace* pudo haberse originado en Norte- o Centroamérica, durante el Mioceno (Drew & Sytsma, 2012).

Audibertia, el clado hermano de *Calosphace*, tiene una distribución restringida a la provincia florística de California, que presenta un clima similar al Mediterráneo; mientras que la mayoría de las especies de *Calosphace* están distribuidas en los bosques de montaña tropical. El levantamiento de las cordilleras mesoamericanas y de Los Andes constituyeron paisajes clave, que proveyeron una gran cantidad de nichos y que pudieron haber permitido al ancestro de *Calosphace* (o de alguno de sus clados menos inclusivos) explorar los hábitats montanos. Ninguna de estas hipótesis ha sido probada de manera formal y en ellas se basa este trabajo. Tratando de responder una pregunta central: ¿Por qué hay tantas salvias en el Neotrópico?

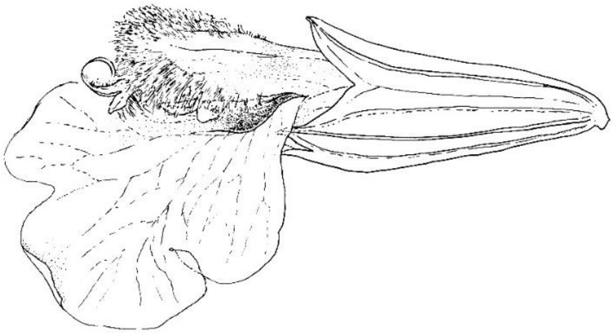
Objetivos

1) Generar una hipótesis filogenética robusta de *Salvia* subgénero *Calosphace*, incluyendo un muestreo representativo de taxa.

2) Estimar los tiempos de divergencia de los linajes dentro del subgénero y explorar posibles correlaciones de dichos eventos de divergencia con escenarios paleogeográficos o paleoecológicos.

3) Analizar si existen cambios significativos en las tasas de diversificación en *Calosphace* y determinar su asociación con factores intrínsecos y/o extrínsecos.

CAPÍTULO I. DOCUMENTANDO LA DIVERSIDAD VEGETAL DEL NEOTRÓPICO: RESULTADOS DE LAS EXPLORACIONES BOTÁNICAS EN MÉXICO



Salvia semiscaposa Epling ex Fragoso & Mart.Gord. (Ilustración: T. Jiménez)

I.I. Fragoso-Martínez, I.*, Martínez-Gordillo, M. & De Luna, E. 2015. *Salvia semiscaposa* (Lamiaceae) a new species from Nanchititla, México. *Phytotaxa* 219(1): 58-68. DOI: 10.11646/phytotaxa.219.1.4

I.II. Martínez-Gordillo, M., **Fragoso-Martínez, I.***, García-Peña, M.R. 2016. A new species of *Salvia* section *Uliginosae* (Lamiaceae), from Oaxaca, Mexico. *Phytotaxa* 245(3): 216-222. DOI: 10.11646/phytotaxa.245.3.4

I.III. Martínez-Gordillo, M., **Fragoso-Martínez, I.***, Salas-Morales, S. 2016. *Salvia robertoana* (Lamiaceae), a new species from Oaxaca, México. *Phytotaxa* 269(4): 271-278. DOI: 10.11646/phytotaxa.269.4.2

I.IV. González-Gallegos, J., **Fragoso-Martínez, I.***, González-Adame, G., Martínez-Ambriz, E., López-Enríquez, L.I. 2018. *Salvia ozolotepecensis*, *S. patriciae* and *S. sirenis* (Lamiaceae), three new species from Miahuatlán district, Oaxaca, Mexico. *Phytotaxa* 362(2): 143-159. DOI: 10.11646/phytotaxa.362.2.2

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Salvia semiscaposa (Lamiaceae) a new species from Nanchititla, Mexico

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Abstract

A new species of *Salvia* section *Lavanduloideae* from the Estado de México is described and illustrated. *Salvia semiscaposa* is a procumbent plant morphologically similar to *S. scaposa* and *S. helianthemifolia*. It differs from the former by having mostly obovate leaf blades, more than six flowers per verticillaster and posterior calyx lobes narrower and apiculate. On the other hand, *S. helianthemifolia* differs from the new species by the presence of an erect stem, ovate leaves and trichomes on the calyx surface distributed only on the veins.

Resumen

Se describe e ilustra una nueva especie de *Salvia* sección *Lavanduloideae* del Estado de México. *Salvia semiscaposa* es una planta procumbente morfológicamente similar a *S. scaposa* y *S. helianthemifolia*. De la primera difiere por presentar generalmente hojas obovadas, más de seis flores por verticilastro y lóbulos posteriores del cáliz más estrechos y apiculados. Por otro lado*, S. helianthemifolia* difiere de la especie nueva por la presencia de un tallo erecto, hojas ovadas y tricomas del cáliz presentes solamente en las venas.

Key words: multivariate analyses, *Calosphace*, geometric morphometrics

Introduction

Salvia Linnaeus (1753: 23) is the second most diverse genus in Mexico (Villaseñor 2004), with more than 300 species, of which 75% are endemic (Martínez-Gordillo *et al*. 2013). These numbers are continuously growing, as more species had been recently described by Klitgaard (2007), Turner (2008, 2013), Bedolla-García *et al.* (2011), Martínez-Gordillo & Lozada-Pérez (2011), González-Gallegos *et al.* (2012a, 2012b, 2013), Iltis *et al*. (2012), Fragoso-Martínez & Martínez-Gordillo (2013), González-Gallegos (2013), González-Gallegos & Castro-Castro (2013), González-Gallegos & Aguilar-Santelises (2014) and Lara-Cabrera *et al*. (2013).

Phylogenetic studies based on molecular data suggest that *Salvia* is paraphyletic and that only the Neotropical subgenus *Calosphace*, is monophyletic (Walker *et al*. 2004). *Calosphace* is the most species-rich subgenus of *Salvia* with ca. 600 species (Epling 1939, Santos 1995). The countries that hold most of the diversity of *Calosphace* are Mexico (310 spp.; Martínez-Gordillo *et al*. 2013) and Peru (81 spp.; Zarucci 1993). The Neotropical sages are distributed mainly along the mountain chains of Mesoamerica and South America, being especially diverse in montane tropical forests (Espejo & Ramamoorthy 1993). In Mexico the endemism of *Salvia* subgenus *Calosphace* is high in the Sierra Madre Oriental, Sierra Madre Occidental, and Trans-Mexican Volcanic Belt (Ramamoorthy & Elliot 1998).

The great diversity of *Calosphace* has been classified in more than 100 sections (Epling 1939, 1940, 1941, 1947, 1951, Epling & Játiva 1966, Ramamoorthy 1984a, Ramamoorthy & Elliot 1998). *Salvia* section *Lavanduloideae* Epling (1939: 34) is a group of 12 species endemic to Mexico, except *Salvia lavanduloides* Kunth (1817: 287), which has been

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reported also from Guatemala to Costa Rica (Klitgaard 2012). This group includes herbaceous perennial plants, with 3-veined posterior calyx lobes, stamens included in the galea and a concave anterior stigmatic branch (Epling 1939). The only revision available for *Lavanduloideae* is the one of Epling (1939), where the section was first described. Later, Epling described three new species but these were never validly published. One species is indeed different to all known in section *Lavanduloideae*, which is now described and validated here with the name given originally by Epling. The new species is similar to *S. scaposa* Epling (1939: 35) and *S. helianthemifolia* Bentham (1833: 254), thus we present a morphometric analysis, which helps to differentiate these species.

Materials and methods

Herbarium work

On December of 1954 in an expedition conducted by the Commission of botanic explorations of the Estado de México, Eizi Matuda collected specimens of a scapose-like sage from Sierra de Nanchititla. Epling, in an unpublished manuscript, recognized one of these specimens (*Matuda 31980*) as the type of a new species. The specimen remained in the collection of MEXU herbarium identified erroneously as *Salvia remota* Bentham (1848: 304). To verify the locality of the new species and to collect additional specimens for the description, two expeditions to Sierra de Nanchititla were made in the years of 2011 and 2012. For the new species we retrieved distribution and morphological data from a total of 17 specimens from the collections at FCME and MEXU herbaria.

Geometric morphometrics

A morphometric comparison of shapes of leaves was undertaken to differentiate the new species from two morphologically similar species: *S. scaposa* and *S. helianthemifolia*, and also its sister species: *S. rzedowskii* Ramamoorthy (1984b: 139). Digital images were acquired from ten specimens of the new species (21 leaves), 14 specimens of *S. helianthemifolia* (33 leaves), 15 specimens of *S. rzedowskii* (28 leaves) and 28 specimens of *S. scaposa* (58 leaves), all including a ruler for scale. The leaf blades were photographed from the herbarium sheets laying flat under a digital camera (Olympus Camedia c-740 U or a Canon EOS Digital Rebel 6M) fixed on a Bencher copy stand. Digital photography was achieved with equipment available at the Laboratorio de Morfometría, Instituto de Ecología (INECOL) and at the Laboratorio de Microcine, Facultad de Ciencias (UNAM).

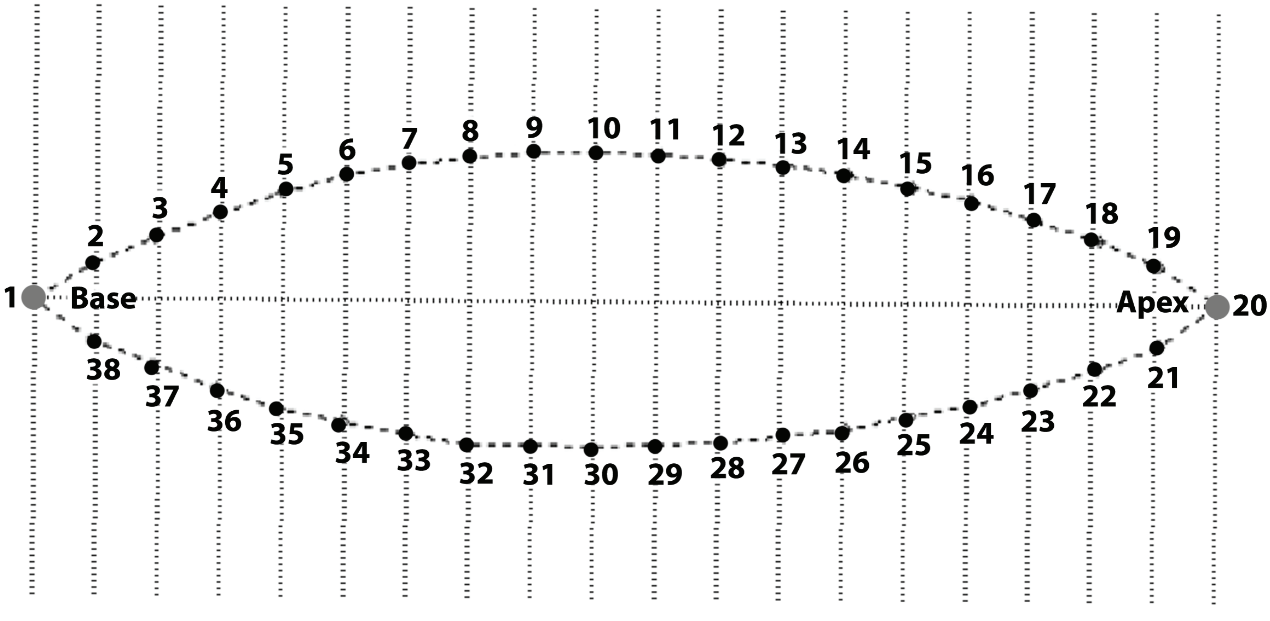

FIGURE 1. Landmarks (gray) and semi-landmarks (black) selected to describe the shape of the leaf blade using a comb with 20 rays.

The shape of the leaf blades was registered with strings of semi-landmark points. We used a comb of 20 rays as a graphical tool to place the same number of points along a curve segment (Sheets 2004a). Combs were added to the photographs using MakeFan6 from the IMP series (Sheets 2004b). The shape of the leaf blades was sampled with a configuration of 38 points: two landmarks (base and apex) and 36 semi-landmarks (Fig. 1).

Two dimensional landmark configurations were digitized in tpsDig 2.16 (Rohlf 2010). The x, y coordinates were processed and analyzed with different programs from the IMP series (Sheets 2004b). Procrustes superpositions were carried out in CoordGen and semi-landmarks were slid in SemiLand. Comparison of shapes among the new species, *S. rzedowskii*, *S. helianthemifolia* and *S. scaposa*, as a priori groups, was performed by canonical discriminant analysis of the partial warp scores (PWs) using the CVAGen6 program (http://www2.canisius.edu/~sheets/morphsoft.html) developed by Sheets (2004b). Our question was whether there is a difference among the four average shapes of the species, as a priori groups. The tests of significance of the canonical variate axes were based on the Wilks's lambda value. The CVA scores obtained on CVAGen6 were plotted using the packages e1071 (Meyer *et al*. 2014) and ggplot (Wickham 2009) in R (R Core Team 2014).

Taxonomy

Salvia semiscaposa Epling ex Fragoso & Mart.Gord. *sp. nov*. (figs. 2, 3)

Salvia scaposae affinis sed foliis subsessilis, ovatis obovatis ad medium attenuatum usque ad basim, corollae infero labio longiore.

Type:—MEXICO. Estado de México, por la carretera a Cañadas de Nanchititla, cerca de 6 km al W, antes de la desviación al parque, 1765 m, 18°52'46.3"N, 100°23'13.8"W, 10 November 2011, *Fragoso-Martínez & Martínez-Gordillo 17* (holotype FCME!, isotype MEXU!).

Decumbent perennial herbs; 0.5–1 m tall, with several stems arising from a woody caudex; stems appressed-hirsute; internodes shortened towards the base, 1.5–8.5(–10) cm long. Leaves developed towards the base, subsessile, petioles 0.1–0.7 cm long, appressed-pubescent; basal leaf blades ovate, upper leaf blades obovate, 2.5–8 × 1–2 cm, apex obtuse, base attenuated, margin crenate, both surfaces appressed-hirsute, lower surface pale. Inflorescences terminal, spiciform, simple or in fascicles of three, sometimes the inflorescence can be secund; peduncles 2–10.5 cm long; spikes 4–15(–20) cm long, verticillasters 0.5–2 cm apart. Bracts minute, 1.5–3.5 × 0.9–1.2 mm, foliaceous, ovate, deciduous early at the development of the inflorescence. Flowers 6–10 per whorl, pedicels 2–5 mm long, divaricate to reflexed, appressed-hirsute. Calyx bilabiate, purple, tube 3.5–5 mm long, appressed-hirsute; calyx lobes obtuse-apiculate, posterior lobe 3-veined, slightly shorter than the anterior ones, lobes 1.5–2.5 mm long. Corolla lilac or purple, tube straight, same size or slightly longer than the calyx tube, 5.5–7 mm long; posterior lobe of the corolla galeate, with the external surface pubescent, 2–3 m long; anterior lobe 5–7(–8) mm long, reflexed. Stamens included; filaments 1.2–2.4 mm long; connectives 1.5–2 mm long, with an acute ventral tooth; thecae 0.75–1.5 mm long. Style 7.5–9.5 mm long, pubescent near the branches, anterior branch rounded at the tip and concave; posterior branch curled backward, longer than the anterior. Mericarp ovoid, smooth, 1.5–2 mm long.

Distribution and habitat:—*Salvia semiscaposa* is endemic to Mexico and is known only from the Sierra de Nanchititla, Estado de México, near the state limits with Guerrero and Michoacán (Fig. 4). This mountain range extends to Guerrero, where this species might inhabit as well. It has been collected in oak forests, pine-oak forests, and xeric shrub lands, usually found on moderately exposed slopes, occurring at 650–2000 m elevation. In all the localities visited, the abundance of this species was moderate to high.

Phenology:—Flowering and fruiting from September to January.

Etymology:—"*semiscaposa*" is the epithet that Epling assigned to this taxa on his unpublished manuscript, it is related to the habit of the plant, which is not entirely scapose because the stems are long but decumbent (Figs. 2A, 3A), conversely to the erect stems in *Salvia scaposa*.

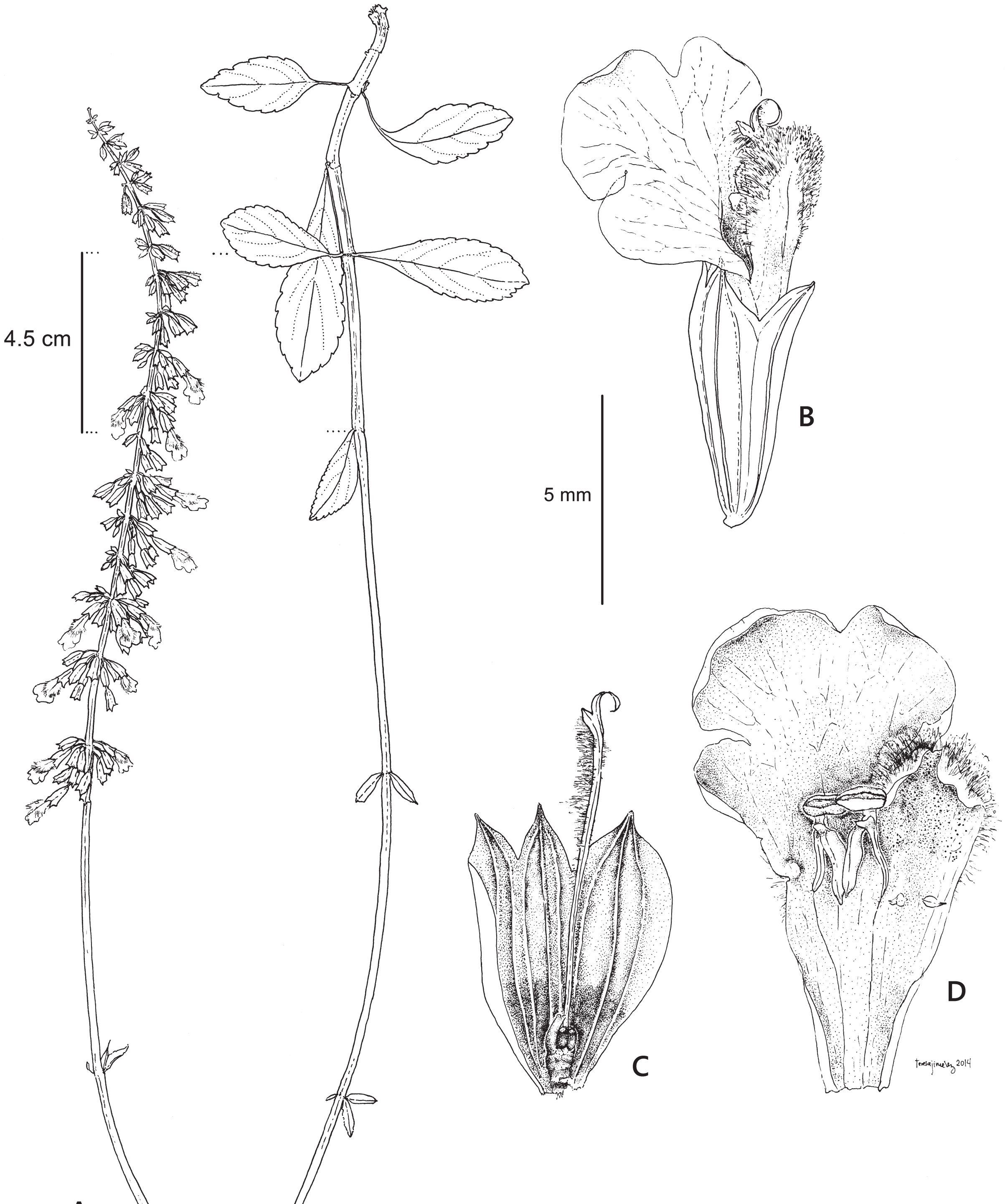

FIGURE 2. *Salvia semiscaposa*. A) Branch with inflorescence. Flower: B) Lateral view; C) Dissected calyx with style, gynobase and gland; D) Dissected corolla with gubernaculum and staminodes. Illustration drawn from the holotype (FCME) by Teresa Jiménez.

Additional specimens examined:—MEXICO, Estado de México: por la carretera a Cañadas de Nanchititla, cerca de 6 km al W, antes de la desviación al parque, 1765 m, 18° 52’46.3”N, 100°23’13.8”W, 10 November 2011, *Fragoso-Martínez & Martínez-Gordillo 18* (FCME!); arroyo en la primera puerta del camino del Parque Ecológico Sierra de Nanchititla, que va hacia el mirador de la Cañada, 1665 m, 18°51’23.9”N, 100°25’30.3”W, 10 November

2011, *Fragoso-Martínez & Martínez-Gordillo 19* (FCME!); alrededores del mirador a la cascada del parque ecológico Sierra de Nanchititla, 1503 m, 18°49'21.8"N, 100°25'32.3"W, 10 November 2011, *Fragoso-Martínez & Martínez-Gordillo 26* (FCME!); por la carretera que va de Cañadas de Nanchititla a Villa Luvianos, ca. 2 km al E de la desviación al parque, 1795 m, 18°52'10.5"N, 100°24'48.3"W, 11 November 2011, *Fragoso-Martínez & Martínez-Gordillo 31* (FCME!); ca. 6.4 km al E de la desviación al Parque Sierra de Nanchititla, por la carretera a Villa Luvianos, 1779 m, 18°52'47.5"N, 100°23'5.8"W, 11 November 2011, *Fragoso-Martínez & Martínez-Gordillo 32* (FCME!); ca. 19–20 km al E de la desviación al Parque Sierra de Nanchititla, por la carretera a Villa Luvianos, 1800 m, 18°52'55.779"N, 100°18'14.8428"W, 11 November 2011, *Fragoso-Martínez & Martínez-Gordillo 52* (FCME!); 8 km del Salitre, 1900 m, 18°52'54.5"N, 100°18'52.1"W, 6 December 2012, *Fragoso-Martínez et al. 123* (FCME!); 3 km antes de llegar al Salitre desde Nanchititla, 1700 m, 18°53'17"N, 100°17'39"W, 6 December 2012, *Fragoso-Martínez et al. 127* (FCME!); Cerro de La Culebra, Luvianos, Progreso, 1300 m, 7 September 1954, *Matuda et al. 31507* (MEXU!); La Junta, Dto. Valle de Bravo, 650–900 m, 11 September 1954, *Matuda et al. 31640* (MEXU!); Cañada de Nanchititla, 1800 m, 4–12 December 1954, *Matuda s.n.* (MEXU!), *31980* (MEXU!); 52 km al SW de Nanchititla, 2000 m, January 1973, *Medrano et al. 5046* (MEXU!); Sierra de Nanchititla a lo largo de cañada, 1930 m, 20 January 1973, *Medrano et al. 5162* (MEXU!); 1.7 km antes de la entrada al parque estatal Sierra de Nanchititla, 1813 m, 18°52'16.9"N, 100°24'52.7"W, 21 November 2012, *Rojas et al. 2424* (FCME!); La Cascada, parque estatal Sierra de Nanchititla, 1490 m, 18°49'24.6"N, 100°25'31.8"W, 21 November 2012, *Rojas et al. 2480* (FCME!).

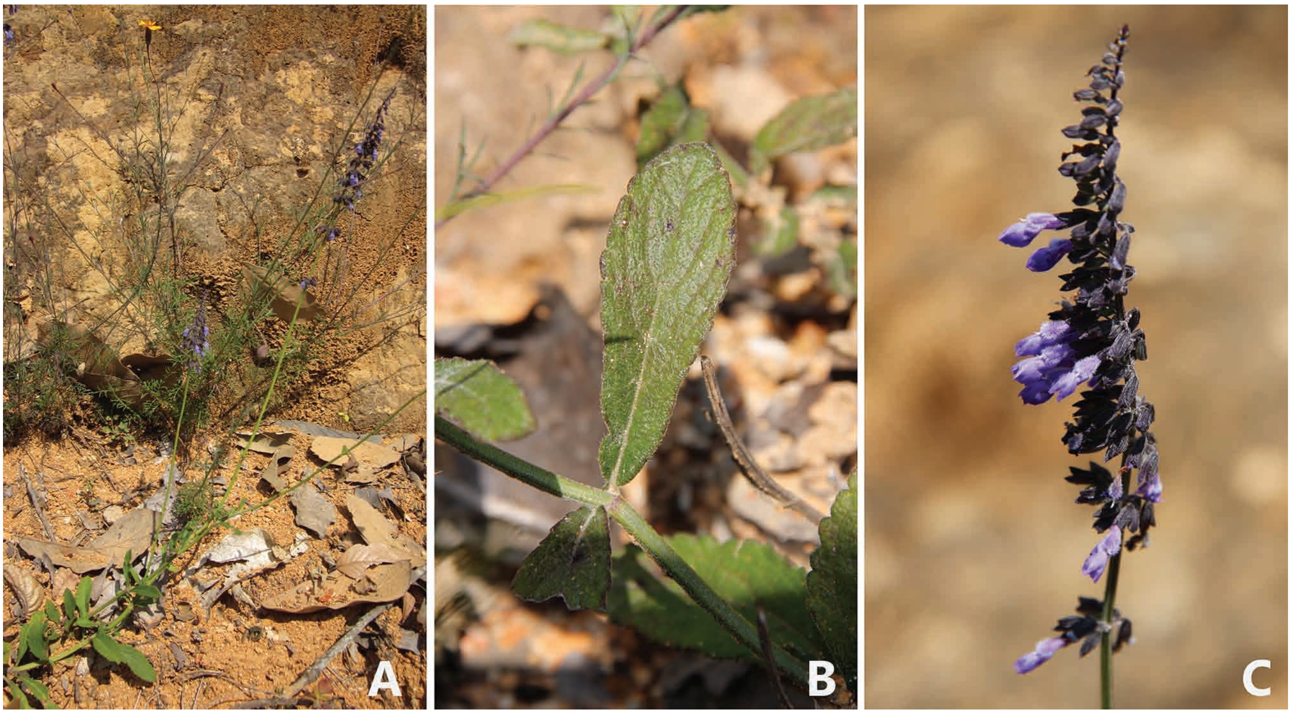

FIGURE 3. *Salvia semiscaposa*. A) Plant habit, B) Leaf blade detail, C) Inflorescence detail. (Photographs by I. Fragoso).

Discussion

The new species is a member of section *Lavanduloideae*. In a preliminary phylogeny of the section obtained from a parsimony analysis that combined morphological data (cualitative, cuantitative and geometric morphometrics data) as well as two molecular markers (the nuclear ribosomal internal transcribed spacer or ITS and the plastid *trnL-trnF* intergenic spacer) (Fragoso-Martínez 2014), *S. semiscaposa* is sister to *S. rzedowskii*. These species share three substitutions on the ITS region, corolla and leaf proportions as synapomorphies. Morphologically, *S. rzedowskii* is different from *S. semiscaposa*; the main differences are the erect habit, the white corollas, and the strongly acute calyx lobes in *S. rzedowskii* (Table 1). Instead, *S. semiscaposa* has a decumbent habit (Fig. 3A), purple corollas (Fig. 3C) and obtuse-apiculate calyx lobes (Fig. 2C). Both species are the sister group to a clade which includes three scapose-like species: *S. scaposa*, *S. teresae* Fernald (1900: 506), and S. *heterofolia* Epling & Mathias (1957: 310).

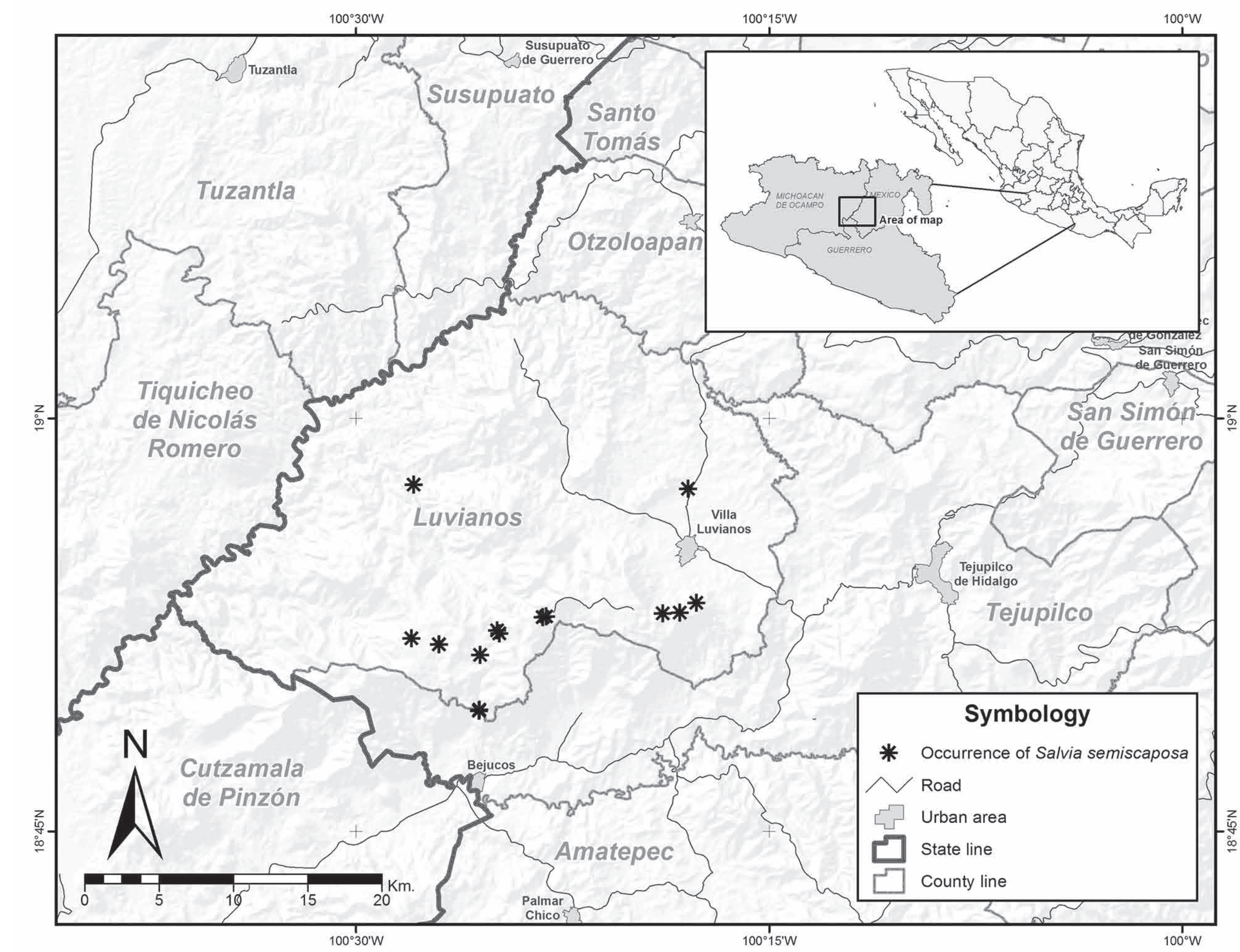

FIGURE 4. Distribution of *Salvia semiscaposa* in Mexico.

TABLE 1. Differences between *Salvia semiscaposa*, *S. rzedowskii, S. scaposa* and *S. helianthemifolia*.

	S. semiscaposa	*S. rzedowskii*	*S. scaposa*	*S. helianthemifolia*
Stem	long and decumbent	long and erect	short and erect	short and erect
Leaf and calyx indumentum	appressed-hirsute	appressed-hirsute	appressed-hirsute on the veins	appressed-hirsute on the veins
Leaf shape	obovate to ovate	elliptical	elliptical	ovate
Number of flowers per verticillaster	more than 6 flowers	more than 8 flowers	2–4(–6) flowers	6(–8)–10 flowers
Distance between verticillasters	0.5–2 cm	0.2–2 cm	0.5–4.5 cm	0.8–3.5 cm
Calyx position	divaricate to reflexed	usually divaricate	reflexed	reflexed
Posterior calyx lobes shape	narrow, apex apiculate	narrow, apex acute	broad, obtuse-mucronate	narrow, apex apiculate
Corolla color	lilac or purple	white	lilac	purple, occasionally white

Morphologically *Salvia semiscaposa* resembles *S. scaposa*. Both species share a similar habit and inflorescence morphology. Nevertheless, the stem in the latter species is short and erect, while it is usually longer and procumbent in the former (Fig. 3A). Some populations of *Salvia scaposa* have developed distal leaves (e.g. Taxco and Tetipac populations) but the typical form is similar to *S. semiscaposa* in having only the leaves at the base well developed (e. g. Sultepec and Zacualpan populations). Other differences between the two species are the indumentum on leaves and calyx; *S. scaposa* has both surfaces sparsely covered with short appressed trichomes whereas *S. semiscaposa* is moderately to densely covered with long appressed-hirsute trichomes (Table 1). Moreover, the leaf shape toward branch apex in *S. scaposa* is elliptical whereas *S. semiscaposa* has obovate leaves (Table 1, Figs. 3B and 6). Concerning the inflorescences of both species, *S. semiscaposa* can be distinguished by the presence of more than six flowers per verticillaster, whereas there are only 2–4, occasionally 6 flowers in *S. scaposa*, with a greater distance between the verticillasters (Figs. 5A and 5B, Table 1). Lastly the posterior calyx lobes of *S. semiscaposa* are narrower than those of *S. scaposa*, and the tip is apiculate (Table 1).

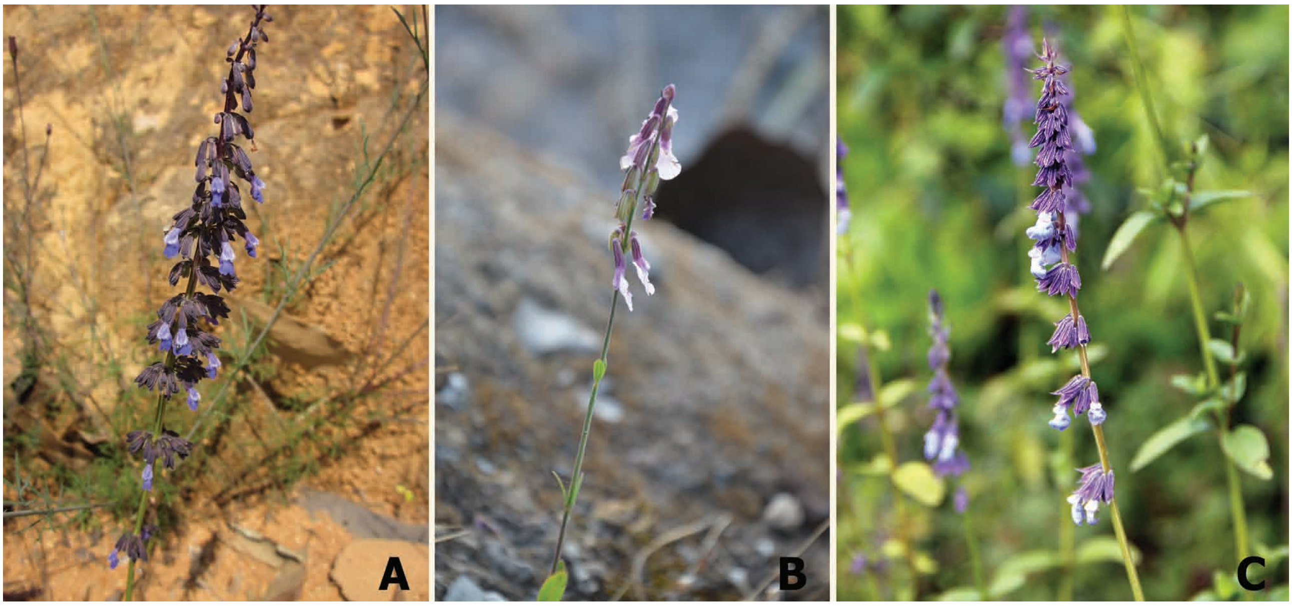

FIGURE 5. Inflorescence morphology of: A) *Salvia semiscaposa*; B) *S. scaposa* and C) *S. helianthemifolia.* (Photographs by I. Fragoso and E. Martínez).

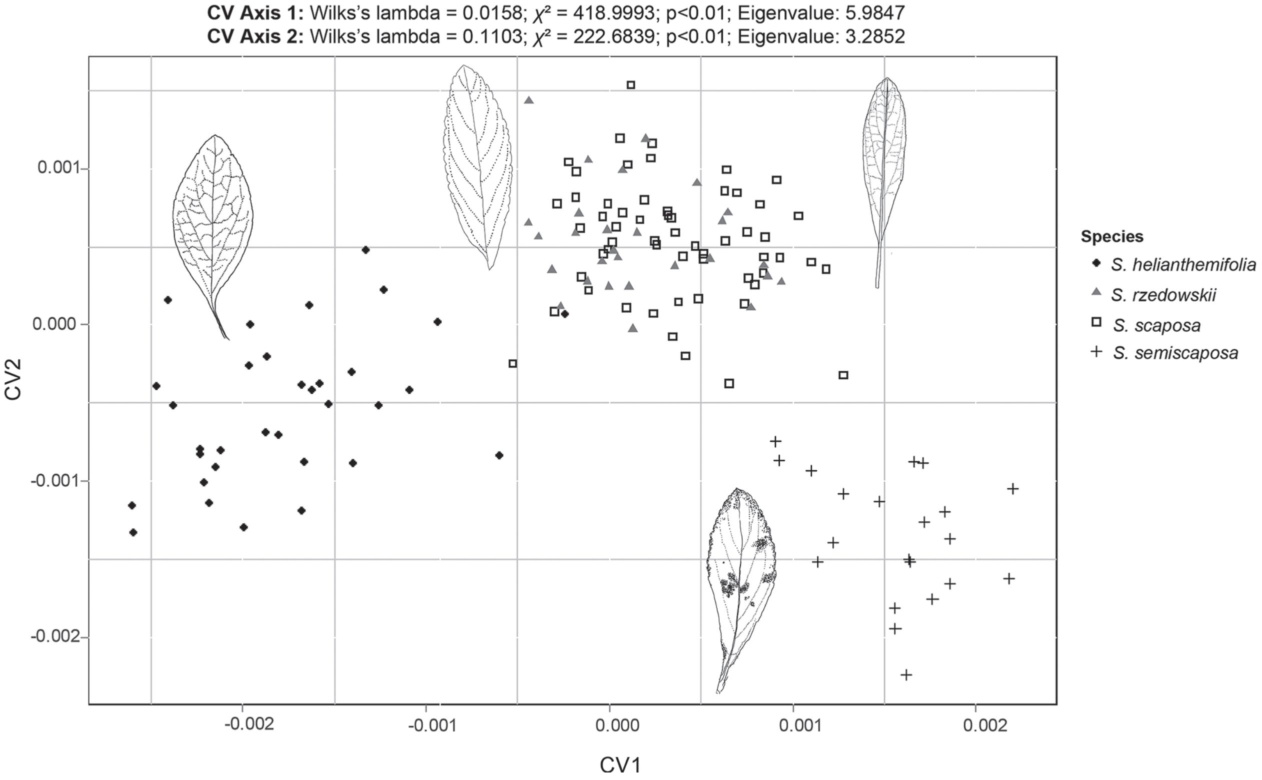

FIGURE 6. Scatterplot of canonical variates (CV), CV1 *vs*. CV2 from the geometric morphometric analysis of n= 140 leaf blade configurations of landmarks and semilandmarks. Illustrations of the structures (not in scale) for each species are shown for comparison purposes.

S. helianthemifolia is another species that resembles *S. semiscaposa* primarily in the inflorescence morphology. The main difference between these species is stem orientation, the former is an erect herb whereas *S. semiscaposa* is a decumbent herb. Also the shape of the leaf blades differs significantly, being ovate in *S. helianthemifolia* and obovate in the new species (Table 1, Fig. 6). Regarding the inflorescence morphology, characters like the distance between verticillasters and calyx orientation overlap between both species (Figs. 5A and 5C); however, there is a tendency in *S. helianthemifolia* to have a greater length between verticillasters (up to 3.5 cm *vs*. 2 in S. *semiscaposa,* Table 1). Finally, the surface of the calyces of both species is appresed-hirsute, but in *S. helianthemifolia* the trichomes are only distributed on the veins.

The discriminant analyses of geometric morphometric data showed the leaf blade shapes are significantly different among three groups: *Salvia semiscaposa, S. helianthemifolia* and a group formed by two species with similar leaf blade shape: *S. scaposa* and *S. rzedowskii* (Fig. 6). The differences in shape between the latter group and the new species are chiefly in the middle of the leaf to the apex, where *S. semiscaposa* is broader and thus has an obovate leaf shape (Fig. 6) while in *S. scaposa* and *S. rzedowskii* the leaf blade is narrower and elliptical (Fig. 6). Conversely, in *S. helianthemifolia* the central portion of the leaf is broader than in *S. semiscaposa* resulting in an ovate shape (Fig. 6). In the group assignments tests from the CVA, none of the specimens of *S. semiscaposa* were misclassified; however a leaf blade from one specimen of *S. scaposa* was classified as similar to the new species (Table 2). Also most of the specimens of *S. helianthemifolia* (96.96%) were assigned correctly. On the other hand, the leaf blades of *S. rzedowskii* and *S. scaposa* have the same shape (elliptic-oblong) which causes the specimens represented by points in the scatterplot to overlap (Table 2, Fig. 6). This suggests leaf blade shape is a good identification marker of *S. semiscaposa* but only in a comparison with specimens of the other three species (Table 2, Fig. 6).

TABLE 2. Group assignment test based on Mahalanobis distances in the space of the significant canonical variates axes, the original groups are along rows and the CVA groups along columns.

	S. semiscaposa	*S. scaposa*	*S. rzedowskii*	*S. helianthemifolia*
S. semiscaposa	21	0	0	0
S. scaposa	1	31	26	0
S. rzedowskii	0	9	19	0
S. helianthemifolia	0	0	1	32

Key to the species of section *Lavanduloideae* from type locality of the new species

1. Pedicels tilted downwards, mature calyces reflexed 2
1. Pedicels erect or spreading, mature calyces ascendant or divaricate 5
2. Plants with erect stems .... *Salvia helianthemifolia*
2. Plants with procumbent stems or scapose habit 3
3. Distal leaves evenly developed along the stems, leaves elliptical-lanceolate to linear; inflorescences spiciform .... *Salvia heterofolia*
- Distal leaves scarcely developed on the stems, basal leaves obovate or ovate; inflorescences scapose 4
4. Herbs 0.4–1 m tall; leaf surfaces moderately to densely covered with long appressed-hirsute trichomes; verticillasters with more than 6 flowers each .... *Salvia semiscaposa*
- Herbs 0.2–0.4 m tall; leaf surfaces sparsely covered with short appressed trichomes; verticillasters with 2–6 flowers each .... *Salvia scaposa*
5. Plants with exclusively white flowers; calyx lobes caudate, 2–5 mm long .... *Salvia rzedowskii*
- Plants with blue or purple flowers; calyx lobes acute to obtuse, 1–2.5 mm long 6
6. Leaf blades ovate-elliptical, glabrate; compact inflorescences (central verticillasters no more than 3 mm apart); pedicels inconspicuous .... *Salvia stachyoides*
- Leaf blades oblong-elliptical or elliptic-lanceolate, hirsute to pubescent; lax or interrupted inflorescences (central verticillasters more than 0.5 mm apart); pedicels conspicuous 1–2.5 mm long 7
7. Interrupted inflorescences, central verticillasters 1–3.5 cm apart; calyx surface lanate, calyx lobes acute.......... *Salvia moniliformis*
- Lax inflorescences, central verticillasters 0.5–1.5 cm apart; calyx surface pubescent, calyx lobes obtuse, apex mucronate .... *Salvia lavanduloides*

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Appendix. Specimens photographed for geometric morphometric analyses

***Salvia helianthemifolia*:—**MEXICO, Guanajuato. Cerro Zamorano, parte alta, 2800 m, 24 October 1988, *Rzedowski 47803* (MEXU!). Hidalgo. “El Cirio”, aproximadamente 1.2 km al W de San Nicolás, 1680 m, 20°19’19”N, 98°12’6”W, 5 September 1993, *Luna et al. 1469* (FCME!); Cerro de las Ventanas, 7 km al N de Pachuca, 2900 m, 25 December 1973, *Rzedowski 31572* (FCME!). Estado de México. Cerro del Huilote, Parque Nacional Lagunas de Zempoala, 2590 m, 14 January 1984, *Aguilar ZC-43* (FCME!); rumbo al llano de Los Tres Gobernadores, por San Juan Xoconusco, 2740 m, 19°22’30”N, 100°14’46”W, 20 March 2005, *Cornejo et al. 1029* (FCME!); parque Nevado de Toluca, 5 km al E de Mesón Viejo, por la carretera Toluca-Altamirano, rumbo a Toluca, 3003 m, 19°10’31.88”N, 99°51’34.16”W, 11 November 2011, *Fragoso-Martínez 100* (FCME!); aprox. 14 km rumbo a Oxtotilpan carr. Toluca-Oxtotilpan, 3176 m, 19°11’3.09”N, 99°51’21.8”W, 21 November 2012, *Fragoso-Martínez 110* (FCME!); crucero-Agua Blanca, 13 January

1936, *Hinton 8828* (MEXU!); Cerro Pelón camino a la colonia, 2620 m, 19°22'10"N, 100°15'54"W, 13 November 2005, *Salinas et al. 938* (FCME!); parque Nevado de Toluca, Ejido El Varal, 3090 m, 19°3'57"N, 99°56'27"W, 21 March 1996, *Villers 11* (MEXU!). Michoacán. Cerro Prieto, aprox. a 5 km al SE de Huajúmbaro, 2760 m, 17 September 1985, *Almazán et al. 582* (FCME!); Llano Largo, aprox. a 1.5 km al NE de Los Azufres, 2940 m, 19 March 1986, *Almazán et al. 641* (FCME!); Cañada de San Pedro, aprox. a 3 km al NW de San Pedro Jácuaro, 2300 m, 6 January 1986, *Almazán et al. 879* (FCME!); Laguna Larga, Los Azufres, 2800 m, 6 December 1986, *Zamudio 5087* (FCME!).

***Salvia rzedowskii*:—**MEXICO, Estado de México. km 6 carr. Zacualpan-Mamatla, 2000 m, 18°41'36.6"N, 99°47'53.6"W, 20 November 1981, *Castilla & Tejero 1539* (MEXU!); Nanchititla 3 km del Salitre, 5 October 1986, *Esquivel & Galicia s.n.* (MEXU!); 5 km de la Goleta hacia Sultepec, 2234 m, 18°41'44"N, 100°5'5.2"W, 22 November 2012, *Fragoso-Martínez 119* (FCME!); camino a Sultepec, 3 km antes de San José del Potrero, 2265 m, 18°44'56.6"N, 100°1'54.9"W, 22 November 2012, *Fragoso-Martínez 121* (FCME!); 6 km de la Goleta hacia Sultepec, 2175 m, 18°41'50.3"N, 100°4'57.5"W, 7 December 2012, *Fragoso-Martínez 129* (FCME!); cerro entre Sultepec y Amatepec, 2200 m, 31 December 1953, *Matuda et al. 30092* (MEXU!); La Corona, Zacualpan, 1 February 1954, *Matuda et al. 30333*, 7–8 December 1963, *Matuda et al. s.n*. (MEXU!); en barranca, cerca de Amatepec, 1500 m, 29 December 1953, *Matuda et al. 30036* (MEXU!); Cerro del Loro, cerca de Sultepec, 29 December 1962, *Paray 3348* (MEXU!); rumbo a Zacualpan, 2327 m, 18°39'39.4"N, 99°46'52.7"W, 18 October 2012, *Rojas et al. 1930, 1990* (FCME!); 1 km de las Peñas, 2186 m, 18°44'55.5"N, 100°0'6.1"W, 19 October 2012, *Rojas et al. 2003, 2038* (FCME!); La Ciénega, 3 km al S de Sultepec, 2350 m, 10 March 1973, *Rzedowski 30326* (MEXU!).

***Salvia semiscaposa*:—**MEXICO, Estado de México. Por la carretera a Cañadas de Nanchititla, cerca de 6 km al W, antes de la desviación al parque, 1765 m, 18°52'46.3"N, 100°23'13.8"W, 10 November 2011, *Fragoso-Martínez & Martínez-Gordillo 17, 18* (FCME!); alrededores del mirador a la cascada del parque ecológico Sierra de Nanchititla, 1503 m, 18°49'21.8"N, 100°25'32.3"W, 10 November 2011, *Fragoso-Martínez & Martínez-Gordillo 26* (FCME!); arroyo en la primera puerta del camino del Parque Ecológico Sierra de Nanchititla, que va hacia el mirador de la Cañada, 1665 m, 18°51'23.9"N, 100°25'30.3"W, 10 November 2011, *Fragoso-Martínez & Martínez-Gordillo 19* (FCME!); ca. 19–20 km al E de la desviación al Parque Sierra de Nanchititla, por la carretera a Villa Luvianos, 1800 m, 18°52'55.7"N, 100°18'14.8"W, 11 November 2011, *Fragoso-Martínez & Martínez-Gordillo 52* (FCME!); ca. 6.4 km al E de la desviación al Parque Sierra de Nanchititla, por la carretera a Villa Luvianos, 1779 m, 18°52'47.5"N, 100°23'5.8"W, 11 November 2011, *Fragoso-Martínez & Martínez-Gordillo 32* (FCME!); por la carretera que va de Cañadas de Nanchititla a Villa Luvianos, ca. de 2 km al E de la desviación al parque, 1795 m, 18°52'10.5"N, 100°24'48.3"W, 11 November 2011, *Fragoso-Martínez & Martínez-Gordillo 31* (FCME!); Cañada de Nanchititla, 1800 m, 4–12 December 1954, *Matuda s.n.* (MEXU!), *31980* (MEXU!); La Junta, Dto. Valle de Bravo, 650–900 m, 11 September 1954, *Matuda et al. 31640* (MEXU!).

***Salvia scaposa*:—**MEXICO, Guerrero. Cerro Huizteco, 2200 m, 19 September 2008, *Cano 1842* (FCME!); 16.5 km al NW de Taxco, camino a Tetipac, 2280 m, 25 January 1986, *Castelo 513* (FCME!); camino templo al Viento, parque Cerro El Huizteco, 2340 m, 6 September 1985, *Castillo et al. 138* (FCME!); 2 km al SW de la entrada al Parque Cerro El Huizteco, 2380 m, 18°35'56"N, 99°36'33"W, 7 September 1985, *Castillo et al. 175* (FCME!); cerca de la cañada El Limón, Campo Morado, 1248 m, 18°11'52.25"N, 100°9'50"W, 17 July 2006, *Cruz-Durán et al. 6288* (FCME!); filo de Cerro Tepehuaje, Campo Morado, 1393 m, 18°12'35.928"N, 100.9°0'23.57'W, 19 July 2006, *Cruz-Durán et al. 6420* (FCME!); Cerro del Huizteco, cerca del monumento al Viento, 2500 m, 12 October 1996, *Domínguez-Licona 71* (FCME!); a 7 km de Taxco, rumbo a Tetipac, 1980 m, 30 September 1984, *Fonseca 860* (FCME!); Camino templo al Viento, parque Cerro El Huizteco, 2340 m 6 September 1985, G*onzález et al. 121* (FCME!); a 7 km de Tetipac, rumbo a Taxco de Alarcón, 1940 m, 10 December 1996, *Domínguez-Licona 170* (FCME!); 14 km al NE de Taxco, rumbo a Tetipac, 18°35'47.2"N, 99°37'13.3"W, 25 January 1986, *López 169* (FCME!); aproximadamente 2.5 km al NO de Taxco, camino a Casahuates-Tetipac, 2120 m, 31 October 1984, *Lorea 3310* (FCME!); El Huizteco, 2400 m, 19 November 1998, *Macedonio 448* (FCME!); Parque el Huizteco, 2367 m, 18°36'21.4"N, 99°36'21.4"W, 29 September 2010, *Martínez 5540* (FCME!); 29 September 2010, *Martínez 5681* (FCME!); Coxcatlán, 1877 m, 18°30'10"N, 99°28'2.2"W, 30 September 2011, *Morales 486* (FCME!); Cerro Huizteco, 2250 m, 2 October 1995, *Ortega 1* (FCME!); Agua de Obispo, *Palma 249* (FCME!); camino templo al Viento, 1 km de la entrada parque Cerro El Huizteco, 2320 m, 6 September 1985, *Rendón 110* (FCME!); El Huizteco, 2400 m, 19 November 1998, *Reyna 527* (FCME!); Casahuates, aproximadamente a 2 km de la entrada, 2350 m, 14 November 1987, *Terán & Matías 256* (FCME!). Estado de México. 5 km de la Goleta hacia Sultepec, 2234 m, 18°41'44"N, 100°5'5.2"W, 22 November 2012, *Fragoso-Martínez et al. 120* (FCME!); camino a Puerto Oscuro, 2383 m, 18°34'8.56"N, 99°40'7.3"W, 24 September 2012, *Rojas et al. 1472* (FCME!), *1476* (FCME!); 1 km a las Peñas, 2186 m, 18°44'55.5"N, 99°10'6.1"W, 19 October 2012, *Rojas et al. 1994* (FCME!).

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A new species of *Salvia* section *Uliginosae* (Lamiaceae), from Oaxaca, Mexico

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Abstract

A new species from Oaxaca is described and illustrated. *Salvia tetramerioides* belongs to section *Uliginosae* as it shares with the species of this section the presence of a 5-veined, 3-mucronate posterior calyx lobe and a laterally flattened style. However, the morphology of the inflorescence of the new species differs from all the previously described taxa in the section; although the vegetative morphology resembles that of *Salvia laevis*. The new species is distinguished by the dense inflorescence, the peduncle longer than the rachis and broadly ovate, persistent bracts, with decussate phyllotaxy and two flowers per node. A key is provided to distinguish it from the species of the section found in Oaxaca.

Key words: *Calosphace*, Tehuacán-Cuicatlán

Resumen

Una nueva especie de Oaxaca se describe e ilustra. *Salvia tetramerioides* pertenece a la sección *Uliginosae* porque comparte con las especies de este grupo la presencia del labio posterior del cáliz 5-venado, 3-mucronado y un estilo lateralmente aplanado. Sin embargo, la morfología de la inflorescencia de la nueva especie difiere de todos los taxa previamente descritos en la sección, aunque la morfología vegetativa se asemeja a *Salvia laevis*. La especie nueva se distingue por la inflorescencia densa, con el pedúnculo más largo que el raquis y brácteas ampliamente ovadas, persistentes con filotaxia decusada y dos flores por nudo. Se presenta una clave para distinguirla de las especies de la sección que se encuentran en Oaxaca.

Introduction

Salvia Linnaeus (1753: 23) is the most diverse group of Lamiaceae, a family with a cosmopolitan distribution (Harley *et al.* 2004). The most outstanding feature of the genus is the 2-stamen androecium with an elongated connective. Generally, the posterior branch of the connective (regarding the filament) is modified into a structure that is involved in pollen deposition on the pollinator (Claßen-Bockhoff *et al*. 2003). Due to the singularity of the masculine structure the genus has been considered monophyletic, with four subgenera: *Salvia* Bentham (1876: 1195), *Leonia* Bentham (1876: 1196), *Sclarea* Bentham (1876: 1195) and *Calosphace* Bentham (1833: 198). However, molecular studies (Walker *et al*. 2004, Walker & Sytsma 2007) recovered *Salvia* as paraphyletic, which implies that the modification of the androecium has arisen independently at least three times during the evolution of the group (Walker *et al.* 2004). *Salvia* subgenus *Calosphace* is distributed exclusively in America, although some of its species have been naturalized in other parts of the world. *Calosphace* is a group with about 600 species (Santos 1995), more than 50% of them are distributed in México and 75.5% of the Mexican *Calosphace* are endemic to the country, where they thrive mainly in montane forests (Martínez-Gordillo *et al.* 2013). Oaxaca is the state with the greatest biological diversity in Mexico due to its latitudinal position and the uneven orography of its territory, which provides a great diversity of microenvironments (García-Mendoza 2011). In this state, at least 84 species of *Salvia* can be found (García-Peña &

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Medina 2011), nine of which belong to the section *Uliginosae* (Epling) Epling (1939: 54). This section includes 28 Mexican species (Turner 2009) that are mostly herbs, with the posterior lobe of the calyx 3-mucronate, 5–7-veined, blue corollas, a more or less deltoid, geniculate gubernaculum and a flattened pubescent style, widened at apex (Epling 1939). Among the collections made for the Tehuacán-Cuicatlán Flora project, which includes parts of the states of Puebla and Oaxaca, some unidentified specimens were determined as members of *Salvia* sect *Uliginosae* based on morphological characters, but could not be referred to any of the current species in that group. Hence, this taxon is distinguished as a new species and differs from the other species of *Uliginosae* by the presence of a dense and compact inflorescence, with a peduncle longer than the rachis, and persistent, broadly ovate bracts [decussate, resembling those of the genus *Tetramerium* Nees (1844: 147; Acanthaceae)], and a blue corolla with the inferior lobe at least three times longer than the posterior lobe.

Taxonomy

Salvia tetramerioides Mart.Gord., Fragoso & García-Peña, *sp. nov.* (Fig. 1)

Species insignis inflorescentiis suis a speciebus nobis notis bene distincta; differt pedunculis 4.2–10 cm longis, rhachidibus 0.6–1.9 cm longis, bracteis decussatis ample ovatis, ciliatis, floribus duobus per nodum, corollarum labio antico 2.2–3.4 mm, *labio postico 8.4–8.9 mm.*

Type:—MEXICO. Oaxaca: Zapoquila, Montaña Verde, ladera sur del Cerro Chicamole, norte de Guadalupe Membrillos, 1 October 2001, *Tenorio & Kelly 21182* (Holotype MEXU!).

Annual herbs, erect stem, 30–40 cm tall, slightly branched; stems pilose with multicelullar trichomes. Leaf blades triangular-lanceolate to lanceolate, 1.7–3.5 × 0.8–1.4 cm, apex acute, margin serrate, base cuneate, upper surface hirsute, lower surface pubescent with appressed hairs, mostly on the nerves, brown spherical glands; petioles 0.1–0.8 cm long. Inflorescences terminal, spiciform, dense, 10.2–14 cm long; peduncle (4.2–) 9.5–10 cm long; rachis (0.6–) 0.8–1.9 cm; occasionally in the mature inflorescence the proximal nodes 0.6–1.1 cm apart. Bracts persistent, two per node, 5–9.1 × 4.1–6.9 mm, decussate, widely ovate, upper and lower surfaces glabrous, apex caudate, margin entire, densely ciliate with multicelullar trichomes, 1–1.5 mm long, base cuneate, purple. Flowers 2 per node; pedicel 1 mm long or less, erect, hirsute. Calyx bilabiate, green, tube 4–5.6 mm long, pilose with appressed hairs, mostly on the nerves, glabrous within; posterior lip 2–3 mm long, 5-veined, 3-mucronate, the middle tooth longer than the laterals, margin ciliate; anterior lip 2 mm long, shorter than the posterior, apex acuminate, margin ciliate. Corolla lilac or blue, tube 3.9–5.3 mm long, straight, the same size or slightly longer than the calyx tube; posterior lip 2.2–3.4 mm long, hirsute, with brown spherical glands, anterior lip 8.4–8.9 mm long, trilobate at the apex, extended. Stamens included in the posterior lip, filament and connective 1.7–2.6 mm long, connective geniculate, with a dorsal tooth near the joint with the filament, ventral surface of the gubernaculum with minute trichomes, geniculum lobate at the apex; filament ending in a tooth; thecae 1.2–1.5 mm long. Nectary horn shorter than the mericarps; style bilobate, 6.4–6.9 mm long, dorsally pubescent, posterior branch at least three times longer than the anterior branch, the anterior branch short and laterally flattened. Mericarps ovoid, 1.6 × 0.9 mm, smooth, brown.

Distribution, habitat and phenology:—*Salvia tetramerioides* is endemic to Zapoquila, in Oaxaca. It has been collected in oak forests and mixed *Quercus*-*Juniperus* forests at 2000 to 2310 m elevation. Flowering and fruiting from September to November.

Etymology:—*tetramerioides* denotes the morphological similarity of the inflorescences of the new *Salvia* species to the inflorescences of the species of *Tetramerium* from the Acanthaceae family.

Discussion:— The most notorious feature of the new species are the broadly ovate floral bracts in a congested disposition, that resemble those of the species from section *Membranaceae* (Bentham) Epling (1939: 143), which are reniform and persistent. Nevertheless, when examined in detail, it is noted that the new species has characteristics that are not found in the species of section *Membranaceae*, such as the posterior lip of the calyx 3-mucronate and a laterally flattened style; this combination of characteristics are distinctive of the taxa from section *Uliginosae.* Some of the most important characters that were used for species identification in this section are the habit of the plant and the presence of subterranean structures for perennation. Within section *Uliginosae* two series were recognized by Epling (1939), the first one comprises species with ovate or obovate leaf blades and petioles shorter than 1 cm long; on the other hand the other series contains species with deltoid, rhomboid or linear leaf blades and petioles of 0.8–2 cm long (Epling, 1939).

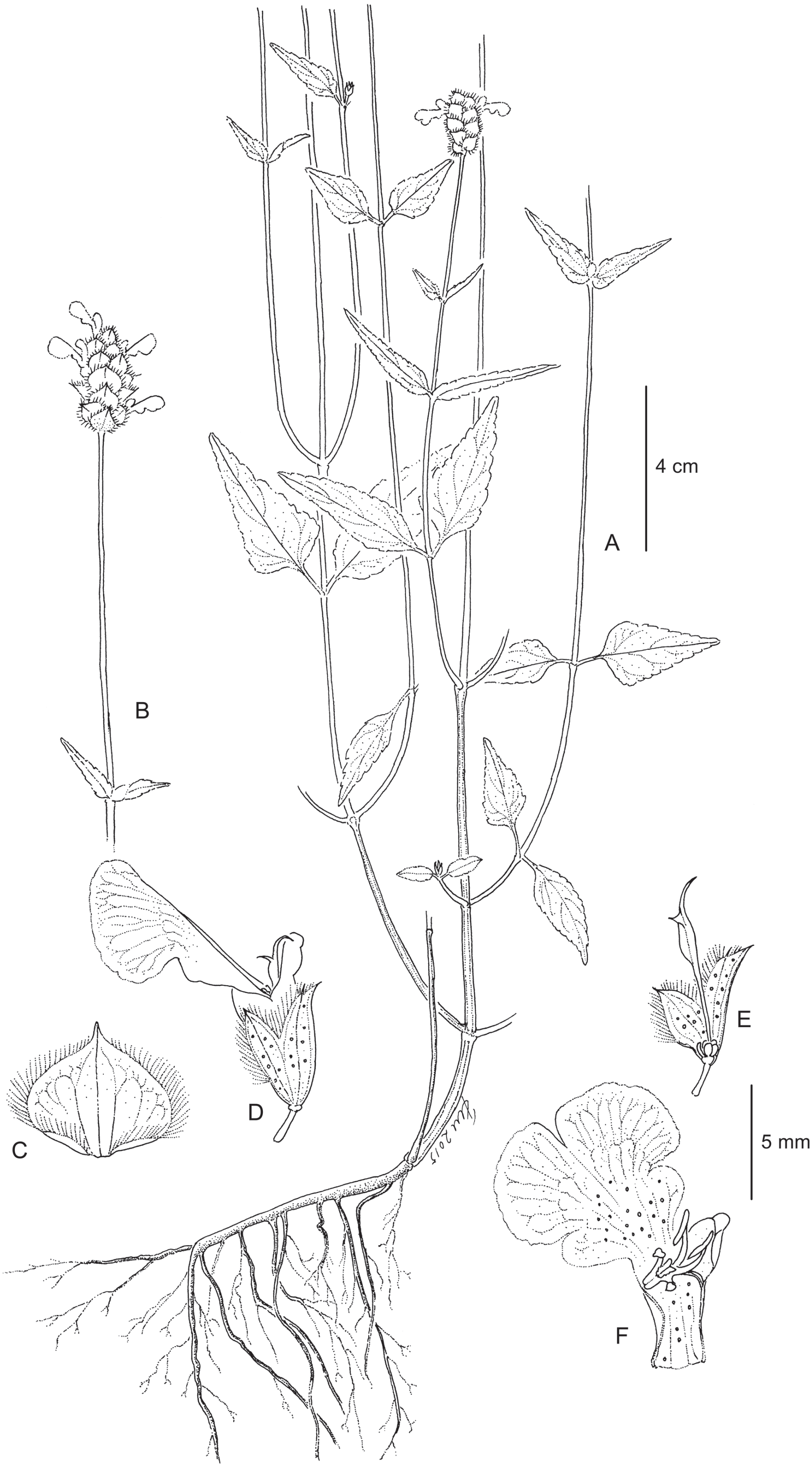

FIGURE 1. *Salvia tetramerioides*. A) Plant with branches and inflorescence. B) Inflorescence with peduncle. C) Bract. D) Lateral view of the flower. E) Dissected calyx with style, gynobase and nectary horn. F) Dissected corolla with gubernaculum and staminodes. Illustration drawn from *Tenorio et al. 18100* (MEXU) by Ramiro Cruz Durán.

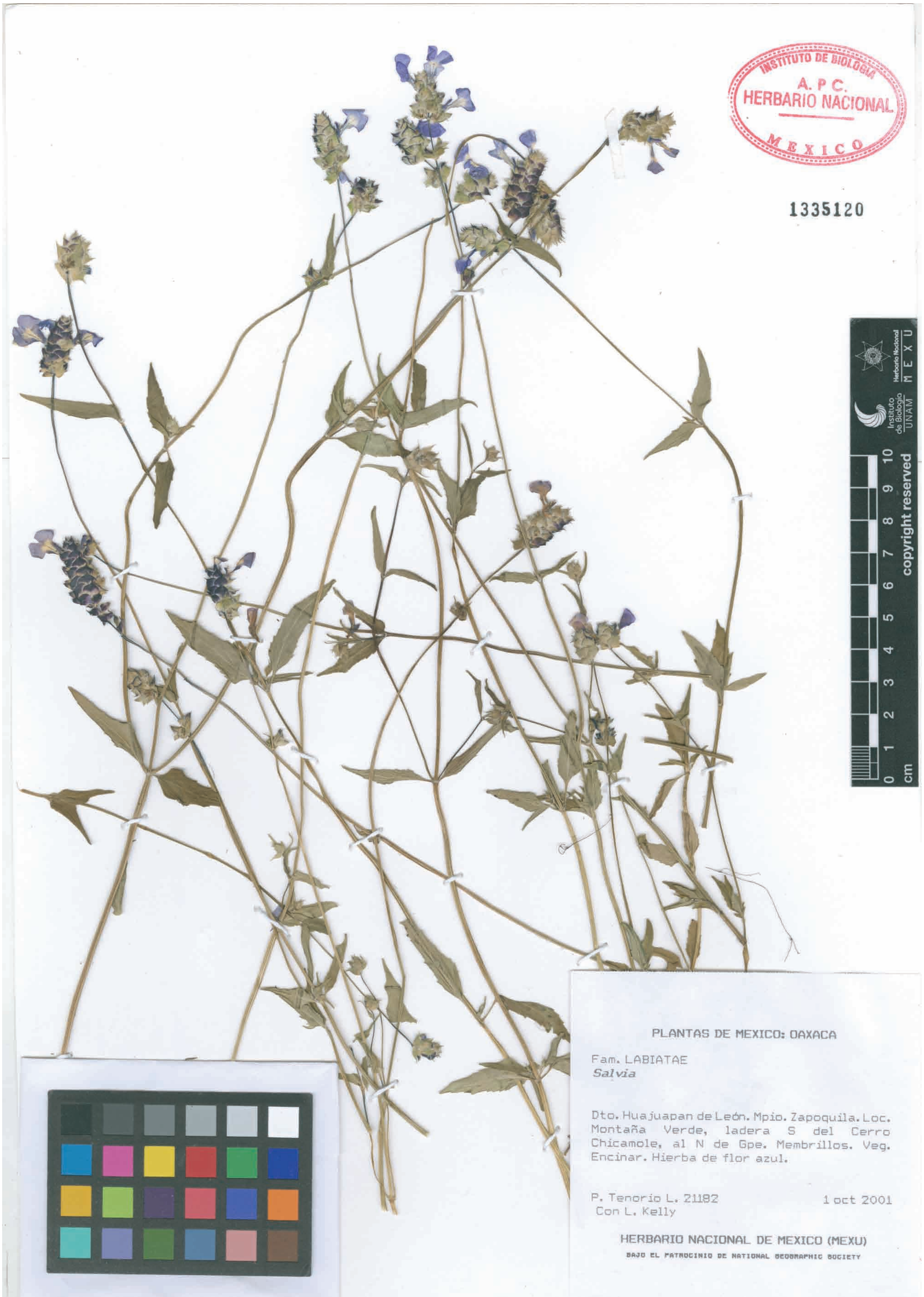

FIGURE 2. *Salvia tetramerioides*, scanned image of the holotype at the MEXU herbarium.

TABLE 1. Comparative table between *Salvia tetramerioides* and four morphologically similar species of section *Uliginosae*.

Character	*S. tetramerioides*	*S. pusilla*	*S. laevis*	*S. setulosa*	*S. prunelloides*
Life form	herb	herb	herb	suffruticose	herb
Stem	erect	postrate	erect	erect	procumbent
Duration	annual	perennial	perennial	perennial	perennial
Plant size (m)	0.3–0.4	0.15–0.35	0.1–0.5	0.3–1	0.3
Underground perennation structures	absent	present	present	unreported	present
Pubescence on the stem surface	pubescent	setose	glabrous	setose	pubescent
Glandular trichomes on the stem surface	absent	present	absent	absent	absent
LEAF					
Size (cm)	1.7–3.5 × 0.8–1.4	0.8–1.5 × 1	4–8 × 0.2–1.2	2–5 × 1–3	2–8 × 1–3.5
Shape	triangular-lanceolate	deltate-ovate	lanceolate or linear	deltate-ovate	rhombic
Apex	acute	acute	acute	obtuse	obtuse
Margin	serrate	crenulate	serrulate	crenate-serrate	crenate-serrate
Base	cuneate	cuneate	attenuate	truncate-cuneate	cuneate
Pubescence in the upper surface	hirsute	pubescent	glabrous	pubescent	pubescent
Pubescence in the lower surface	pubescent	pubescent	glabrous	glabrescent	pubescent
Petiole length (cm)	0.1–0.8	0.3–0.7	0–0.3	0.05–0.2	0.05–0.15
INFLORESCENCE					
Peduncle size (cm)	4.2–10	2.6–8.2	6.6–16.4	3.5–10.5	6.5–9.8
Length (cm)	10.2–14	4.5–8.6	9.2–21.2	12.2–25.6	12.5–16
Distance between nodes (cm)	0.6–1.1	0.5–2.8	1.7–4.2	0.5–3.6	0.2–2.5
Number of flowers per node	2	1–3	3	4–8	3–6
BRACT					
Length (mm)	5–9.1	2.1-3.6	8–12	1.6–6.5	4.5–9
Shape	broadly ovate	ovate	ovate	ovate	ovate
Duration	persistent	persistent	deciduous	late-falling	deciduous
Pubescence	glabrous	glabrescent	glabrous	glabrous	pubescent
Apex	caudate	acute	caudate	caudate	acute
FLOWER					
Calyx length (mm)	4–5.6	3–5	5–6.5	6–10	5–8
Corolla color	blue or lilac	blue or purple	blue	blue	blue
Corola tube length (mm)	3.9–5.3	3.5–4	5–7.5	6.5–8	6.5–8
Length of the posterior corolla lip (mm)	2.2–3.4	2.5–3	3–4.5	5–5.5	5–6
Length of the anterior corolla lip (mm)	8.4–8.9	4.5–5	8–10	7–8	10–13

Following these criteria, *Salvia tetramerioides* can be placed in the second series with *S. laevis* Bentham (1833: 251), which shares with the new species the shape of the leaf blade. Albeit it differs from it mainly by being a perennial herb (vs. annual), with the presence of subterranean structures (vs. absence), 3 flowers per node (vs. 2 flowers per node) and caducous bracts when fruiting (vs. persistent bracts in fruiting) (Table 1). Other morphologically similar species to *S. tetramerioides* are: 1) *S. pusilla* Fernald (1900: 495), that shares the presence of bracts during fruiting but differs chiefly in characters such as the presence of subterraneous perennation structures, glandular indumentum, and an acute bract apex (Table 1); 2) *S. setulosa* Fernald (1901:499) which occasionally presents bracts during fruiting but differs primarily in the presence of 3–8 flowers per node and a longer flower than the new species (Table 1); 3) *S. prunelloides* Kunth

(1818:289) that also shares the absence of glandular trichomes on the stem and pubescent lower surface of the blade; but can be differentiated by being an herbaceous perennial plant with rhomboid leaves and the presence of 3–6 flowers per node, with bracts absent during the fruiting stage (Table 1).In the phylogenetic tree obtained by Jenks *et al.* (2013) section *Uliginosae* is polyphyletic; hence, our decision of placing *S. tetramerioides* within this section is provisional, since it is evident that in the near future a complete reevaluation of Epling’s sections (Epling 1939, 1940, 1941, 1944, 1947, 1951, Epling y Jativa 1966) will be a necessary to ease the study of this species-rich subgenus. A key is proposed here to distinguish it from the species of the sect. *Uliginosae* growing in Oaxaca.

Additional specimens examined:—MEXICO. Oaxaca: Zapoquila, La Zotolera, E de Guadalupe Membrillos, 2000 m, 4 November 1991, *Tenorio et al. 18100* (MEXU!); Guadalupe Membrillos, 2310 m, 18º1’ N, 97º33’ W, 10 September 2001, *Tenorio & Alvarado 20848* (MEXU!); Rincón del Capulín, entre los cerros Quiote Blanco y La Zotolera, SE de Guadalupe Membrillos, 1 October 2001, *Tenorio & Kelly 21136* (MEXU!).

Key to the species of *Salvia* subgenus *Calosphace* section *Uliginosae* from Oaxaca, México

1.	Bracts persistent, 2 flowers per node	*S. tetramerioides*
-	Bracts early or lately deciduous, 3–6 flowers per node	2
2.	Plants mainly with basal leaves, broadly ovate	*S. nana*
-	Plants mainly with caulinar leaves with varied shapes but not broadly ovate	3
3.	Corolla tube 3.5–5 mm long, posterior lip up to 2.5 mm	*S. pusilla*
-	Corolla tube 6–8 mm long, posterior lip > 3 mm	4
4.	Leaves lanceolate, lower surface densely pubescent	*S. textitlana*
-	Leaves rhomboid, ovate, deltoid or deltoid-ovate, lower surface glabrescent, except in the veins	5
5.	Petiole 5–8 cm long, stems with appressed trichomes	*S. tricuspidata*
-	Petiole 0.5–3 cm long, stems with erect trichomes	6
6.	Stems with long glandular trichomes, both blade surfaces also with glandular trichomes	*S. villosa*
-	Stems with short or long trichomes, eglandular, both blade surfaces with eglandular trichomes	7
7.	Anterior corolla lip 10–13 mm long, blades rhomboid to narrowly ovate	*S. prunelloides*
-	Anterior corolla lip 7–8 mm long, blades deltate or deltate-ovate	8
8.	Bracts with the apex long-acuminate	*S. setulosa*
-	Bracts with the apex acute	9
9.	Blades with margins serrate-crenate; rhizomatous plants	*S. oreopola*
-	Blades with margins crenate; non-rhizomatous plants	*S. glechomifolia*

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Salvia robertoana (Lamiaceae), a new species from Oaxaca, Mexico

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Abstract

A new species of *Salvia* belonging to *Salvia* sect. *Penellia* from the state of Oaxaca is here described and illustrated. Only two species are included within this section: *S. pennelli* and the new species, *S. robertoana*. Both species are perennial herbs, with a 3-veined upper calyx lip, included stamens in the galea, late deciduous bracts, corolla tube epapillate inside, pubescent style and violet flowers. However, *S. robertoana* is distinguished from *S. pennellii* by its larger, elliptic to elliptic-oblong leaf blades (vs. blades lanceolate, oblong-lanceolate to oblong ovate) with tomentulose upper surface and pale, tomentose lower surface, and shortly decurrent base; usually longer inflorescence and shorter corolla tube and lower lip. A comparative table and an identification key of *Salvia* sect. *Pennellia* are provided.

Resumen

Una nueva especie de *Salvia* perteneciente a *Salvia* sect. *Penellia*, del estado de Oaxaca, se describe e ilustra. Sólo dos especies se incluyen dentro de esta sección, *S. pennelli* y la nueva especie *S. robertoana*. Ambas especies son hierbas perennes, con el lóbulo superior del cáliz 3-nervado, estambres inclusos en la gálea, brácteas caducas tardíamente, el interior del tubo de la corola sin papilas, estilo pubescente y flores violeta. No obstante, *S. robertoana* se distingue de *S. pennellii* por sus láminas foliares más grandes, elípticas a oblongo-elípticas (vs. láminas lanceoladas, oblongo-lanceoladas a oblongo ovadas) con el haz tomentuloso y envés pálido, tomentoso, y base cortamente decurrente; inflorescencia usualmente más larga y tubo de la corola y labio inferior más cortos. Se presenta un cuadro comparativo y una clave de identificación para las especies de *Salvia* sect. *Pennellia*.

Introduction

Salvia Linnaeus (1753: 23) is the most diverse genus of the Lamiaceae, with nearly 1,000 species distributed worldwide (Harley *et al*. 2004); as such, it has been traditionally subdivided into four subgenera: *Salvia* Bentham (1876: 1195), *Sclarea* Bentham (1876: 1195), *Leonia* Bentham (1876: 1196) and *Calosphace* (Bentham 1833: 198, 245; Epling 1939: 3). Among these subgenera, *Calosphace* has the highest species-richness, is endemic to the American Continent, and has its main center of diversification in Mexico (Jenks *et al*. 2013). Martínez-Gordillo *et al.* (2013) listed 307 species of *Salvia* for Mexico, with an endemism of 75.5%. Recently, several new species have been described (Bedolla-García & Zamudio 2015, Fragoso-Martínez & Martínez-Gordillo 2013, Fragoso-Martínez *et al*. 2015, González-Gallegos 2013, 2015, González-Gallegos & Aguilar-Santelises 2014, González-Gallegos & Castro-Castro 2013, González-Gallegos *et al.* 2013, Lara-Cabrera *et al.* 2013, González-Gallegos & López-Enríquez 2016, Martínez-Gordillo *et al.* 2016, Turner 2013), and currently the number of described species is 331, most of them belonging to *Salvia* subg. *Calosphace*. The most recent revision of *Salvia* subg. *Calosphace* was made by Epling (1939), who divided the subgenus into 91 sections. Later, several sections were described (Epling 1940, 1941, 1944, 1947, 1951, Epling & Játiva 1966, Epling & Mathias 1957), and currently 105 sections, most of them monotypic, are included in this subgenus.

In Mexico, Oaxaca is the state with the highest number of angiosperms (Villaseñor 2014), as well as the one which

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embraces more *Salvia* species. The physiography of this state, due to its geological evolution, explains the diversity of soil types and, consequently, types of vegetation (Centeno-García 2004). The combination of these features underlies the magnitude of plant richness in this state. *Salvia* is the second-most diverse genus in Oaxaca, with 84 species (García-Mendoza 2011), and an endemism of 16.6%. As part of an ongoing project of our research group on the inventory of Lamiaceae species in Mexico (Fragoso-Martínez & Martínez-Gordillo 2013, Fragoso-Martínez *et al*. 2015, Martínez-Gordillo *et al*. 2016), herbarium specimens of *Salvia* from Oaxaca, kept in the National Herbarium of Mexico (MEXU, Thiers 2016), were examined. Some specimens could not be assigned to any of the known species, and are here proposed as a new species of *Salvia* sect. *Pennellia* Epling (1939: 211). This section is distributed in the Sierra Madre Oriental in Mexico. It previously comprised only *Salvia pennellii* (1939: 2011), a perennial herb, with 3-veined upper calyx lips, stamens included in the galea, epapillate inner corolla tube, pubescent style and violet-colored flowers.

Taxonomy

Salvia robertoana Mart.Gord. & Fragoso, *sp. nov.* (Figs. 1, 2).

Salvia pennellii affinis sed foliis ellipticis vel elliptico-oblongis, laminis 8.1–12 cm longis, 3–3.75 cm latis, tomentulosis supra, albido-tomentosis subtus, breviter decurrentibus base, inflorescentiis longioribus, plerumque corollae tubo et labio infero brevioribus differt.

Type:—MEXICO. Oaxaca: Putla, Santa Cruz Itundujia: El Mirador, 9.54 km en línea recta de Santa Cruz Itundujia, 2849 m, 16º48’10”N, 97º36’33”W, 1 August 2008, *K. Velasco 3061* (holotype MEXU!).

Herbs unbranched, 0.3–0.9 m tall, stems tan brown colored, densely pubescent with short, multicellular trichomes. Leaf blades elliptic to oblong-elliptic, 8.1–12 × 3–3.75 cm, base decurrent, margin serrulate, involute, apex acute to short-acuminate, upper surface green, bullate, tomentulose, with short sparse trichomes, lower surface pale, tomentose, densely covered with multicellular trichomes, lower side involute; petioles 0.6–3.6 cm long, pubescent. Inflorescences terminal, (8–)18–30 cm long; peduncle 2–5.6 cm long, internodes up to 2.3 cm long, 6 or more flowers per node; bracts persistent at anthesis, 0.8–1.7 cm long, ovate-caudate, margin cuneate, apex caudate to shortly-acuminate, upper surface glabrescent, lower surface densely tomentose, whitish, with parallel venation. Calyx 9–11 mm long, upper lip 3-veined, densely pubescent, with simple trichomes and spherical glands on the outer surface, mainly along the veins, pubescent inner surface, with antrorse trichomes. Corolla purple, 16–18 mm long, tube 10–12 mm long, ventricose, sometimes slightly curved, papillae absent; upper lip 6–7 mm long, lower lip (4–)6–8 mm long. Stamens inserted near the mouth of the corolla, filaments 2.7–3.5 mm, connective straight, 4.5–5.2 mm, abaxially with a retrorse tooth. Style pubescent, 1.4–1.6 cm long; gland of the gynobase larger than the mericarps. Mericarps oblong, 1.5–2.1 mm, brown, smooth.

Distribution, habitat and phenology:—The new species is thus far known from the state of Oaxaca, in the region of the Mixteca Alta (Fig. 3), in Pine-Oak forest and xeric shrublands. It has been collected at 2540–2849 m elevation. Flowering and fruiting in August.

Etymology:—The specific epithet *robertoana* honors Roberto Carreño-Colorado, an enthusiast in botany who has spent time and resources supporting research on the Mexican Lamiaceae.

Discussion:—*Salvia robertoana* is a small herb, unbranched, with showy inflorescences, due to size and bluish tones of the calyx and bracts. These features, in addition to the size of the corolla, the lower lip slightly longer than the upper lip, and the presence of a functional lever mechanism, suggest that the new species fits into a melittophilous pollination syndrome (Wester & Claben-Bockhoff 2011). In Table 1, *Salvia robertoana* is compared with *S. pennellii*, which due to its morphological similarity is considered its most closely related species. Although they share traits such as the habit and duration (perennial herbs), as well as the presence of lately deciduous bracts, violet corollas with stamens inserted in the galea and an epapillate corolla tube, *S. robertoana* can be distinguished by its larger, elliptic to elliptic-oblong leaves, which are tomentose and whitish on the lower side and tomentulose on the upper side, with a shortly decurrent base, longer inflorescences, and usually shorter corolla tube. *Salvia pennellii* is distributed in northern Mexico (Fig. 3), where it has been collected in the mountainous zones of San Luis Potosí and Tamaulipas, at 1600 to 2870 m elevation, in oak forests, conifer forests and xeric shrublands, growing on dry and cold limestone slopes (José García, comm. pers.). This species is cultivated as ornamental for the beauty of its flowers and its resistance to low humidity levels (José García, comm. pers.). On the other hand, *S. robertoana* is endemic to the Mixteca Alta region of the state of Oaxaca, at elevations of 2540 m or higher, where pine and mixed forests predominate.

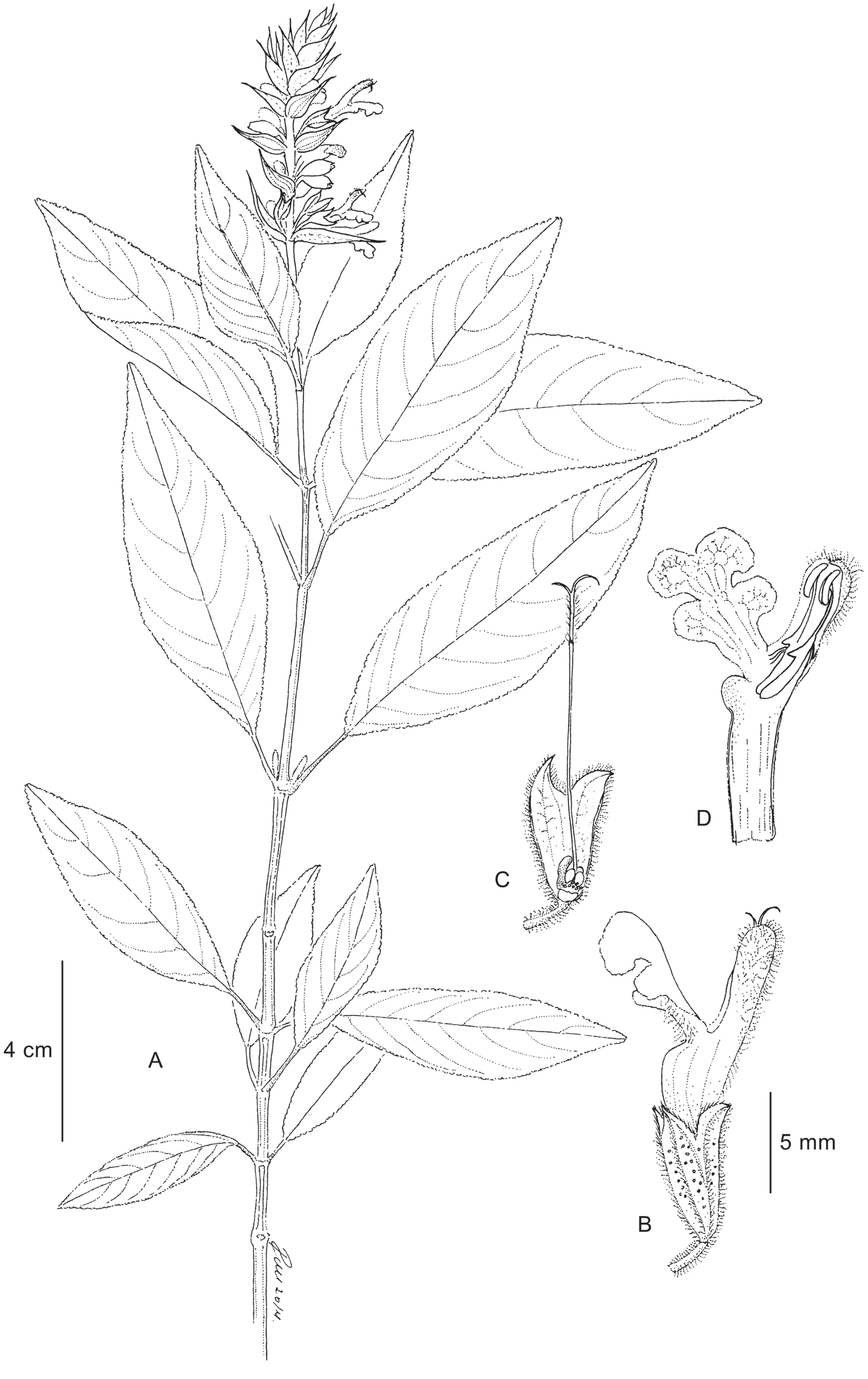

FIGURE 1. *Salvia robertoana*. A) Branch with leaves and inflorescence. B) Lateral view of the flower. C) Dissected calyx with style, mericarps and gland of the gynobase. Dissected corolla with androecium. Illustration drawn from the type by Ramiro Cruz Durán.

FIGURE 2. Scanned image from the holotype of *Salvia robertoana*.

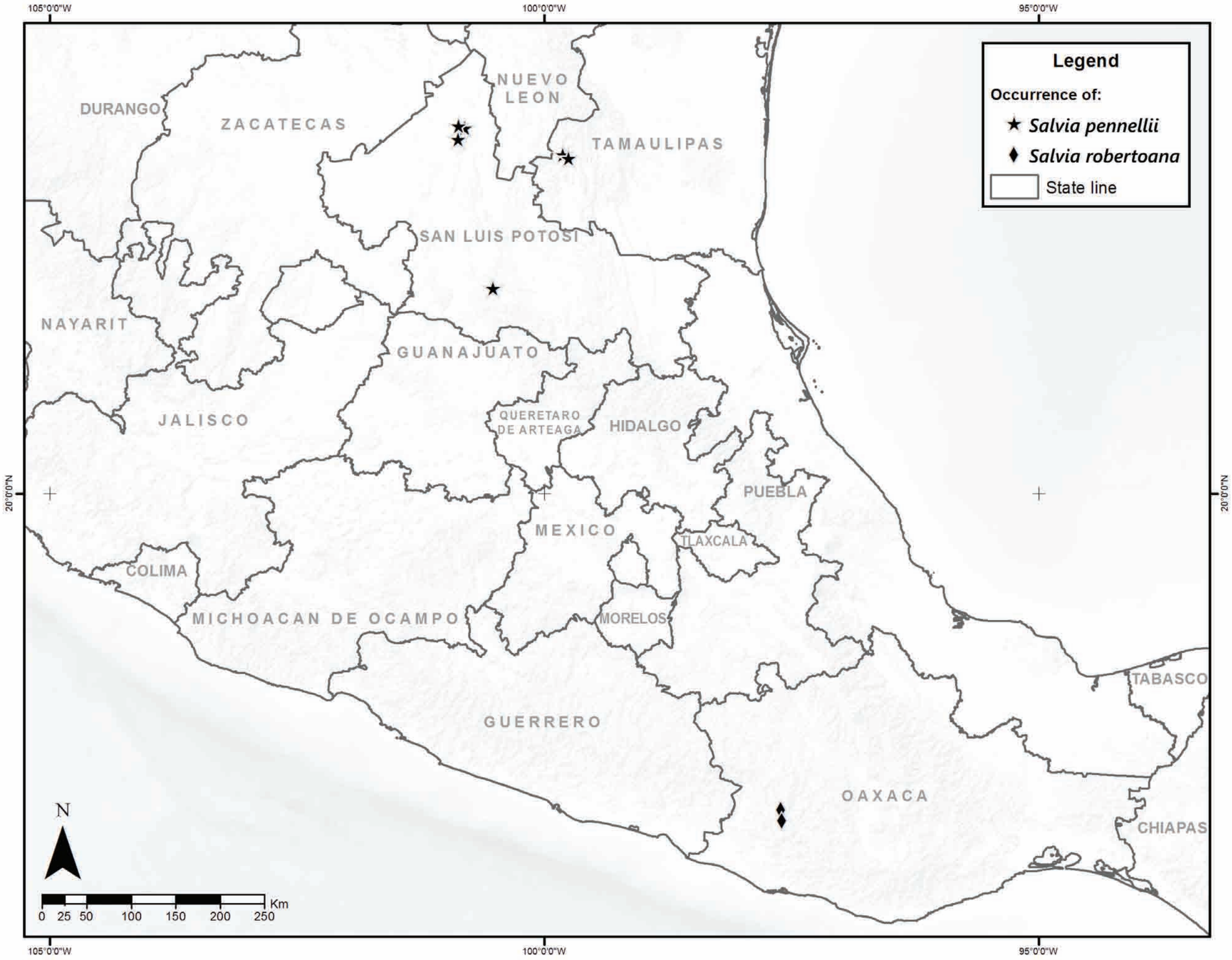

FIGURE 3. Distribution map of the species of *Salvia* subg. *Calosphace* sect. *Pennellia*.

Key to the species of *Salvia* subg. *Calosphace* sect. *Pennellia*

1. Perennial herb, branched; stem color purple, leaf blades lanceolate, oblong-lanceolate to oblong-ovate, margin crenate-serrate, lower leaf surface hirtellous, green; lower corolla lip 7–10 mm long ....*S. pennellii*

\- Perennial herb, unbranched; stem color tan brown, leaf blades elliptic or oblong-elliptic, margin serrulate, lower leaf surface tomentose, whitish; lower corolla lip 4–8 mm long ....*S. robertoana*

Additional specimens examined:

Salvia robertoana:—MÉXICO. Oaxaca: Putla, Santa Cruz Itundujia: La Torre, en el cerro Yucucasa, 2.85 km en línea recta de Independencia, 2540 m, 16º41’36.3” N, 97º35’45.8” W, 5 August 2009 (fl, fr), *K. Velasco 4143* (MEXU!).

Salvia pennellii:—MÉXICO. San Luis Potosí: Sierra de Catorce, al N del cerro Los Chatos, al E de Santa Rita y cerro La Cuchilla ca. 15 km al E de la Estación Wadley, 2 km al E de Tierras Negras, 2870 m, 9 October 1979, *J. García et al. 1310* (MEXU!); ca. 170 km N of Cd. San Luis Potosí in Sierra de Catorce on barron overgrazed hills SE of Real de Catorce near old La Presa Mine, 2500 m, 23°43’N, 100°52’W, 18 September 1980, *J. Henrickson & P. Bekey B18584* (MEXU!); 15 km al S de Cedral, 2300 m, 30 September 1954, *J. Rzedowski 4896* (SLPM!); km 55 carretera San Luis-Río Verde, 1800 m, 11 September 1954, *J. Rzedowski 4468* (SLPM!); Sierra de Catorce, en la parte superior de Arroyo el Pastor, al N de Cerro Los Chatos y al E de Santa Rita y cerro la Cuchilla, ca. 14 km al E de Est. Wadley por vía corta, 2750–2900 m, 23°35’–23°36’N, 100°52’15”W, 9 October 1979, *T. Wendt et al. 2234* (MEXU!). Tamaulipas, Bustamante: 6 km al SW de Bustamante, 1950 m, 15 August 1972, *F. Medrano et al. 4645* (MEXU!); camino de Felipe Angeles al ojo de agua de San José, 1600 m, 5 October 1985, *L. Robles et al. 1606* (MEXU!).

TABLE 1. Morphological comparison between *Salvia pennellii* and *S. robertoana*

Character	*Salvia pennellii*	*Salvia robertoana*
Habit	perennial herb, branched	perennial herb, unbranched
Size (m)	0.4-0.8	0.3–0.9
Stem color	purple	tan brown
LEAF BLADE		
Petiole length (cm)	0.5–1.2	0.6–3.6
Shape	lanceolate, oblong-lanceolate to oblong-ovate	elliptic to elliptic-oblong
Size (cm)	(2–)5–7 × 1.3–2.3	8.1–12 × 3.3–7.5
Apex	acute	acute to short-acuminate
Margin	crenate-serrate	minutely serrulate
Base	truncate to slightly attenuate	shortly decurrent
Pubescence in the upper surface	hirtellous	tomentulose
Pubescence in the lower surface	hirtellous	tomentose
Lower surface colour	green	whitish
INFLORESCENCE AND FLOWERS		
Inflorescence length (cm)	15–22	(8–)18–30
Number of flowers per node	6 or more	6 or more
Bract shape	ovate-caudate	ovate-caudate
Bract length (cm)	0.7–1.2	0.8–1.7
Calyx tube length (mm)	7.5–11	9–11
Calyx pubescence	hirtellous, long multicellular trichomes	densely hirtellous, long multicellular trichomes
Corolla tube length (mm)	(9–)–13	10–12
Corolla colour	violet, deep violet or blue	violet
Length of the upper corolla lip (mm)	5–6	6–7
Length of the lower corolla lip (mm)	7–10	4–8
Width of the lower corolla lip (mm)	4.7–6	4.5–5.2

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Salvia ozolotepecensis, *S. patriciae* and *S. sirenis* (Lamiaceae), three new species from Miahuatlán district, Oaxaca, Mexico

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Abstract

Salvia ozolotepecensis, *S. patriciae* and *S. sirenis* (Lamiaceae) from Oaxaca, Mexico, are described and illustrated as three new species. The first one is characterized by its glandular-capitate pubescence in stems, leaves and inflorescences, mostly persistent floral bracts, entire and 5-veined upper calyx lip, red corolla, epapillate inside, included stamens, and connective ornate with opposed retrorse and antrorse teeth bordering an incision. It is most similar to *S. perlonga* and *S. praestans* to which it is compared. The second can be distinguished in having ovate to ovate-deltoid leaf blades, entire, acute and 7-veined upper calyx lips, red corollas, internally ornate near the base with two slender linguiform papillae, upper corolla lip shorter than the lower one, exserted stamens and glabrous style. This new taxon is morphologically similar to the species belonging to *Salvia* sections *Cardinales*, *Charantia*, *Incarnatae*, *Flexuosa*, *Fulgentes*, *Iodophyllae* and *Pedicellata*; although, it cannot be unambiguously assigned to one of these. In contrast, *S. sirenis* clearly belongs to *S.* sect. *Scorodonia*; amongst the species therein, the new species can be differentiated by the concolorous leaves, rounded to slightly oblique at base, 6–8-flowered floral nodes, longer floral bracts, pedicels, calyces and corollas, and 5 or 7-veined upper calyx lip. Also, it represents an addition to Mexican *Salvia* with white corollas. The molecular markers ITS, *trn*L-*trn*F and *trn*H-*psb*A were sequenced for two of the three new species, and their phylogenetic position is discussed.

Resumen

Se describe e ilustra como tres especies nuevas a *Salvia ozolotepecensis*, *S. patriciae* y *S. sirenis*, provenientes de Oaxaca, México. La primera de ellas se caracteriza por la presencia de pubescencia glandular-capitada en tallos, hojas e inflorescencias, brácteas florales generalmente persistentes, labio superior del cáliz entero y 5-nervado, corola roja y epapilada en el interior, estambres inclusos y conectivo ornamentado con un par de dientes opuestos entre sí al borde de una pequeña hendidura. Esta especie es más similar a *S. perlonga* y *S. praestans*, contra las que se compara. La segunda puede distinguirse por sus láminas foliares ovadas a ovado-deltoides, labio superior del cáliz entero, agudo y 7-nervado, corolas rojas, ornamentadas en el interior con un par de papilas delicadas, labio superior de la corola más corto que el inferior, estambres exsertos y estilo glabro. El taxon nuevo es semejante por su morfología a las especies de las secciones *Cardinales*, *Charantia*, *Incarnatae*, *Flexuosa*, *Fulgentes*, *Iodophyllae* y *Pedicellata*; aunque, no puede asignarse sin ambigüedad a alguna de ellas. En contraste, *S. sirenis* pertenece con claridad a la sect. *Scorodonia*; entre las especies que abarca, la especie nueva puede diferenciarse por las hojas concoloras, redondeadas a poco oblicuas en la base, nodos florales con 6–8 flores, brácteas florales, pedicelos, cálices y corolas más largos, y el labio superior del cáliz con 5 o 7 venas. Además, ésta representa una adición a las salvias mexicanas con corolas blancas. Los marcadores moleculares ITS, *trn*L-*trn*F y *trn*H-*psb*A fueron secuenciados para dos de las tres especies nuevas, y su posición filogenética es discutida.

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Introduction

Mexico is one of the nations with the largest *Salvia* diversity (Alziar 1988, Hedge 1992, Walker *et al.* 2004), 306 species inhabit in the country and 77 % are endemic (Martínez-Gordillo *et al.* 2017). A wide proportion of these species are concentrated in Oaxaca, 94, which ranks the first amongst the other Mexican states (unpublished data). In fact, Oaxaca is the richest in overall vascular plants with 8903–10299 species (García-Mendoza & Meave 2011, Villaseñor 2016). The magnitude of the diversity in Oaxaca is explained by the spatial and historical complexity of its territory, which is translated into heterogeneity promoting diversification and providing a wide habitat spectrum to be occupied (García-Mendoza *et al.* 2004).

In recent years, several new species of *Salvia* have been described from Oaxaca: *Salvia clarkcowanii* Turner (2008: 141), *S. pericona* Turner (2009a: 260), *S. robertoana* Martínez-Gordillo & Fragoso-Martínez (2016b: 272), *S. tetramerioides* Martínez-Gordillo *et al.* (2016a: 217), *S. textitlana* Turner (2009b: 454) and *S. tilantongensis* González-Gallegos & Aguilar-Santelises (2014: 13). The above testifies that our knowledge on the diversity of the flora from Oaxaca and on the genus *Salvia* is a field still in construction. More efforts are necessary, and as long as the herbarium collections are more thoroughly curated, and remote areas are explored, new findings will come to light. Here we contribute on this aspect with the description and illustration of three new species that were discovered while verifying *Salvia* determinations of specimens from Oaxaca in GBH herbarium: *Salvia patriciae* and *S. sirenis*, and when trying to collect the first two: *S. ozolotepecensis*.

Although an updated phylogenetic framework for *Salvia* subgenus *Calosphace* (Bentham 1833: 198) Epling (1939: 4) was published recently (Fragoso-Martínez *et al.* 2018), only ca. one third of the species of this subgenus are represented in it, and the newly described Mexican taxa are underrepresented. Also, the latter study, along with previous efforts to provide a phylogeny for the subgenus (Jenks *et al.* 2013), revealed a highly artificial infrageneric classification in *Calosphace,* with only a few of Epling's (1939) sections resulting monophyletic. As a consequence, assigning new taxa to non-monophyletic sections has become controversial. In addition to the morphological comparison of the new species with similar taxa, in this study we provide their phylogenetic position, by obtaining DNA sequences for three molecular markers and analyzing them in combination with previous datasets (Fragoso-Martínez *et al.* 2018).

Materials and methods

Herbarium and field work:—The three new taxa were identified as such by using identification keys and descriptions authored by Epling (1939) for *Salvia* subgen. *Calosphace,* and the complementary literature cited in González-Gallegos *et al.* (2013), and contrasting against herbarium specimens of similar species and types of these from JSTOR Global Plants (2017). Also, we conducted two series of botanical explorations in order to collect more material of each species and to better interpret and describe morphological variation. The first collections were made in November 2015, and the second in November 2017, both in Miahuatlán district, Oaxaca. At least three duplicates were obtained for each collection number and these are distributed mainly in the following herbaria: CIIDIR, IBUG and MEXU. The specimens were prepared and processed according to the standard methods described in Lot & Chiang (1986). Silica dried foliar tissue and pictures of living plants were also taken during field exploration, except for *Salvia sirenis*, which was not found in the field and not included in the phylogenetic analysis because it could not be extracted DNA of good quality from the herbarium specimens.

Taxon sampling:—To advance our knowledge in the phylogenetic position of some of the recently described species of Mexican salvias; in addition to the three new taxa described here, we included other six species that have been described in the last three years (Table 1). These species were placed in *S.* sections *Angulatae* (Epling 1935: 67) Epling (1939: 234), *Brandegeia* Epling (1939: 314) (Bedolla-García & Zamudio 2015), *Lavanduloideae* Epling (1939: 34) (Fragoso-Martínez *et al.* 2015) and *Uliginosae* (Epling 1935: 52) Epling (1939: 54) (Bedolla-García & Zamudio 2015, Martínez-Gordillo *et al.* 2016). However, one of these species, *Salvia madrigalii* Zamudio & Bedolla-García (2018: 77)*,* could not be placed in an existing section due to its particular characteristics (Zamudio & Bedolla-García 2018). To assess the phylogenetic position of these taxa, the obtained DNA sequences were combined with the currently most comprehensive dataset for *Calosphace* (Fragoso-Martínez *et al.* 2018), which includes 245 species representing 85 sections.

Molecular methods:—DNA extraction from silica gel-dried tissue followed Fragoso-Martínez *et al.* (2017). Amplification of the ITS, *trn*H-*psb*A and *trn*L-*trn*F regions for the species in Table 1 was carried out in 15 µl reactions, following the PCR profiles and primer combinations described in Fragoso-Martínez *et al.* (2018). Sanger sequencing was performed at the Laboratorio de Biología Molecular de la Biodiversidad y la Salud, Instituto de Biología, Universidad Nacional Autónoma de México.

Sequence edition, alignment and analyses:—Sequences were assembled and edited with Geneious version 10 (http://www.geneious.com, Kearse *et al*. 2012). The alignments of each region were made using the online version of MAFFT (Katoh & Standley 2013), followed by minor manual adjustment in PhyDE (Müller *et al*. 2005). The concatenated matrix is 2246 base pairs (bp) long, from which 718 bp belong to the ITS region, 505 bp to the *trn*H-*psb*A intergenic spacer (IGS), and 970 to the *trn*L-*trn*F region. Previous to phylogenetic inference, model selection was achieved using the phylogenetic aware terrace data structure (Chernomor *et al.* 2016). The models selected were GTR+F+I+G4 for the ITS matrix, TVM+F+G4 for the *trn*H-*psb*A IGS, and TIM+F+G4 for the *trn*L-*trn*F region. The maximum likelihood analysis was carried out using the IQ-TREE algorithm (Nguyen *et al.* 2015) in the IQ-TREE web server (Trifinopoulos *et al.* 2016). The phylogenetic trees were drawn using FigTree version 1.4.2 (Rambaut 2014). To ease visualization of the phylogeny and given branch heterogeneity, the outgroup was pruned from the resulting phylogram using the phytools package (Revell 2012) in R (R Core Team 2014). Using the same software, the pruned tree was transformed to ultrametric using the "extend" method and the branch lengths were scaled to and arbitrary scale of 0 at the tips and 1 at the root of *Calosphace*. A complete version of the tree along with unmodified branch lengths can be consulted in Supplementary Material 1.

TABLE 1. Newly sequenced species of recently described Mexican salvias.

		GenBank accession numbers		
Taxon	**Voucher information**	**ITS**	***trn*H-*psb*A**	***trn*L-*trn*F**
Salvia calderoniae	*S. Zamudio et al. 16545* (IEB)	MH574780	MH613774	MH594469
Salvia madrigalii	*S. Zamudio et al.* 17060 (IEB)	MH574781	MH613775	MH594470
S. ozolotepecensis	*I. Fragoso-Martínez et al*. 380 (MEXU)	MH574774	MH613776	MH594471
Salvia patriciae	*I. Fragoso-Martínez et al*. 363 (MEXU)	MH574775	MH613777	MH594472
Salvia semiscaposa	*I. Fragoso-Martínez & M. Martínez Gordillo* 26 (MEXU)	MH574776	MH613778	MH594473
Salvia tetramerioides	*P. Tenorio 18100* (MEXU)	MH574777	MH613779	MH594474
Salvia xolocotzii	*S. Zamudio et al. 16450* (IEB)	MH574778	MH613780	MH594475
Salvia zamoranensis	*S. Zamudio et al. 16091* (IEB)	MH574779	-	MH594476

Taxonomic descriptions

Salvia ozolotepecensis J.G.González & Fragoso, *sp. nov.* (Figs. 1 & 2)

Differt a S. perlonga foliis ovatis vel ovato-lanceolatis (oblongo-lanceolatis), brevioribus (2.7–10.5 vs. 10–15 cm longis), bracteis floralibus perstatis vel sub-perstatis (vs. deciduis), calycibus brevioribus (7.2–9 vs. 13–14 mm longis), corollarum tubis brevioribus [9.9–12.3 vs. (13–)14.5–19 mm longis], corollarum labiis superis brevioribus (6.9–11.2 vs. 14–16.7 mm longis), corollarum labiis inferis brevioribus (6.9–10.4 vs. 18–21 mm longis), staminibus inclusis (vs. exsertis 3–4 mm) et connectivis brevioribus (12.5–13.3 vs. 20–22 mm longis).

Type:—MEXICO. Oaxaca. Miahuatlán district. Mun. San Juan Ozolotepec: 1.3–1.5 km al NW de San Juan Ozolotepec por la brecha a Santo Domingo Ozolotepec, 16.142ºN, 96.26ºW, 2229 m, 27 November 2017, *J.G. González-Gallegos, G. González-Adame, A. Casasola-González & M. Cruz-Aguilar 2317* (holotype CIIDIR!, isotypes HUAA!, IBUG!, IEB!, MEXU!, OAX!).

Perennial herb, erect to subscandent, 0.6–2 m tall; stems hirsute with glandular-capitate hairs. Leaves with petioles (0.5–)1.4–2.7 cm long, pilose with eglandular hairs; blade ovate to ovate-lanceolate, 2.7–10.5 × 1.5–5.7 cm, acute to acuminate at apex, truncate to subcordate at base, margin serrate, rugose above (especially in immature leaves), pilose in both faces, pubescence denser beneath. Inflorescence in racemes 7–25 cm long, with 3–12 floral nodes, each with 4–8(–10) flowers, the lowermost 1.3–2.3 cm apart from each other; floral axis pilose with eglandular hairs. Floral bract persistent (or the majority of them persisting after anthesis), ovate to lanceolate, 6.3–15.9 × 2.7–6 mm, apex acuminate to caudate, base truncate to cordate, margin entire, pilose. Flowers with pedicels 1.4–2.3 mm long, up to 7.9 mm long in fruit, short pilose. Calyx 7.2–9 × 3.9–5.1 mm, up to 11.2 × 7 mm in fruit, pilose with glandular-capitate hairs and hispidulous outside, short hispidulous inside, lips subequal, 1.9–2.8 mm long, the upper one entire and 5-veined. Corolla red, short pilose with the hairs dorsal and distally concentrated, upper surface of the lower lip glabrous; tube 9.9–12.3 × 3.2–4.9 mm, ventricose, straight or slightly invaginated towards the base, epapillate inside; upper lip 6.9–11.2 mm long, lower 6.9–10.4 × 6.3–9 mm (the lower usually longer than the upper one). Stamens included, filament 2.9–4.1 mm long, connective 12.5–13.3 mm long, ornate at midportion with a retrorse and antrorse teeth bordering an incision, thecae 2.5–2.8(–3.2) mm long; a pair of filiform staminodes above and behind filament insertion. Gynobasic horn 0.6–0.7 mm long, style 16.1–22.6 mm long, included (except by the branches) or up to 1 mm exserted, short pilose towards the apex, lower stigmatic branch acute and shorter than the upper one. Mericarp ovoid, 2.2–2.5 × 1.3–1.5 mm, light brown and irregularly marbled with a darker tone, glabrous and smooth.

Common name:—In both *Hunn 243* (OAX!) and *Hunn 1978* (OAX!), it is stated that this plant is known as guizh-dzing in Zapotec language.

Etymology:—The name of this new *Salvia* was coined in honor to the localities of Miahuatlán district bearing the name of Ozolotepec, especially to the town and people of San Juan Ozolotepec. On this way, we recognize the help and hospitality of them while conducting fieldwork to collect the new species.

Distribution, habitat and phenology:—*Salvia ozolotepecensis* is an endemic species from Oaxaca, Mexico (Fig. 3). The known distribution of this taxon is restricted to Miahuatlán district in the municipalities of San Juan Mixtepec, San Juan Ozolotepec and Santo Domingo Ozolotpec; although, it is very likely that it would be eventually found in nearby municipalities. It grows in pine-oak forest from 1960–2473 m elevation. Flowering and fruiting probably from August to February.

Notes:—According to the indumenta composed by simple hairs, persistent floral bracts, 5-veined calyx tube, red corolla, included stamens and non-geniculate connective, *Salvia ozolotepecensis* should be placed within *S.* sect. *Brandegeia*. However, the placement of *S. ozolotepecensis* within this section would be somewhat unusual in terms of geographical distribution since the three species in the section inhabit western and northern Mexico: *Salvia angustiarum* Epling (1939: 315) in Jalisco, Nayarit and Zacatecas; *S. blepharophylla* Brandegee in Epling (1939: 314) in Nuevo León, San Luis Potosí and Tamaulipas; and *S. oresbia* Fernald (1900: 536) in Nuevo León and San Luis Potosí; whereas *S. ozolotepecensis* is located in the southern region. From *S.* sect. *Brandegeia*, and following the identification key provided by Epling (1939), the new species is morphologically most similar to *S. oresbia*; however, it differs from this in having taller stems (0.6–2 *vs.* 0.2–0.8 m), longer leaves (2.7–10.5 *vs.* 1–2.5 cm), usually longer petioles [(0.5–)1.4–2.7 *vs.* up to 1 cm], pilose leaves above and beneath (*vs.* glabrous), shorter pedicels (1.4–2.3 *vs.* 3–7 mm long), shorter (7.2–9 *vs.* 10–16 mm) and pubescent calyces (pilose with glandular-capitate hairs and hispidulous *vs.* glabrous), and shorter corolla tube (9.9–12.3 *vs.* 14–16 mm).

In general morphology, and free from the constrictions in the identification key, *Salvia ozolotepecensis* is also similar to *S. praestans* Epling (1940: 530), the only species in *S.* sect. *Hintoniana* Epling (1940: 530), and *S. perlonga* Fernald (1900: 546), also monotypic from *S.* sect. *Nelsonia* Epling (1947: 516). *Salvia ozolotepecensis* differs from the first by its mostly persistent floral bracts (*vs.* deciduous), shorter calyces [(7.2–8.9 *vs.* 13.2–14.8(–17) mm long], shorter corolla tube (9.9–12.3 *vs.* 1–19 mm long), internally naked towards the base (*vs.* rugose or ornate with two papillae), shorter upper corolla lip (6.9–11.2 *vs.* 17–20.1 mm long), smaller lower corolla lip (6.9–10.4 × 6.3–9 *vs.* 22.4–29 × 18.3–24 mm), included stamens (*vs.* exserted by 2.1–4 mm), shorter connective (12.5–13.3 *vs.* 27.5–30 mm long), shorter style (16.1–22.6 *vs.* 40–41.2 mm long), and smaller mericarps (2.2–2.5 × 1.3–1.5 *vs.* 2.5–2.7 × 1.5–1.6 mm). It can be distinguished from *S. perlonga* in having ovate to ovate-lanceolate leaf blades (*vs.* oblong-lanceolate) and shorter (2.7–10.5 *vs.* 10–15 cm long), persistent floral bracts (*vs.* deciduous), shorter calyx (7.2–9 *vs.* 13–14 mm long), shorter corolla tube [9.9–12.3 *vs.* (13–)14.5–19 mm long], naked inside (*vs.* ornate with a couple folds inside towards the base), shorter upper (6.9–11.2 *vs.* 14–16.7 mm long) and lower (6.9–10.4 *vs.* 18–21 mm long) corolla lips, included stamens (*vs.* exserted), and shorter connective (12.5–13.3 *vs.* 20–22 mm long).

Furthermore, morphological evidence supports a stronger relationship with *S. perlonga* than to any other species including those in *S.* sect. *Brandegeia* in spite of matching sectional diagnostic characters. The peculiar connective ornamentation exhibited in the new species (opposing retrorse and antrorse teeth bordering an incision at connective midportion) is also present in *S. perlonga* as it can be seen in the corolla dissection available in the isotype kept at UC (*Salvia perlonga* UC1943339, JSTOR Global Plants 2018). This condition is uncommon in Mexican *Salvia*, perhaps only similar in some species from *S.* section *Uliginosae* (Epling 1935: 52) Epling (1939: 54), which embraces very distinctive species in terms of the trimucronate upper calyx lip, conspicuous and abundant glandular dots throughout the calyx, geniculate connective and swollen upper portion of the style.

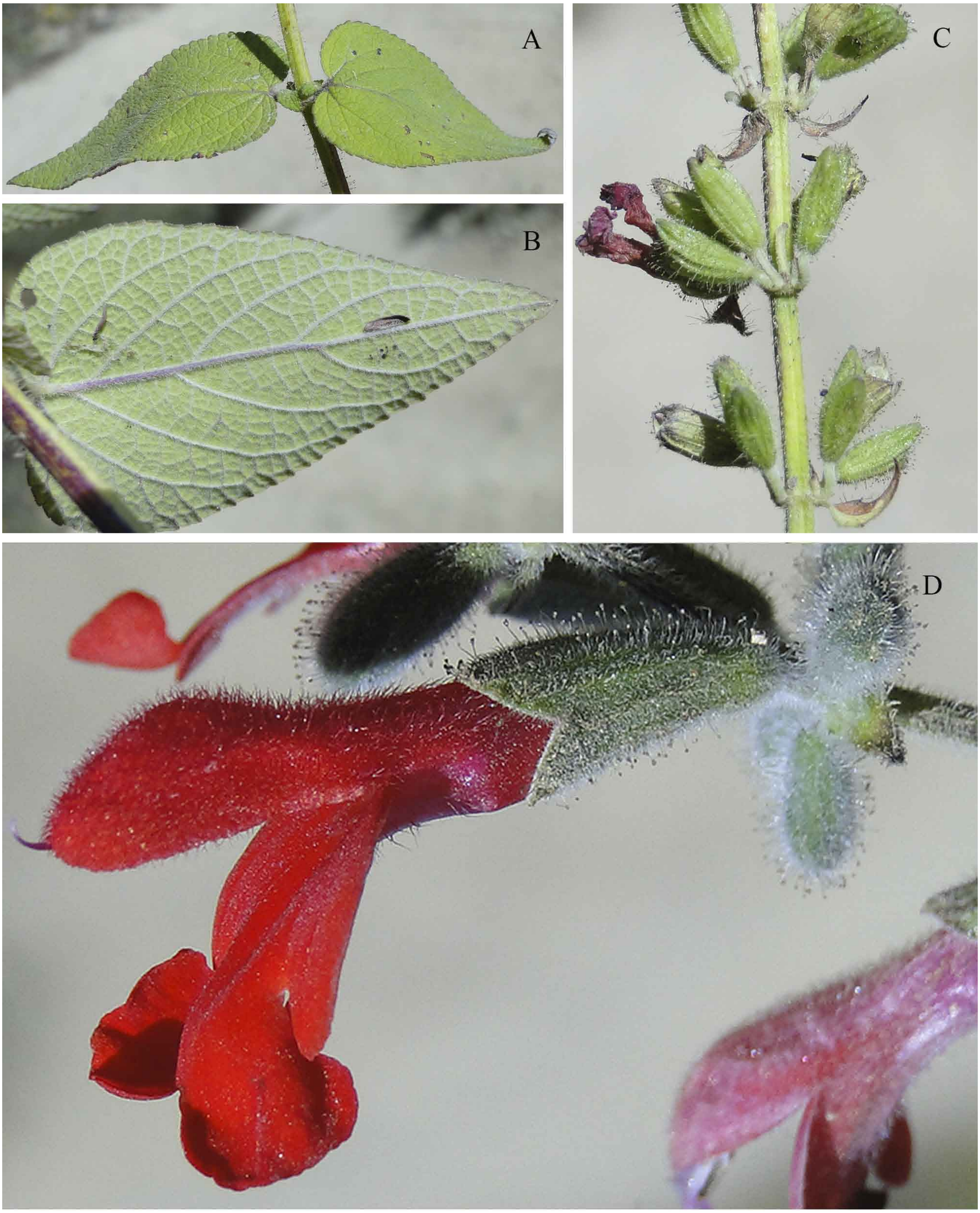

FIGURE 1. *Salvia ozolotepecensis*. **A** Leaves. **B** Leaf blade underside. **C** Inflorescence portion showing persistent floral bracts. **D** Flower (Photographs taken by G. González-Adame from type locality).

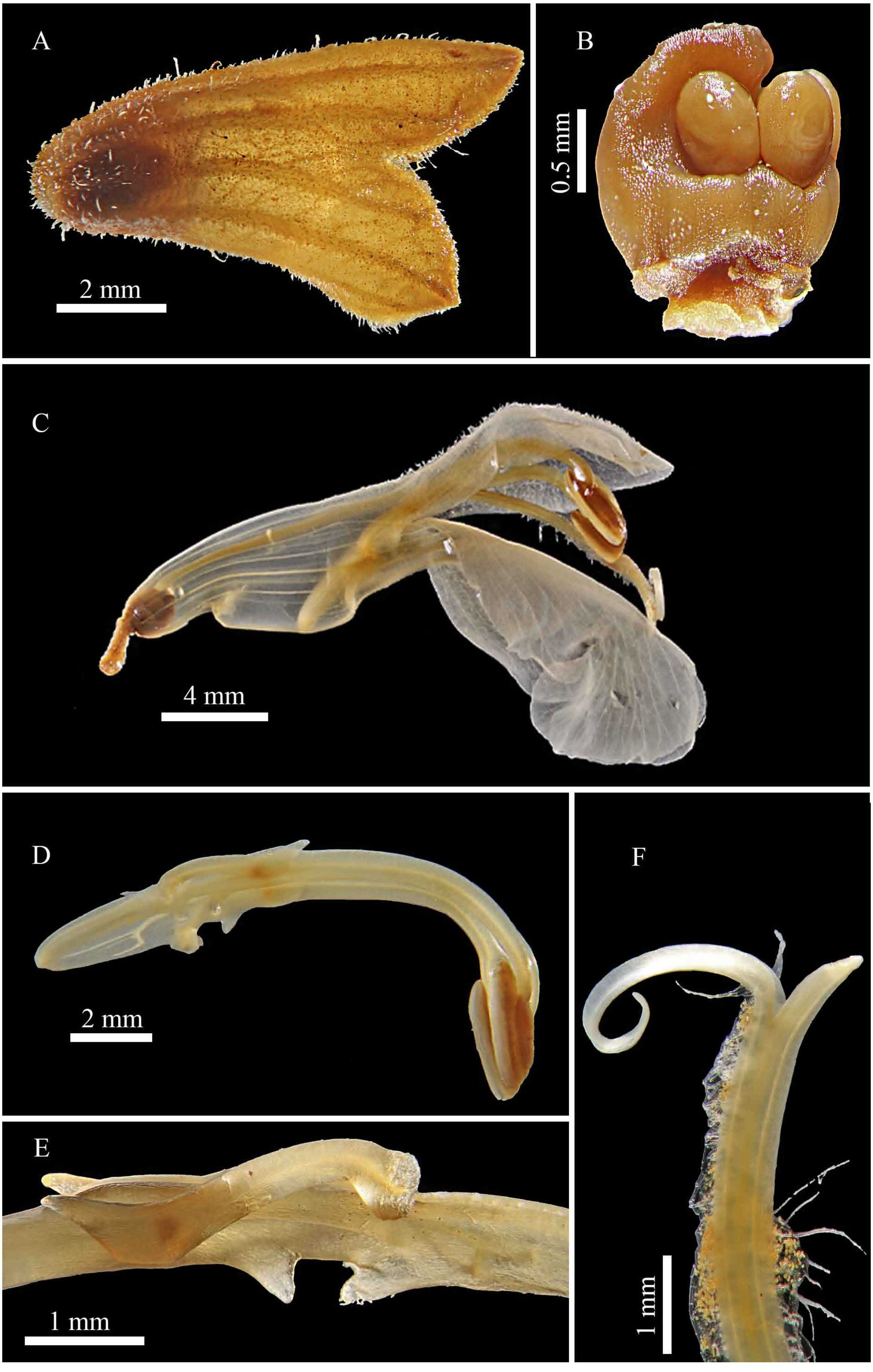

FIGURE 2. *Salvia ozolotepecensis*, floral details. **A** Calyx. **B** Ovary and gynobase. **C** Corola. **D** Stamens. **E** Stamen detail showing connective ornamentation. **F** Style apex [Photographs taken from *I. Fragoso 369* (MEXU!)].

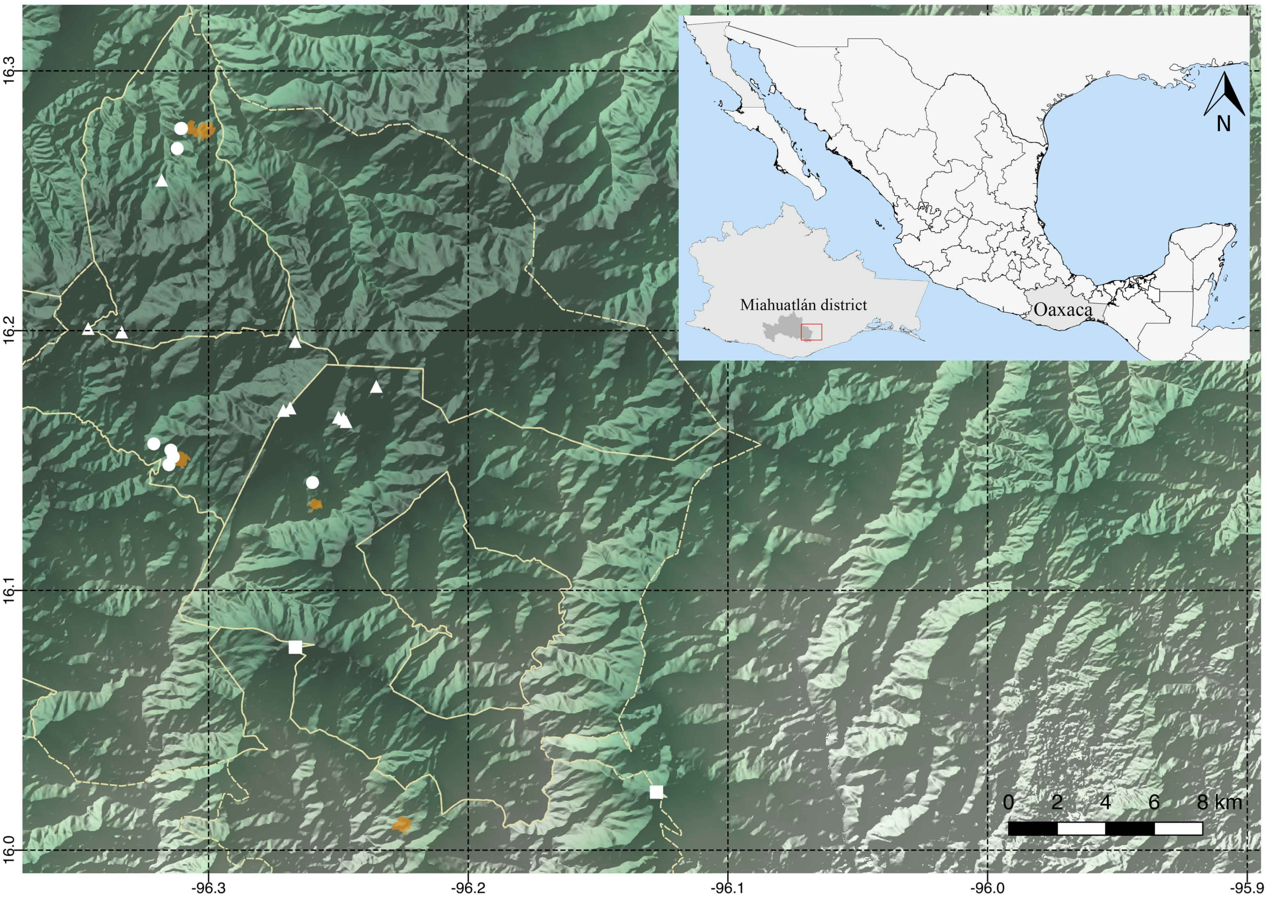

FIGURE 3. Distribution map of *Salvia ozolotepecensis* (white dots), *S. patriciae* (white triangles) and *S. sirenis* (white squares). Upper right corner showing the location of Oaxaca and Miahuatlán district; the red rectangle highlights the territory magnified in the rest of the figure. Sand lines correspond to municipalities boundaries within Miahuatlán district. Orange shadows represent the towns of, from top to bottom, San Juan Mixtepec, Santo Domingo Ozolotepec, San Juan Ozolotepec and Santiago Xanica.

Due to the above, *Salvia ozolotepecensis* cannot be assigned unambiguously to any of the recognized sections, it is more likely that in the near future, once *Salvia* classification is restructured, this new taxon together with *S. perlonga* and *S. praestans* will belong to the same section. These observations are supported by the molecular data; as shown in figure 4, both *S. perlonga* and *S. praestans* form a clade that also includes the new species (100 % Bootstrap Support; BS). This subclade is nested in the Scorodonia clade I (Fragoso-Martínez *et al.* 2018), which is a morphologically heterogeneous group where the only common characters are the presence of glandular indumentum, ovate-acuminate lately deciduous (or persistent) bracts, 5–7 veined upper calyx lip, ventricose and clearly or slightly invaginated corolla tube, longer lower corolla tube than the upper one, and pubescent style. Within this clade, the subclade in which *S. ozolotepecensis* is placed, contains red-flowered species, sister to blue-flowered. In light of these results, it would be important to re-evaluate the staminal morphology of the remaining species of the clade; as has been made with the Uliginosae clade (Barrera-Aveleida 2017), to uncover a common pattern in otherwise morphologically different species.

Additional specimens examined (paratypes):—MEXICO. Oaxaca. Miahuatlán district. Mun. San Juan Mixtepec: NE edge [from San Juan Mixtepec], 1960 m, 14 October 1996, *E. Hunn 243* (OAX!); 1.3 km WSW from San Juan Mixtepec, 16°17'N, 96°18'W, 2200 m, 12 August 1998, *E. Hunn 1889* (MEXU!); west edge of town [San Juan Mixtepec], 2050 m, 29 January 2000, *E. Hunn 1978* (OAX!). Mun. Santo Domingo Ozolotepec: 0.7 km de Santo Domingo Ozoltepec por la terracería a San José del Pacífico, 16°8'54"N, 96°18'55"W, 2343 m, 29 November 2015, *I. Fragoso-Martínez et al. 366* (MEXU!); 1.8 km de Santo Domingo Ozoltepec por la terracería a San José del Pacífico, 16°9'23"N, 96°19'16"W, 2373 m, 29 November 2015, *I. Fragoso-Martínez et al. 369* (MEXU!); La Cruz, Santo Domingo Ozoltepec, 16°9'5"N, 96°18'49"W, 2473 m, 29 November 2015, *I. Fragoso-Martínez et al. 380* (MEXU!); 1.5–2 km al NO de Santo Domingo Ozolotepec, por la brecha a San José del Pacífico, 16º9'14.3"N, 96º18'52.4"W, 2421 m, 26 November 2017, *J.G. González-Gallegos et al. 2314* (CIIDIR!, IBUG!, MEXU!).

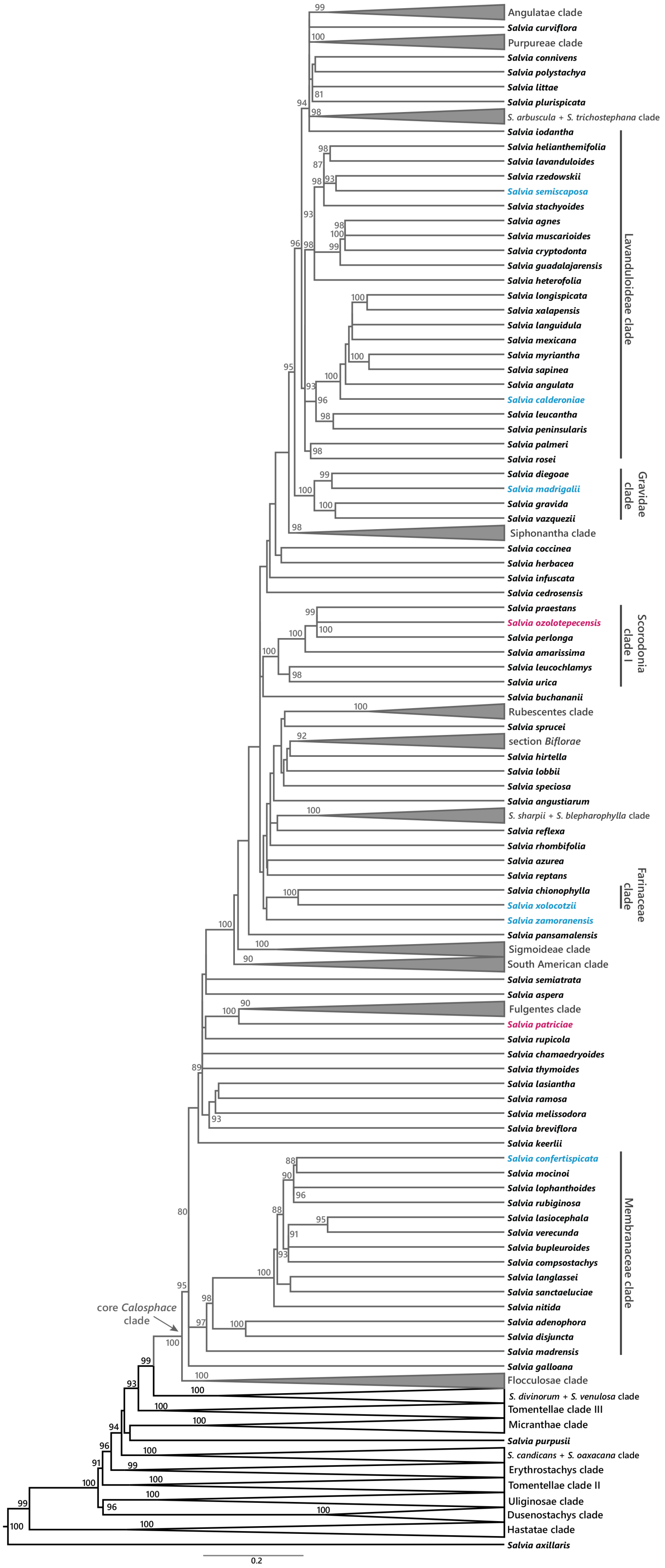

FIGURE 4. Phylogenetic relationships of *Salvia* subgenus *Calosphace* based on three molecular markers (nrITS, trn*H*-psb*A* and trn*L*-trn*F*) and an extended taxon sampling including the species of Table 1. Bootstrap values ≥80% are shown above the branches. Species names in blue and pink correspond to the new species published earlier and herein, respectively. The clades remaining unchanged with the new taxon additions have been collapsed to ease visualization. An expanded version of the phylogeny can be seen in Supplementary materials.

Salvia patriciae J.G.González & E.Martínez-A., *sp. nov.* (Figs. 5 & 6)

Salvia stolonifera primo adspectu maxima simile, sed corolla tubis angustatioribus (3.5–6.5 vs. 7–9.5 mm latis), corollarum labiis superis brevioribus [(5.9–)7.8–10 vs. 12–15.5 mm longis] et inferis brevioribus vel longitudo quasi eadem (vs. longioribus vel quasi eadem), papillis erectis (vs. recurvatis) et brevioribus [1–1.4 vs. 6–7(–10) mm longis], staminibus exsertis (vs. inclusis), connectivis longioribus [(18.4–)27.6–40 vs. 21.6–25 mm longis], et stylis prope ad medium pilosulis (vs. pilosulis infra stylos ramarum).

Type:—MEXICO. Oaxaca: Miahuatlán district. Mun. San Juan Ozolotepec: Quiexobra [Cerro del Agua], 3550 m, 15 October 1995, *G.B. Hinton 26159* (holotype CIIDIR!, isotype GBH!).

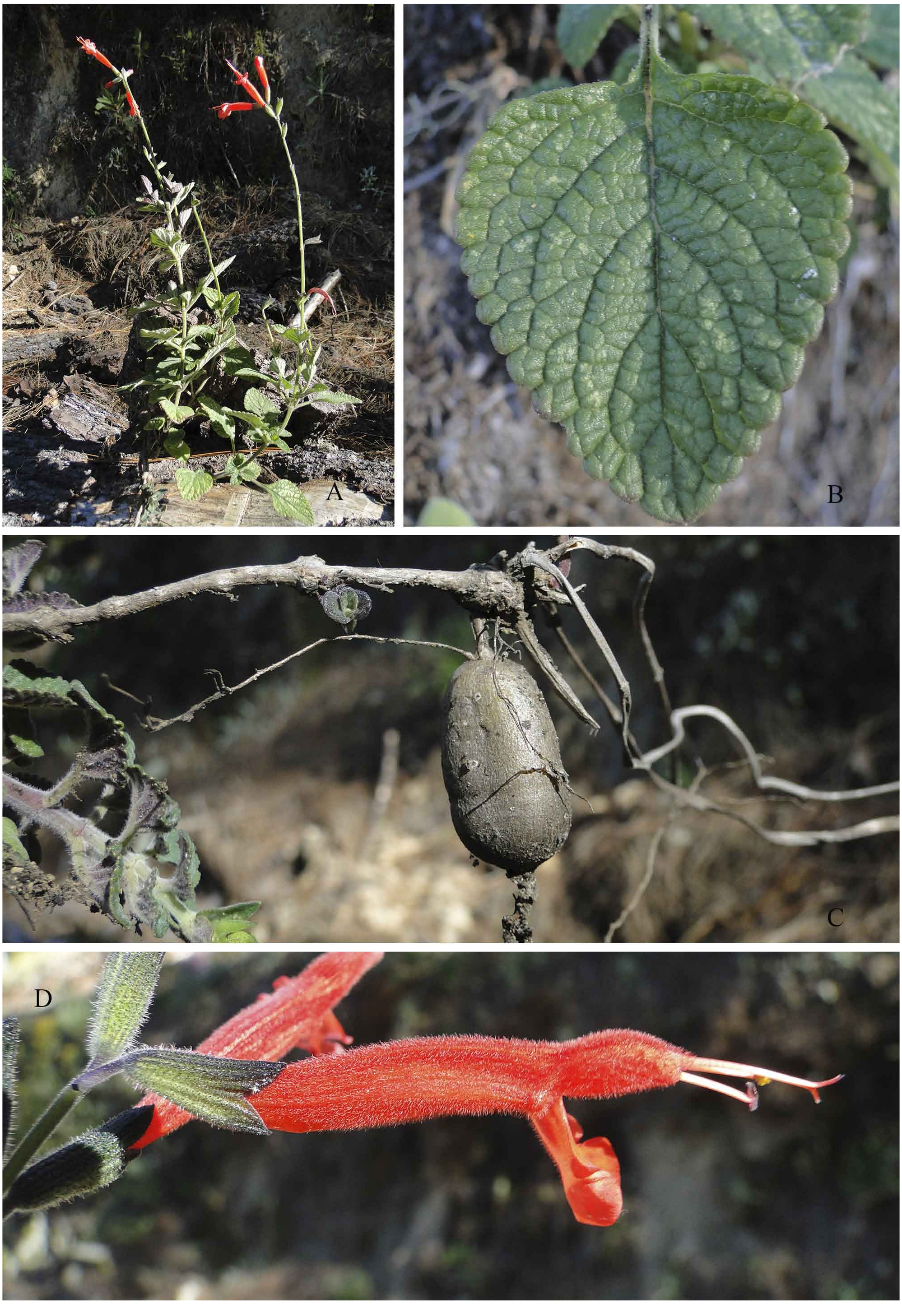

FIGURE 5. *Salvia patriciae*. **A** General habit. **B** Leaf blade, upperside. **C** Tuber and roots. **D.** Flower (Photographs taken by G. González-Adame from Cerro Quiexobra [Cerro del Agua]).

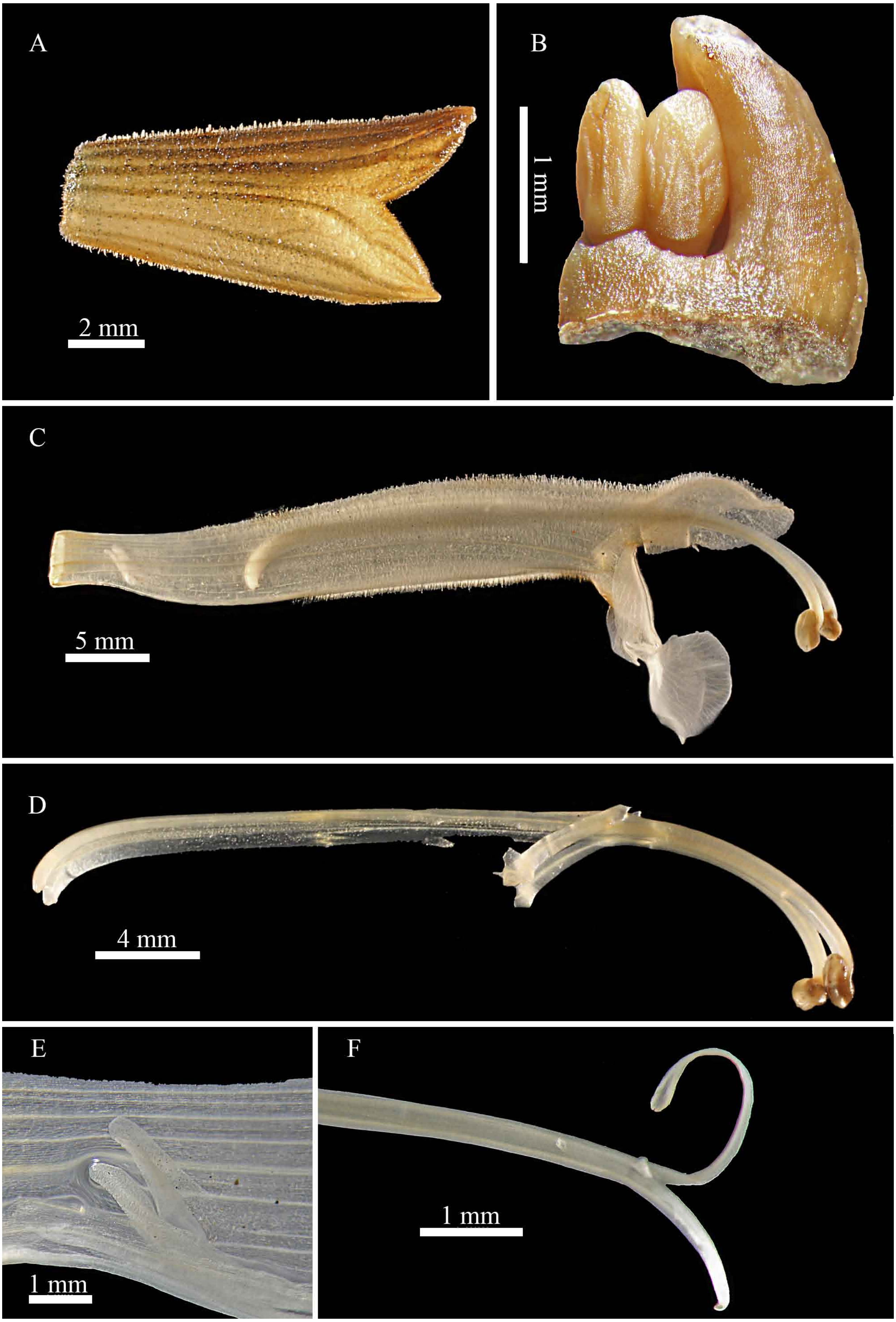

FIGURE 6. *Salvia patriciae*, floral details. **A** Calyx. **B** Ovary and gynobase. **C** Corolla. **D** Stamens. **E** Corolla tube papillae. **F** Style apex [Photographs taken from *I. Fragoso 363* (MEXU!)].

Perennial herb or subshrub, erect, 20–50 cm tall; stems short pilose; developing stolons and tubers. Leaves with petioles (0.3–)1.2–2.7 cm long, short pilose; blade deltoid to ovate-deltoid, 2.5–5.1 × 1.6–4 cm, acuminate to acute at apex, cuneate at base, margin crenate to crenate-serrate, pilose in both faces and covered with amber glandular dots, pubescence longer and denser beneath. Inflorescence in racemes (5.2–)9–22 cm long, with 3–8 floral nodes, each with 2–4 flowers, the lowermost 1.4–2.1(–4.4) cm apart from each other; floral axis with sparse simple hairs and more abundant tiny glandular-capitate. Floral bract soon deciduous, rhomboid-elliptic to lanceolate, 3.9–6.1 × 1.2–1.5 mm long, apex short caudate, base cuneate and then truncate, margin entire, pubescence as in the floral axis. Flowers with pedicels (2.9–)3.6–5.5 mm long, sparsely pilose and with dense tiny glandular-capitate hairs. Calyx (9.3–)10.2–12 × 3.2–5.2 mm, up to 8 mm long in fruit, pubescence outside as in floral axis, short hispidulous inside, lips subequal, (2–)2.5–3.4(–4.5) mm long, the upper one entire and 7-veined. Corolla red to orange-red, sparsely short pilose; tube 22.2–31.7 × 3.5–6.5 mm, thin throughout, straight at base and with two slender linguiform papillae inside (1–1.4 mm long); upper lip (5.9–)7.8–10 mm long, lower (5.5–)7–11.5 × (4.5–)7–12.8 mm. Stamens exserted by 3.2–7 mm, filament 3.4–4.5(–6.1) mm long, connective (18.4–)27.6–40 mm long, slightly curved and with and antrorse short tooth near midportion, theca (1.7–)2.3–3.1 mm long; a pair of small staminodes above and behind filament insertion. Gynobasic horn 1.3–1.5 mm long, style (30–)37.7–45 mm long, 7–10.7 mm exserted, sparsely pilose near midportion, lower stigmatic branch acute and shorter than the upper one. Mericarp ovoid, 2.8–3.2 × 1.5–1.8 mm, brownish gray and irregularly punctate with a darker tone, glabrous and smooth

Etymology:—The epithet honors Patricia Hinton, member of an outstanding family of plant collectors in Mexico, which dates back to George B. Hinton (1882–1943). They have contributed greatly to increase the knowledge of the Mexican flora, and in their collecting adventures have explored very remote and inaccessible localities. Their legacy includes 19 type specimens of currently accepted names in *Salvia*.

Distribution, habitat and phenology:—*Salvia patriciae* is an endemic species from Oaxaca, Mexico; known from several localities no more than 6 km apart from each other (Fig. 3). It grows in the mountains of Miahuatlán district in Sierra Madre del Sur from 2655–3550 m elevation. It dwells in pine forests. Flowering from June to November, and fruiting probably from late August to February.

Notes:—Following Epling (1939) identification keys for *Salvia* subgenus *Calosphace* does not lead to a clear assignment to one of the sections. The most morphologically suitable are *Charantia* Epling (1939: 141), *Flexuosa*, *Iodophyllae* Epling (1939: 141) and *Pedicellata* Epling (1949: 518) based on sharing exserted stamens, papillae inside corolla tube base, 5–7-veined upper calyx lip, upper corolla lip longer than the tube and deciduous floral bracts. However, *Salvia patriciae* differs from the species in *S.* sect. *Charantia* by having deltoid to ovate-deltoid leaf blades (*vs.* oblong to oblong-lanceolate) and straight corolla tube at base (*vs.* invaginated). The species in *S.* sect. *Flexuosae* differs by the longer leaves (6–18 *vs.* 2.5–5.1 cm long), usually longer floral bracts (5–25 *vs.* 3.9–6.1 mm long) or upper corolla lip longer or as long as the lower; besides, this section is restricted to South America. *Salvia* sect. *Iodophyllae* possesses only one species, *Salvia iodophylla* Epling (1939: 141), which differs from the new one by its 2-flowered floral nodes (*vs.* 2–4-flowered), 5-veined upper calyx lip (*vs.* 7-veined), lower corolla lip shorter than the upper (*vs.* lower longer than the upper) and entire connective (*vs.* ornate with an antrorse short tooth). Finally, *S.* sect. *Pedicellata* has also only one species, *Salvia palealis* Epling (1940: 519), which differs in having bigger leaves (11–13 × 6–9 *vs.* 2.5–5.1 × 1.6–4 cm), cordate leaf blades (*vs.* cuneate), 12-flowered floral nodes (*vs.* 2–4-flowered), 5-veined upper calyx lip (*vs.* 7-veined), and subequal corolla lips (*vs.* the upper shorter than de lower).

In general aspect, *Salvia patriciae* is more similar to the species in section *S.* sect. *Incarnatae.* At first glance, it looks like *Salvia elegans* Vahl (1804: 238) but with bigger flowers and exserted stamens. Nonetheless, more detailed observations reveal there are other deep discrepancies: entire and acute upper calyx lip (*vs.* trimucronate or caudate), corolla internally ornate with two slender papillae (*vs.* epapillate), lower corolla lip longer than the upper (*vs.* shorter than the upper).

If stamen exsertion is ignored, following the identification key leads to *S.* sect. *Fulgentes* Epling (1939: 273) when trying to identify *Salvia patriciae*. Amongst the species of this group, *Salvia lineata* Bentham (1835: 724) is the morphologically most similar. However, *S. patriciae* can be distinguished because the pilose leaves (*vs.* glabrous), shorter floral bracts (3.9–6.1 *vs.* 6–10 mm long), longer corolla tube (22.2–31.7 *vs.* 19.6–20.4 mm long), exserted stamens (*vs.* included below upper corolla lip), antrorse tooth in connective midportion (*vs.* retrorse) and style short pilose near midportion (*vs.* pilose towards the apex). If the proportion between corolla lips is also ignored (upper one as long or shorter than the lower, *vs.* as long or longer), *S. patriciae* can be related to *S.* sect. *Cardinales* Epling (1939: 295), and within this, to *S. stolonifera* Bentham (1840: 70). Both species are very similar and hard to be separated considering only vegetative characters, except by the leaves that are cordate, cordate and short cuneate to truncate (*vs.* cuneate) at base and slightly papillate beneath (*vs.* smooth) in *S. stolonifera*. However, once floral characters are compared, stronger evidence arises in order to support the recognition of two different species; *S. patriciae* differs in having thinner corolla (3.5–6.5 *vs.* 7–9.5 mm wide), shorter upper corolla lip [(5.9–)7.8–10 *vs.* 12–15.5 mm long] and this as long or shorter than the lower (*vs.* as long or longer), straight vertical (*vs.* recurved and oriented forwards) and shorter papillae [1–1.4 *vs.* 6–7(–10) mm long] inside corolla tube, exserted stamens (*vs.* included), longer connective [(18.4–)27.6–40 *vs.* 21.6–25 mm long], and style short pilose near midportion (*vs.* with the hairs disposed in the apex just before stigmatic branches).

Because of the similarity between *Salvia patriciae* and *S. stolonifera*, this was referred as the latter in the floristic inventory conducted by McDonald (2013) in Cerro Quiexobra. Later, B.L. Turner labeled the specimen *McDonald 3004* (TEX!) as *Salvia macdonaldii*, no date on the label, revealing his intention of publishing this as a new species; however, he did not validate the name.

In summary, *Salvia patriciae* is a distinctive new species sharing characters with the species of several Epling's sections, but which cannot be unambiguously assigned to one of these. However, although the results of the phylogenetic analysis (Fig. 4) do not show a clear assignment to any of the sections, it is shown a close relationship with the Fulgentes clade. This group is a heterogeneous assemblage including some species of 4 different sections: *Cardinales* [as *Holwaya* Ramamoorthy (1984: 323) according to a more recent proposal], *Flocculosae* (Epling 1935: 77) Epling (1939: 153), *Fulgentes* and *Nobiles* Epling (1939: 280) [the sampled species from this section also might belong to *Holwaya* according to dos Santos (1991)]. The species in this clade share 5–7 veined upper calyx lip, red corollas (mostly), ventricose corolla tube and pubescent style [except *S. univerticillata* Ramamoorthy ex Klitgaard (2007: 208) according to the description in *Flora Mesoamericana* (Klitgaard 2012)]. All of these characters are shared with *S. patriciae*; however, the remaining species of the clade have stamens that are included in the upper corolla lip. The differences in the level of exsertion of the stamens have been used by Epling (1939) to separate species into contrasting sections. These results could suggest that the transition from exsertion to inclusion is more common and labile, and/or that there are still a number of species that remain to be sampled. Expanding the taxon sampling in the phylogenetic framework would shed light into the evolution of floral morphological characters and their value as taxonomic diagnostic characters, such as stamen exsertion.

Due to the above, and in face that Epling's classification needs to be rearranged since it is far from natural (Jenks *et al.* 2013), no section is here designated.

Additional specimens examined (paratypes):—MEXICO. Oaxaca. Miahuatlán district. Mun. San Juan Mixtepec: San Juan Mixtepec, 5 km SW of town, 16°20'8"N, 96°19'9"W, 2900 m, 23 June 1997, *E. Hunn 1259* (MEXU!). Mun. San Juan Ozolotepec: 35 km ESE of Miahuatlán, 5 km NE of Santo Domingo Ozolotpec, Cerro Quiexobra, 16º10'N 96º15'W, 3500–3700 m, 3 October 1990, *J.A. McDonald 3004* (TEX!); Quiexobra [Cerro del Agua], 3425 m, 6 August 1996, *G.B. Hinton 26787* (GBH!); Zona de aprovechamiento forestal, camino al Cerro del Agua, 16°10'42.77"N 96°14'7.59"W, 3126 m, 27 November 2017, *G. González-Adame et al. 2830* (Herbario de la Universidad Sierra Juárez!, SERO!); camino de acceso al Cerro del Agua, 16° 9'58.04"N 96°14'52.99"W, 3141 m, 27 November 2017, *G. González-Adame et al. 2831* (Herbario de la Universidad Sierra Juárez!, SERO!); Cerro del Agua, 3.5–4 km al NE de San Juan Ozolotepec, 16º9'53.9"N 96º14'49.5"W, 3128 m, 27 November 2017, *J.G. González-Gallegos et al. 2321* (CIIDIR!, HUAA!, IEB!, MEXU!, OAX!, ZEA!). Mun. Santo Domingo Ozolotepec: Neverías, 2655 m, 3 August 1996, *G.B. Hinton 26651* (GBH!); Las Trancas, 16°11'45"N 96°16'0"W, 3516 m, 8 July 2010, *A. Sánchez M. 2906* (MEXU!, SERO); desviación a Santo Domingo Ozoltepec, 16°12'3"N 96°20'47"W, 2920 m 28 November 2015, *I. Fragoso-M. et al. 363* (MEXU!).

Salvia sirenis J.G.González & G.González, *sp. nov.* (Fig. 7)

A Salvia pericona foliis concoloribus (vs. bicoloribus), foliorum laminae basi truncatis vel leviter obliquis (vs. cordatis), floribus 6–8 in nodis floralibus (vs. 10–12), bracteis floralibus magnioribus [(10.8–)14–15.3 × 7.2–10 vs. 6–8 × 4–6 mm], calycibus magnioribus (11–12.7 × 4.5–6.5 vs. 8–9 × 3–3.5 mm), calcycum labiis superis 5 vel 7-nervatis (vs. 7-nervatis), corollarum tubis longioribus [(12.4–)13.6–15 vs. (8–)10–13 mm longis], corollarum labiis superis longioribus (6.4–7.8 vs. 4–6 mm long) et corollarum labiis inferis longioribus [(5.5–)7–9.2 vs. 4.5–6 mm long) statim dignoscenda.

Type:—MEXICO. Oaxaca. Miahuatlán district. Mun. San Juan Ozolotepec: La Sirena, summit, 2980 m, 23 October 1995, *G.B. Hinton 26385* (holotype CIIDIR!, isotype GBH!).

Shrub, erect, 1 m tall; stems puberulent and short pilose in the nodes. Leaves with petioles 7–13.4 mm long, puberulent and sparsely hispidulous; blade ovate to ovate-lanceolate, coriaceous, (4.6–)7–8 × 3.2–4 cm, acuminate to acute at apex, rounded to slightly oblique at base, margin finely serrate, both faces puberulent, sparsely hispidulous to glabrescent and covered with ocher glandular dots. Inflorescence in racemes 19–27.5(–58) cm long, with 10–14 floral nodes,

HERBARIUM OF G. B. HINTON NO. 26385

LAMIACEAE
Salvia sp.

Determined by

April 1996

HERBARIUM OF G. B. HINTON NO. 26385

Collected by Hinton et al. 1995/10/23

La Sirena, summit. 2980 m.
Miahuatlán, Oax., México.
Rocky pine and oak forest.
Fl. white. 1 m. Small colonies.
Common.

FIGURE 7. Holotype of *Salvia sirenis* kept in CIIDIR.

each with 6–8 flowers, the lowermost 3–3.2(–5.8) cm apart from each other; floral axis hispidulous and with short glandular-capitate hairs and amber glandular dots. Floral bract deciduous, ovate to ovate-rhomboid, (10.8–)14–15.3 × 7.2–10 mm, caudate at apex, truncate at base, margin entire, puberulent and scarcely hispidulous. Flowers with pedicels 2.7–5(–5.7) mm long, up to 7.8 mm in fruit, hispidulous, with short glandular-capitate hairs and amber glandular dots. Calyx 11–12.7 × 4.5–6.5 mm, pubescence outside as in the pedicel, short hispidulous inside, lips subequal, 4.8–5.4 mm long, the upper one entire and 5 or 7-veined. Corolla white, dorse and upper lip sparsely pilose, tube (12.4–)13.6–15 × 4.3–5.4 mm, ventricose at the middle, straight at the base and with two papillae inside; upper lip 6.4–7.8 mm long, lower (5.5–)7–9.2 × (6.8–)9.4–10.6 mm. Stamens inserted, filament 2.2–3.3 mm long, connective 9–10.1 mm long almost straight and with a retrorse acute tooth at midportion, theca 1.8–2.1 mm long; a pair of small staminodes above and behind filament insertion. Gynobasic horn 1.3–1.4 mm long, style 18.2–21.6 mm long, slender throughout, short pilose near the apical portion, lower stigmatic branch acute and shorter than the upper one. Mericarp ovoid, 2–2.1 × 1–1.1 mm, brown and irregularly marbled with a darker tone, glabrous and smooth.

Etymology:—The epithet make reference to the type locality of the species, La Sirena, in its Latinized form.

Distribution, habitat and phenology:—*Salvia sirenis* is an endemic species from Oaxaca, known only from two localities in Miahuatlán district 2572–2980 m elevation (Fig. 3). It inhabits pine-oak forests. Flowering and fruiting probably from September to December.

Notes:—*Salvia sirenis* matches with *S.* sect. *Scorodonia* Epling (1939: 166) in every character according to Epling (1939), Turner (2009a) and Olvera-Mendoza *et al.* (2017) circumscriptions. *Salvia sirenis* is morphologically most similar to *Salvia pericona* Turner (2009: 260) sharing ovate leaves, truncate to rounded at base, no glandular hairs in the calyx, white corollas, ventricose, upper corolla lip longer than the lower one, and short pilose style. However, *Salvia sirenis* differs in having almost concolorous leaf blades (*vs.* bicolored), rounded to slightly oblique at base (*vs.* cordate), 6–8-flowered floral nodes (*vs.* 10–12-flowered), larger floral bracts [(10.8–)14–15.3 × 7.2–10 *vs.* 6–8 × 4–6 mm], larger calyx (11.5–12.7 × 4.5–6.5 *vs.* 6–8 × 4–6 mm), 5 or 7-veined upper calyx lip (*vs.* 7-veined), longer corolla tube [(12.4–)13.6–15 *vs.* (8–)10–13 mm long], and longer upper (6.4–7.8 *vs.* 4–6 mm long) and lower [(5.5*–)*7–9.2 *vs.* 4.5–6 mm long)] corolla lips.

It is interesting that in the last two decades several species with white corollas have been described from Mexico: *Salvia diegoae* Martínez-Gordillo & Lozada-Pérez (2011: 211), *S. madrigalii*, *S. meera* Ramamoorthy ex González-Gallegos *et al.* (2012: 593), *Salvia pericona* and *S. wixarika* González-Gallegos & López-Enríquez (2016: 178). This corolla color is unusual among Mexican species, being present now in 18 of them (González-Gallegos *et al.* 2012, González-Gallegos & López-Enríquez 2016, Zamudio & Bedolla-García 2018), *i.e.* about 6 % of the total.

Additional specimen examined (paratype):—MEXICO. Oaxaca. Miahuatlán district. Mun. Santiago Xanica: Punto Trino en el Cerro Lobo, 16º1’20.2”N 96º7’39”W, 2572 m, 17 December 2003, *J. Pascual 958* (SERO!).

Phylogenetic analyses

In the present study we advanced in the knowledge of the phylogenetic position of recently described Mexican sages including those described herein. According to the results showed in Figure 4 and Supplementary Material 1, these new taxa can be divided into two groups. The first encompasses those taxa that are morphologically congruent with their phylogenetic position: *Salvia ozolotepecensis*, which is similar to *S. perlonga* and *S. praestans*, to which it is closely related; *S. confertispicata* Fragoso-Martínez & Martínez-Gordillo (2013: 2) that was described into the monophyletic *S.* sect. *Membranaceae* (Bentham 1833: 202) Epling (1939: 143); *S. semiscaposa* Fragoso-Martínez *et al.* (2015: 60), another example of a species that was described into a monophyletic section, *Lavanduloideae*; although *S. calderoniae* Bedolla-García & Zamudio (2015: 39) was described into the polyphyletic *S.* sect. *Angulatae*, its phylogenetic placing coincides with the species against it was compared in the protologue; this is the same case with *S. madrigalii*, a species that was not assigned to any section but only its morphological resemblance to *Salvia diegoae* was established.

The second group is represented by the taxa that have been difficult to assign to any of the described sections due to the presence of a combination of diagnostic characters of different sections in only one species. In this group fits one of the species described here, *Salvia patriciae*, that shares characters with at least six sections, and it is phylogenetically related with taxa from *S.* sect. *Flocculosae*, *Fulgentes* and *Holwaya* (Fragoso-Martínez *et al.* 2018). *Salvia xolocotzii* Bedolla-García & Zamudio (2015: 43) was described into *S.* sect. *Uliginosae*, but it is included instead in the core Calosphace clade. It should be noted that in the original description of *S. xolocotzii* (Bedolla-García & Zamudio 2015), the authors related the new taxa to *S.* sect. *Uliginosae* because of the resemblance to *S. galloana* Turner (2009b: 448), but at the same time they enumerated several discrepancies of these with the circumscription of the section. *Salvia galloana* is also in the core *Calosphace* clade; however, it is not closely related to *S. xolocotzii*. Instead, *S. galloana* seems to form part of one of the early-diverging lineages of core *Calosphace,* but the relationships at the backbone of this clade still lack resolution. *Salvia zamoranensis* Zamudio & Bedolla in Bedolla-García & Zamudio (2015: 48) is compared to taxa in *S.* sect. *Brandegeia* but its phylogenetic position is uncertain, suggesting a lack of sampling of taxa from the mid-center and north of Mexico.

We are aware that Epling's classification is largely artificial, but it represented and effort of summarizing the diversity of *Calosphace* into more manageable and practical units that have been useful to the botanists involved with the subgenus. However, with an increasing and continuous description of new Neotropical sages, more than ever a re-evaluation of this classification and a proposal of a new one is needed. This classification could be based on natural units such as clades, and an effort should be made to reflect the complex evolution of *Calosphace* in the New World.

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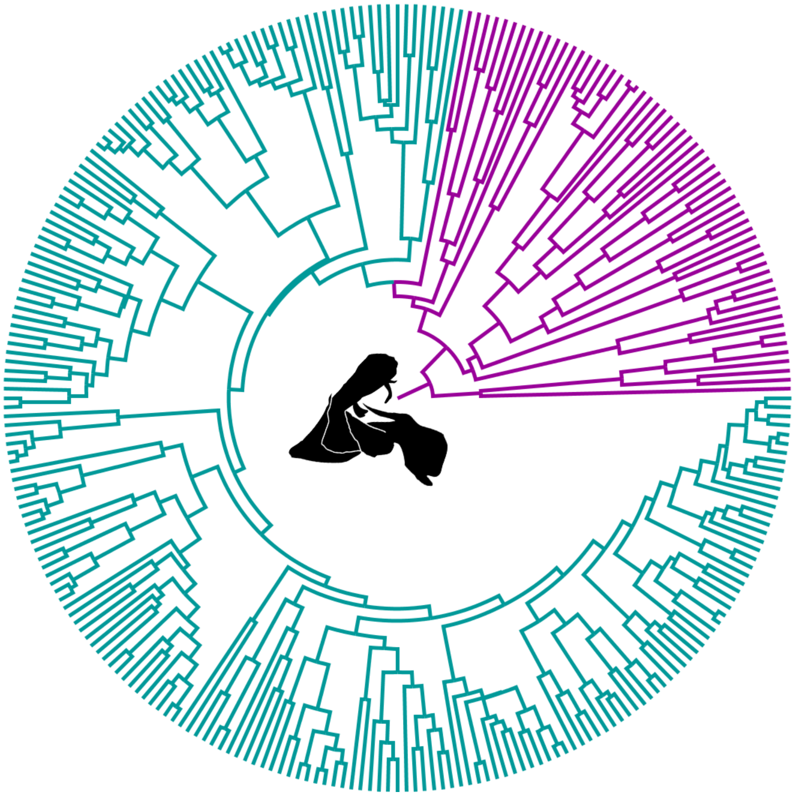
CAPÍTULO II. RELACIONES FILOGENÉTICAS DE LAS SALVIAS NEOTROPICALES



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Phylogeny of the Neotropical sages (*Salvia* subgenus *Calosphace*; Lamiaceae) and insights into pollinator and area shifts

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Abstract. *Salvia* subgenus *Calosphace* (Lamiaceae, Lamiales) is a highly diverse clade endemic to the New World. The phylogenetic relationships of *Calosphace* have been previously investigated using DNA sequences of nuclear ITS region and plastid *psb*A-*trn*H intergenic spacer, but the resulting trees lack resolution and support for many clades. The present paper reassesses the phylogenetic relationships of subgenus *Calosphace*, including a broader taxon sampling, with a special focus on representing previously unsampled sections, and using an additional plastid marker (*trn*L-*trn*F region). Our results show increased resolution and overall patterns of support, recovering ten main clades. Within core *Calosphace*, the most inclusive of these main clades, 17 new subclades were identified. Of the 42 sections for which more than one species was analysed, only 12 are monophyletic. Our biogeographic analysis identified at least twelve migrations to South America from Mexican and Central American lineages, in agreement with previous suggestions of multiple origins of South American *Calosphace* diversity. This analysis also confirmed a colonization of the Antilles by Andean lineages. The reconstruction of ancestral states of pollination syndromes showed multiple shifts to ornithophily from melittophily, and one reversal to the latter.

Keywords: ITS, Neotropics, *trn*H-*psb*A intergenic spacer, *trn*L-*trn*F region, pollination shifts, area shifts.

Introduction

Salvia is the most species-rich genus of the mint family (Lamiaceae, Lamiales). Currently, there are two phylogenetically and taxonomically valid, opposing proposals to tackle its paraphyly: subsume other five small genera in *Salvia* to achieve monophyly (Drew et al. 2017) or split the species originally included in *Salvia* in six genera (Will et al 2015; Will and Claßen-Bockhoff 2017). Of the almost 1,000 species traditionally recognized for *Salvia* distributed worldwide (Harley et al. 2004), ca. 600 belong to the New World *Salvia* subgenus *Calosphace* (Santos 1995). Among the lineages of *Salvia*, *Calosphace* is found in *Salvia* clade II, along with its sister clade, subgenus *Audibertia* (ca. 19 spp.; Walker et al. 2015), which is also endemic to the New World, and the Asian genera *Dorystaechas* and *Meriandra* (Walker et al. 2004; Walker and Sytsma 2007; Will and Claßen-Bockhoff 2017).

Calosphace is characterized by the presence of a linear staminal lever and the absence of fertile posterior thecae (Walker and Sytsma 2007; Will and Claßen-Bockhoff 2017). The species of *Calosphace* are pollinated by bees or hummingbirds, and the ornithophilous syndrome is considered as derived and having arisen at least twice within the clade (Wester and Claßen-Bockhoff 2011). According to Jenks et al. (2013), Mexico and Central America harbour most of the diversity of *Calosphace* (275 spp.), but other important centers of diversity for this subgenus are the Andean region (155 spp.), eastern South America (60 spp.), and the Antilles (45 spp.). These authors considered Mexico as an ancestral center of diversity from where the Andean and Brazilian lineages arose, colonizing South America at least in seven different events.

The most comprehensive revision of subgenus *Calosphace* to date was conducted by Epling (1939), in which he recognized 468 species distributed in 91 sections based mainly on floral morphology. Other species and sections were added in subsequent studies, rendering a total of 99 currently recognized sections (Online Resource 1). The phylogenetic relationships of *Calosphace* were assessed by Jenks et al. (2013), who analysed DNA sequences of the ITS region and the *psb*A-*trn*H intergenic spacer (IGS) of 171 species belonging to 69 sections. Their parsimony analysis detected some strongly supported clades, albeit overall resolution and support were low due to limited sequence variation among species. In their tree, *Calosphace* consists of five clades of mainly Mesoamerican species forming a grade at the base of the subgenus, and a large, poorly resolved clade formed by most of the species sampled, which they referred to as "core *Calosphace*". Many of the currently recognized sections were found to be non-monophyletic but the phylogenetic relationships showed instead a strong geographical structure, which indicates the need of a critical re-evaluation of the sectional classification of *Calosphace*, as pointed out earlier by Espejo and Ramamoorthy (1993). Although the work of Jenks et al. (2013) represents a significant step towards untangling the phylogenetic relationships within this subgenus, further effort is needed in order to achieve a natural sectional classification of the great diversity displayed by *Calosphace*.

In this work, we build upon previous studies to advance in the understanding of phylogenetic relationships within *Calosphace*, emphasising the inclusion of sections that were not sampled in previous analyses. We also increased the sample of characters by producing new sequences for three previously used molecular markers: ITS, *trn*L-*trn*F region and *trn*H-*psb*A IGS (Walker et al. 2004; Walker 2006; Walker and Systma 2007; Jenks et al. 2011, 2013). The aim of this study is to present a phylogenetic framework of *Salvia* subgenus *Calosphace* based on a more comprehensive taxonomic sampling and increased sequence data for assessing its main relationships, and for advancing our knowledge on the phylogenetic position of a number of monotypic sections, which are analysed here for the first time. Additionally, we tested the hypotheses of Jenks et al. (2013) and Wester and Claßen-Bockhoff (2011) of multiple colonization events of South America and the Antilles and multiple origins of ornithophily in *Calosphace,* respectively, using the resulting phylogeny as an explicit historical framework.

Material and methods

Taxon sampling. We analysed a total of 265 species, including 247 species of *Calosphace* that represent 85.8% of its currently recognized sections (Online Resource 1). Outgroup selection was based on a previous study (Walker et al. 2004) and comprised six species of *Salvia* subgenus *Audibertia*; eight species of *Salvia* belonging to clades I and III of Walker et al. (2004); five species of the allied genera *Dorystaechas, Lepechinia* and *Perovskia*; and *Melissa officinalis* as functional outgroup. Seventy-one species were sequenced here for the first time, 16 of them representing Mexican, South American, and Caribbean sections that had not been sampled in previous analyses. Most such sections are monotypic. Our sampling of *Calosphace* includes all the Mexican sections except for monotypic section *Iodophyllae*. Since the sectional classification is based on floral morphology, our increasing sectional coverage also implies a greater representation of the morphological diversity displayed by *Calosphace*. Other sequences analysed here were generated in previous studies and downloaded from GenBank. A list of the species sampled, including GenBank accessions and information on voucher specimens, is provided in Online Resource 2. The nomenclature used follows Ramamoorthy (1984), Govaerts and García-Peña (2007), Wood (2007), Klitgaard (2012), González-Gallegos and Gama-Villanueva (2013), Jenks et al. (2013), Martínez-Gordillo et al. (2013) and González-Gallegos (2015).

Molecular methods. The molecular markers used are three non-coding DNA regions selected on the basis of previous phylogenetic studies of *Salvia*. They include the nuclear ribosomal ITS region, plastid *trn*L-*trn*F region and plastid *psb*A-*trn*H IGS (Walker et al. 2004; Walker and Systma 2007; Jenks et al. 2011, 2013).

DNA extraction was done from herbarium or silica gel-dried specimens using the 2 × CTAB method (Doyle and Doyle 1987) modified as described in Salazar et al. (2003). PCR was carried out in 25 µL reaction volumes using the commercial mix 'Taq PCR Core Kit' (QIAGEN), adding 1 µL of 0.4% aqueous solution of bovine serum albumin (BSA) and, in the case of the ITS region, 0.5 µL of dimethyl sulfoxide (DMSO). The ITS and *trn*L-*trn*F regions we amplified using a PCR profile consisting of an initial denaturation at 94°C for 2 min, followed by 30–35 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 30 s and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. The PCR profile for the *psb*A-*trn*H IGS was as above, except for an annealing temperature of 51°C. The ITS region was amplified with primers ITS5 and ITS4 (White et al. 1990), or alternatively with primers ITS5 and 26SE (Sun et al. 1994). The *trn*L-*trn*F region was amplified either as a single fragment using primers C and F (Taberlet et al. 1991), or as two segments with the primer pairs C–D and E–F (Taberlet et al. 1991). The *psb*A-*trn*H IGS was amplified using primers trnH F (Tate and Simpson 2003) and psbA R (Sang et al. 1997); however, because these primers were problematic for some *Salvia* species, two new primers were designed: S-trnH-F2 (5'-GGCGAACGACGGGAATTGAACCC-3') and S-psbA-R2 (5'-GTGCTAACCTTGGTATGGAAGTT-3'). Sequencing was performed at the Laboratorio de Biología Molecular de la Biodiversidad y la Salud, Instituto de Biología, Universidad Nacional Autónoma de México.

Sequence edition and alignment. Sequences were edited and assembled with Sequencher version 4.10 (Gene Codes Corp., Ann Arbor, MI). Each individual region was aligned using the online version of MAFFT v. 5 (Katoh et al. 2005), followed by manual adjustment using PhyDE (Müller *et al.* 2005). For some species of which ITS and *trn*H-*psb*A IGS sequences were downloaded from GenBank, *trn*L-*trn*F region sequences were not available. These were coded as missing data and account for 7.9% of the positions in the concatenated matrix. The alignments are available from the TreeBase database (http://purl.org/phylo/treebase/phylows/study/TB2:S21390).

Phylogenetic analyses. The nuclear ribosomal (nrITS) and plastid datasets (*trn*H-*psb*A IGS plus *trn*L-*trn*F region) were analysed separately and in combination using maximum likelihood (ML). All analyses were conducted using the IQ-TREE algorithm (Nguyen et al. 2015) on the W-IQ-TREE web server (Trifinopoulos et al. 2016). The matrices were uploaded implementing the 'auto' model selection option, which evaluates 22 substitution models per partition (Chernomor et al. 2016). The models selected were TVM+G4 for the nuclear dataset, TIM+G4 for the *trn*L-*trn*F region, and TVM+G4 for the *trn*H-*psb*A IGS. Nodal support was calculated with 1,000 replicates of ultrafast bootstrap (Minh et al. 2013). The resulting phylogenetic trees were compiled and drawn using FigTree version 1.4.2 (Rambaut 2014), rooting them with *Melissa officinalis,* a member of the sister clade of *Salvia* (Drew et al. 2017). For ease of visualization, and given branch length heterogeneity, the trees were transformed to ultrametric and set to a relative time scale of 0 at the tips and 1.0 at the root using the non-parametric rate smoothing method (Sanderson 1997) implemented in TreeEdit v1.0a10 (Rambaut 2002).

Congruence test. To determine whether the nuclear and plastid loci could be combined in a single phylogenetic analysis, we used the 'Congruence among distance matrices' test (CADM; Campbell et al. 2011). The ML trees resulting from the separate analyses of nuclear and plastid datasets were converted to species pairwise distance matrices. We performed the test using the function 'CADM.global' implemented in the *ape* package (Paradis 2012) of R language (R Core Team, 2016). A Kendall's concordance statistic W value of 0 indicates complete incongruence, while a value of 1 indicates complete concordance (Campbell et al. 2011). The null hypothesis of complete incongruence among distance matrices was tested with 9999 permutations.

Ancestral area reconstruction. To evaluate the colonization hypothesis proposed by Jenks et al. (2013), we carried out an ancestral area reconstruction analysis using the 'Reconstruct Ancestral State in Phylogenies' (RASP) software on the ML tree resulting from the combined analysis, using the Bayesian Binary MCMC Method (BBM) for ancestral states (Yu et al. 2015). The BBM analysis ran 10 Markov chains for 10,000,000 generations, sampling every 1,000 generations and discarding the initial 20% trees as burn-in. State transitions were described with a Jukes-Cantor model, and among-site variation was set to 'equal'. The distribution areas analysed are the four diversity centers mentioned in Jenks et al. (2013)*:* 1) Mexico and Central America, 2) Andean region, 3) the Antilles and 4) eastern South America. Distribution data for the species was obtained from specimens deposited at the herbaria examined: CHAP, CHAPA, ECUAMZ, ENCB, FCME, JBSD, K, IEB, MEXU, OAX, QCNE, SERO, SLPM and XAL, complemented with information from relevant literature, mainly: Wood and Harley (1989), Brako and Zarucchi (1993), Rodriguez (2006), Wood (2007), Klitgaard (2012), Jenks et al. (2013), Martínez-Gordillo *et al*. (2013), Martínez-Gordillo *et al*. (in rev.), Santos (2016). Species with conflicting phylogenetic position between the nuclear and plastid ML trees (BP ≥85) were excluded from the analysis using the pruning tool in RASP.

Character mapping and ancestral state reconstruction. We estimated the ancestral states of pollination syndromes on the combined ML tree using a matrix of pollination syndromes for the species of *Calosphace* based on the classification proposed by Wester and Claßen-Bockhoff (2011) for the New World sages. Each species was coded as: melittophilous (m), ornithophilous (o) or intermediate (i). The 'intermediate' character state was assigned to all species with more than one type of pollinator. However, the intermediate character state is at least in part based on incomplete information about pollinator visits and deduced from intermediate morphological characters; thus, the results should be taken with caution. Character-state history among pollination syndromes was traced in a Bayesian stochastic character mapping approach (Nielsen 2001; Huelsenbeck et al. 2003), using the *make.simmap* function from the *phytools* R package (Revell 2012). Given a phylogeny and discrete character states for extant terminal taxa, this Bayesian method applies a Monte Carlo algorithm to sample the posterior probability distribution of ancestral states on the branches of a phylogeny with a Markov process of evolution using a model where all transition rates were different ("ARD" model) (Revell 2012). We simulated 1,000 ancestral-state histories on the combined ML tree. We used as prior distribution on the root node the actual frequencies of pollination syndrome recorded in *Calosphace* by Wester and Claßen-Bockhoff (2011), i.e. melittophilous=0.59, ornitophilous=0.31, and intermediate=0.09.

Results

Congruence between plastid and nuclear datasets. A high Kendall's concordance statistic (W) in the CADM test indicates congruence between the nuclear and plastid phylogenies (W = 0.878, χ^2= 0.0006, p = 0.001), and on that basis, both were combined in a single phylogenetic analysis. The trees from the separate analyses are available as online resources (Online Resources 3–4). The ML tree resulted from the combined analysis is shown in Figs. 1–4.

Phylogenetic relationships. Both the nuclear and plastid aligned matrices consisted of 265 taxa; the ITS matrix included 713 characters, whereas the plastid matrix had 1,499 characters, 990 corresponding to the *trn*L-*trn*F region and 509 to the *psb*A-*trn*H IGS.

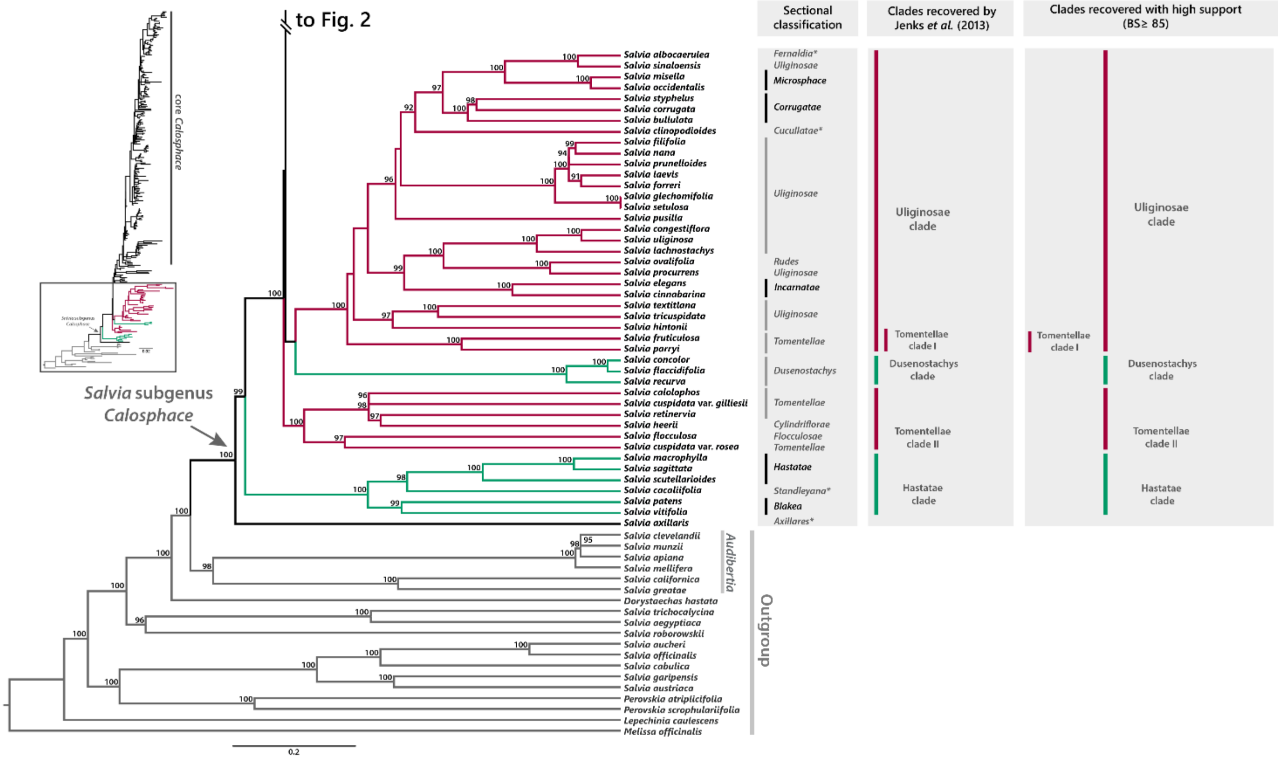

Fig. 1 Phylogenetic relationships of *Salvia* subgenus *Calosphace* based on the combined analysis of nuclear (ITS) and plastid (*trn*L-*trn*F, *psb*A-*trn*H) DNA sequences. The more-inclusive clades are marked with colours. Bootstrap values ≥85% are shown above the branches. In the first column on the right, clade names in boldface refer to monophyletic sections and an asterisk (*) indicates a monotypic section. In the third column two asterisks (**) indicate clades newly recovered and discussed in this study.

The combined phylogenetic tree showed more resolution and support than either of the separate analyses (Figs. 1–4, Online Resources 3–4), both results and discussion will refer to the combined ML tree. In the following, the phylogenetic relationships recovered in the combined analysis are described considering only strongly supported clades (Bootstrap support; BS ≥ 85);clades that are mentioned without BS values indicated in parentheses obtained a BS 100. *Calosphace* is monophyletic, with *Salvia axillaris* sister to all the remaining species of the subgenus (Fig. 1). *Calosphace* includes ten strongly-supported clades (Figs. 1–4): 1) Hastatae clade; 2) Tomentellae clade II; 3) Dusenostachys clade; 4) Uliginosae clade, including Tomentellae subclade I; 5) Erythrostachys clade (BS 92); 6) *Salvia candicans* + *S. oaxacana*; 7) Micranthae clade; 8) Tomentellae clade III; 9) *Salvia divinorum* + *S. venulosa*; and 10) core *Calosphace* (BS 99). The latter comprises 76% of the sampled species and 17 strongly supported clades (Figs. 2–4). Among these clades, the Fulgentes clade is further divided into the subclades Holwaya and Fulgentes.

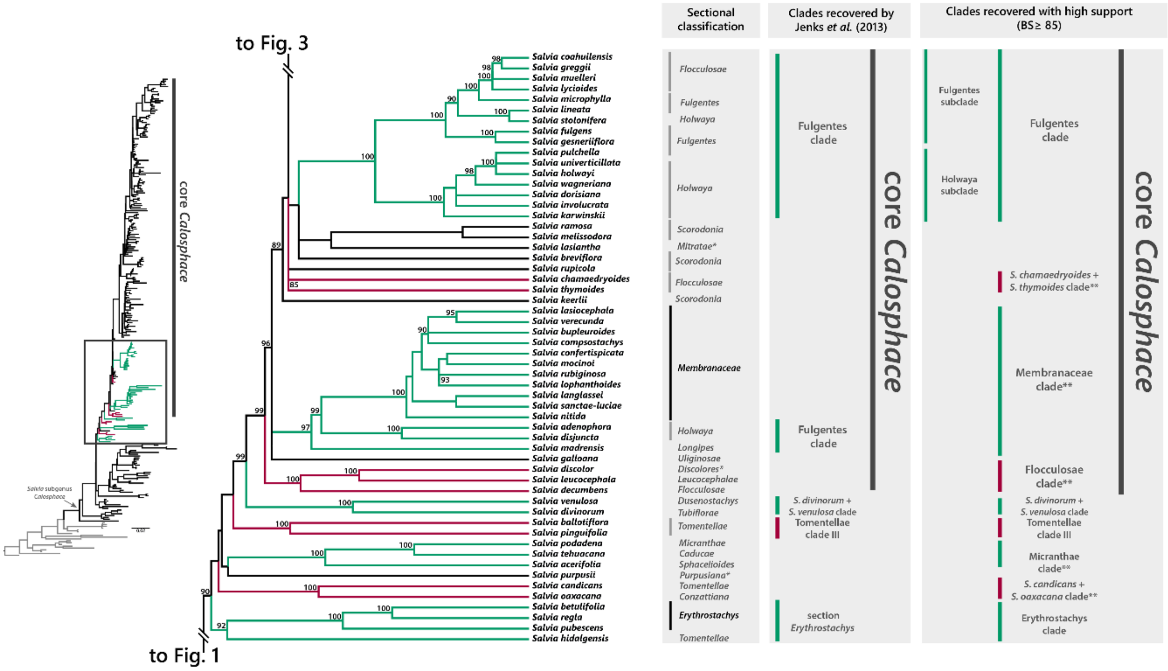

Fig. 2 Continuation of the tree in Fig. 1

Of the 38 non-monotypic sections recognized by Epling and represented in our analyses by two or more species, only 12 were recovered as monophyletic (Table 1). Except for sections *Axillares*, *Cucullatae*, *Fernaldia*, *Purpusiana* and *Standleyana*, most of the monotypic sections are nested within core *Calosphace*.

Table 1. Monophyletic sections recovered by Jenks et al. (2013) and the present study (BS≥85 in the ML tree resulting from the combined dataset).

Epling's sections (% of species sampled from the total)	**Monophyletic sections recovered by**	**Clade of inclusion**
Biflorae (100%)*	Present study	core Calosphace
Blakea (40%)*	Jenks et al. (2013)	Hastatae clade
*Corrugatae** (60%)*	Jenks et al. (2013)	Uliginosae clade
Curtiflorae (50%)*	Present study	core Calosphace
Erythrostachys (60%)*	Jenks et al. (2013)	Erythrostachys clade
*Hastatae** (42.8%)*	Jenks et al. (2013)	Hastatae clade
*Incarnatae** (100%)*	Jenks et al. (2013)	Uliginosae clade
Lavanduloideae (50%)*	Present study	core Calosphace
*Membranaceae** (84.6%)*	Present study	core Calosphace
*Microsphace** (40%)*	Jenks et al. (2013)	Uliginosae clade
Sigmoideae (33.3%)*	Jenks et al. (2013)	core Calosphace
Skeptostachys(50%)*	Jenks et al. (2013)	core Calosphace

In the first column on the right section an asterisk (*) represents monophyly supported only by nuclear ribosomal data; two asterisks (**) indicate monophyly supported by both the nuclear and plastid datasets.

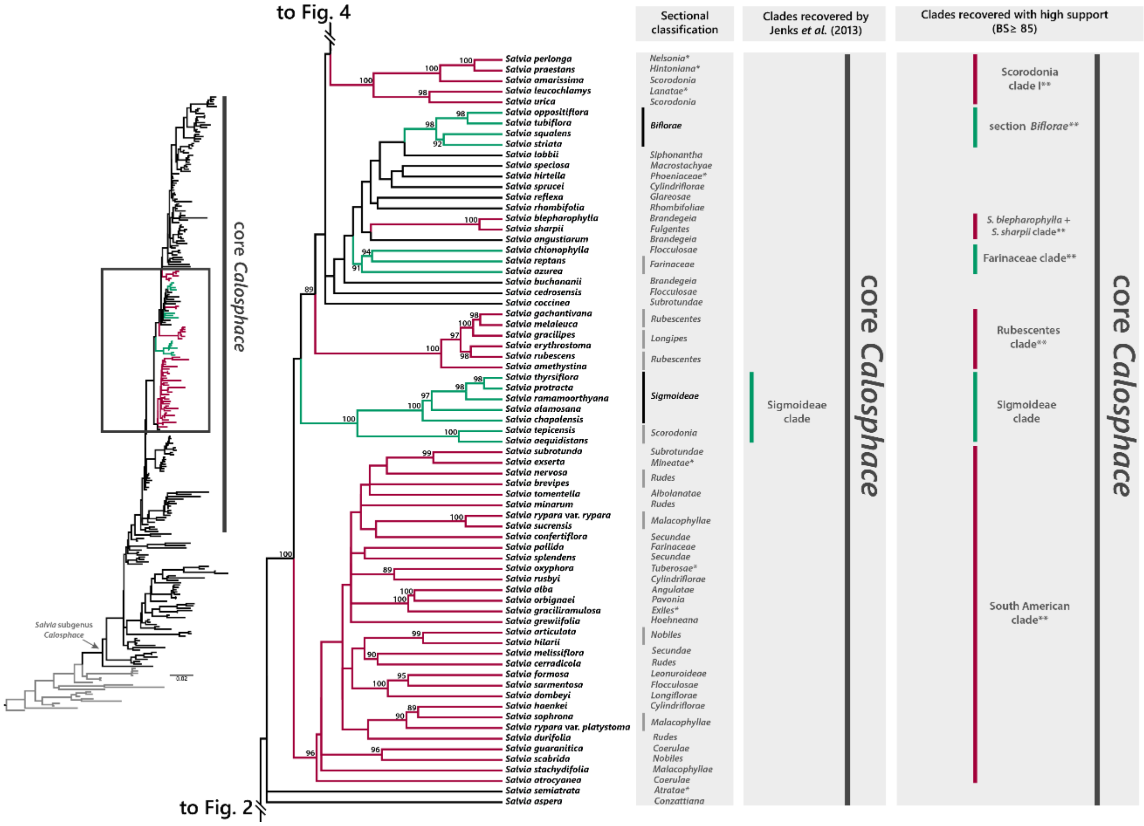

Fig. 3 Continuation of the tree in Fig. 2

Ancestral area reconstruction. The ancestral area reconstruction (Fig. 5; Online Resource 5) indicates a Mexican/Central American origin for *Calosphace* and identified at least twelve independent migrations to South America. It also suggests that there has been exchange between the Andean region and other areas, such as the Antilles and Eastern South America.

Evolution of pollination syndromes. The stochastic character mapping of the pollination syndromes showed thirteen transitions to ornithophily in the following strongly supported clades: 1) Hastatae, 2) Tomentellae II, 3) section *Incarnatae* inside Uliginosae, 4) Erythorstachys, 5) *S. divinorum* + *S. venulosa*, 6) Membranaceae, 7) Fulgentes, 8) South American clade, 9) Rubescentes, 10) Biflorae, 11) Siphonantha, 12) Lavanduloideae; and 13) Angulatae (arrows 1–13 in Fig. 6; Online Resource 6). One reversal to melittophily was found in the Fulgentes clade, involving *Salvia lycioides, S. muelleri* and *S. coahuilensis*, three melittophilous species in a subclade of a bird-pollinated clade (asterisk in Fig. 6). The estimated transition matrix among syndromes indicated that direct reversion from ornithophily to melittophily is unlikely, involving instead an intermediate polymorphic state.

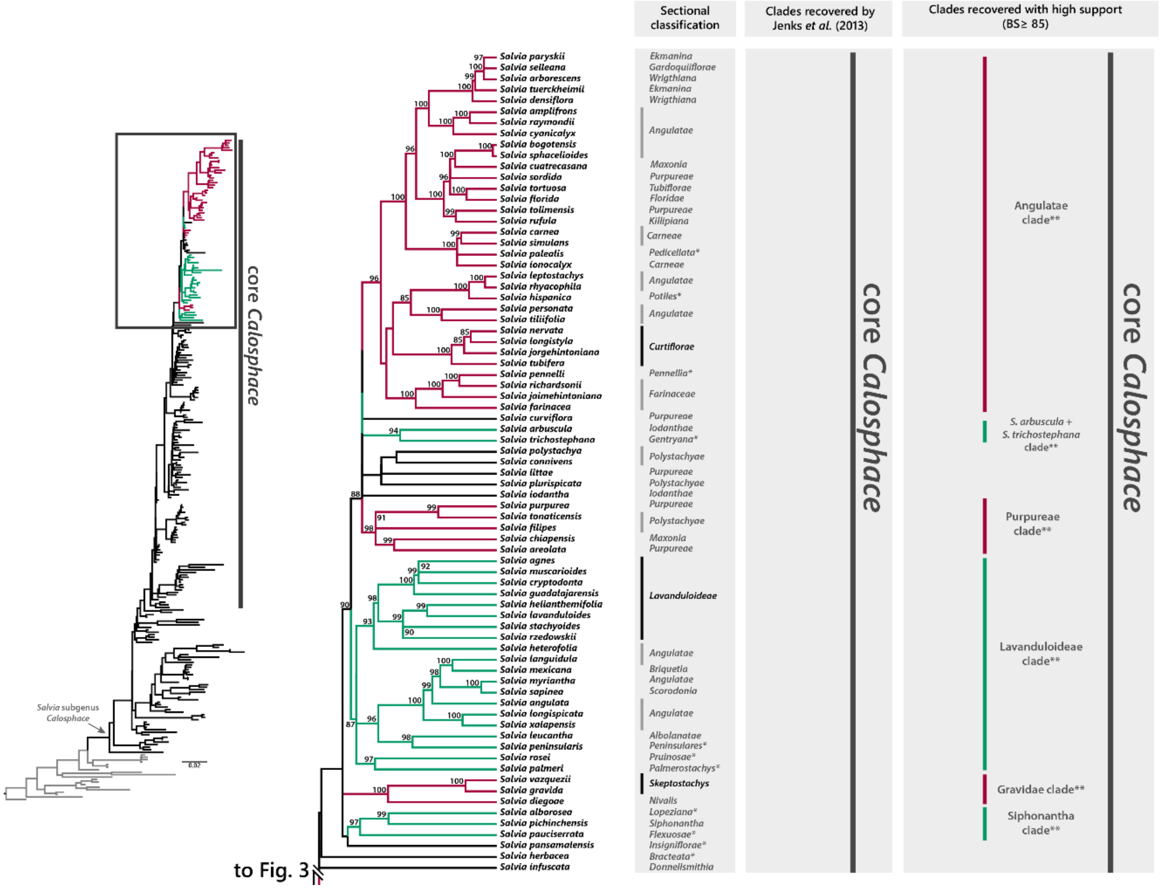

Fig. 4 Continuation of the tree in Fig. 3

Discussion

Phylogenetic relationships

The present study represents a step forward towards a sound phylogenetic framework for *Salvia* subgenus *Calosphace*. Our extended taxon sampling, incorporating the Hispaniolan taxa from the analysis of Zona et al. (2016), includes species from 18 additional sections relative to the previous study of Jenks et al. (2013), for an 86% of sectional coverage. The combination of broader taxon sampling and additional character information resulted in increased resolution and overall clade support, allowing the recognition of three new main clades and several less-inclusive clades.

Resolution within core *Calosphace* was substantially improved. This is a morphologically highly diverse clade that includes most of the species in the subgenus. Jenks et al. (2013) reported a synapomorphic 9 base-pair (bp) deletion in the *psb*A-*trn*H IGS of core *Calosphace*, which was confirmed here in all the newly sequenced species belonging to this group. Additionally, we discovered a 5 bp synapomorphic deletion in the *trn*L intron of the *trn*L-*trn*F region of core *Calosphace*. Although some of the relationships on the 'backbone' of core *Calosphace* remain unresolved, 17 strongly-supported internal clades were identified. Two of these clades, Fulgentes and Sigmoideae, were previously identified by Jenks et al. (2013).

Overall, our topology resembles that of Jenks et al. (2013), excepting their Fulgentes clade, which here was recovered as polyphyletic. The inclusion of *Salvia adenophora*, *S. disjuncta* and *S. madrensis* in the Fulgentes clade was weakly supported (BS <50) in the study of Jenks et al. (2013), whereas our results strongly support (BS 99) a close relationship of these species to section *Membranaceae* (Fig. 2). This relationship was only revealed when taxon sampling was expanded to include species from this section.

In addition to the previously recognized main clades of Jenks et al. (2013), the new Mexican clades *Salvia candicans* + *S. oaxacana* and Micranthae were identified here (Fig. 2). While the Micranthae clade consists of species distributed in a wide variety of habitats, *Salvia candicans* and *S. oaxacana* are restricted to xeric shrublands. Polyphyly of the Fulgentes clade and the new main clades uncovered by our analysis both highlight the importance of increasing taxon sampling in the phylogenetic reconstruction of *Calosphace*, as no species of section *Membranaceae* and the Micranthae clade were included by Jenks et al. (2013).

Epling's infrageneric classification of *Calosphace* was largely based on floral morphology and geographical distribution, and sections were often distinguished by combinations of characters. Sectional non-monophyly might result, for instance, from homoplasy due to similar pressures from pollinators in distantly related clades that are pollinated by the same type of animal. For example, exsertion of stamens from the corolla (i.e. exposure of the upper arm of the connective and thecae out of the upper lip of the corolla; Wester and Claßen-Bockhoff 2006) is a character that is present in species of many clades (e.g. Hastatae, South American clade, Siphonantha, Angulatae, etc.) and is often associated with ornithophily (Wester and Claßen-Bockhoff 2007). Conversely, closely-related species adapted to different pollinators may exhibit striking differences in floral attributed related to pollinator attraction. The latter is exemplified by corolla colouration, a character often used to delimit sections: our results indicate repeated instances of red-flowered species that are sister to species with blue flowers, such as the group that includes blue- and purple-flowered *Salvia coahuilensis, S. lycioides* and *S. muelleri*, nested in an otherwise red-flowered clade (Fulgentes clade, Fig. 2). Nevertheless, shared floral characters can also be synapomorphic for monophyletic sections, e.g. *Incarnatae*, characterised by exserted stamens and red corolla; whereas a combination of exserted and inserted stamens (i.e. stamens secluded in the upper lip of the corolla) diagnose *Curtiflorae*.

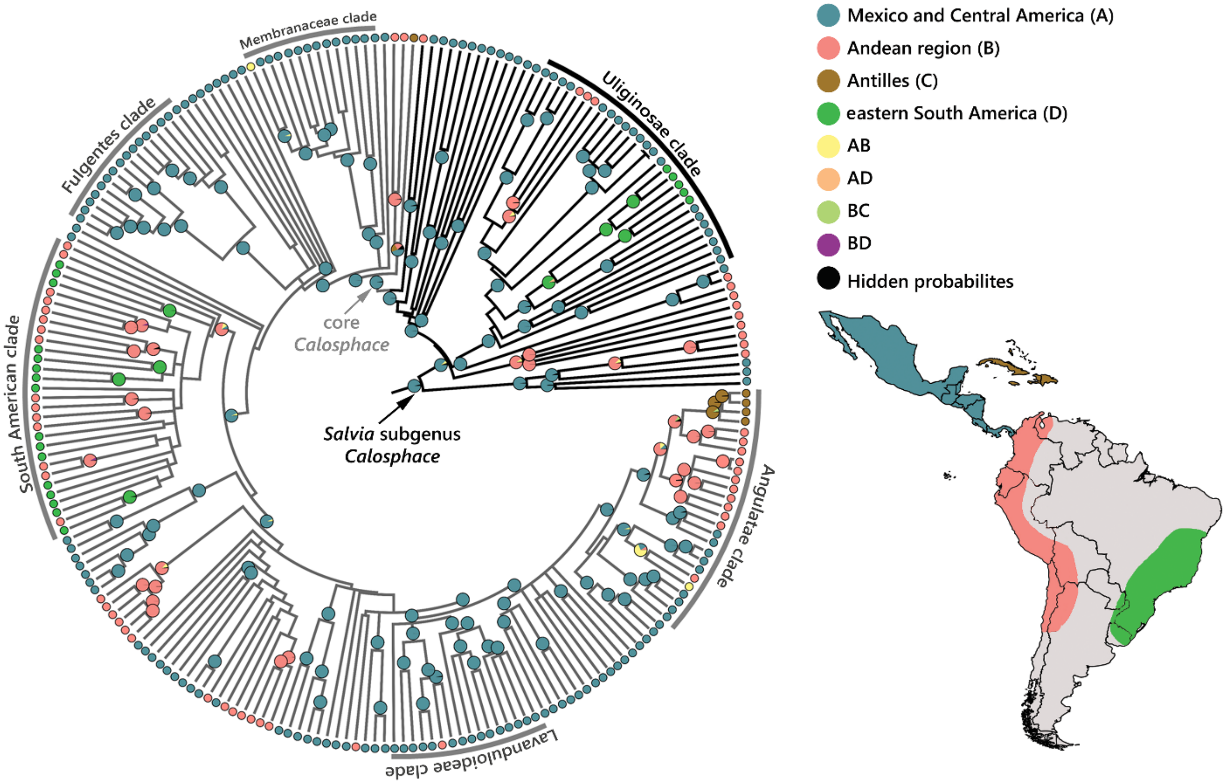

Fig. 5 Ancestral area reconstruction analysis under the Bayesian Binary MCMC (BBM) method for *Salvia* subgenus *Calosphace.* Ancestral areas are displayed for the most-inclusive, highly-supported clades and only probabilities >0.001 are shown in the pie charts. Map modified from John Harvey, Wikimedia Commons (https://upload.wikimedia.org/wikipedia/commons/e/e8/BlankMap-World6-Equirectangular.svg) original text: {PD-USGov-CIA-WF}. Creative Commons License (CC0 1.0) https://creativecommons.org/publicdomain/zero/1.0/deed.en

Ancestral area reconstruction

Our ancestral area analysis corroborates the pattern of geographic differentiation proposed by Jenks et al. (2013), with a Mexican/Central American origin for *Calosphace* and at least twelve independent migrations to South America, mostly to the Andean region (Fig. 5). This analysis also suggests a possible long-distance dispersal from Mexico/Central America to eastern South America within the Uliginosae clade (Fig. 5; Online Resource 5). Walker et al. (2015) estimated that the separation of subgenus *Calosphace* from subgenus *Audibertia*, its sister clade, took place between 11.9–19.1 Mya during the mid-late Miocene. The lower limit of this interval is congruent with the estimated age of the uplift of the Central and Northern Andes (Gregory-Wodzicki 2000). As in other angiosperm groups (*Lupinus*: Hughes and Eastwood 2006; Dipsacales: Moore and Donoghue 2007; *Lepechinia*: Drew and Sytsma 2013; *Bartsia*: Uribe-Convers and Tank 2015; Lobelioideae: Lagomarsino et al. 2016), the Andean uplift could have played an important role in the radiation of *Calosphace,* as it might have served as a 'species pump' and a 'biotic corridor' through which the

Mexican and Andean lineages migrated to eastern South America and the Antilles, respectively, promoting radiations as a result of increased habitat heterogeneity (Hoorn et al. 2010).

Zona et al. (2016) included Caribbean members of *Calosphace* in a phylogeny for the first time and suggested that the Caribbean members of *Calosphace* could have arisen from two independent colonizations, one from the Andean region and the other from Mexico. Our analysis confirms the former and assigns a high probability to the latter, although both Caribbean lineages are more closely related to Andean than Mexican species (Fig. 5; Online Resource 5). The inclusion of Caribbean taxa allowed to uncover a closer relationship between the sampled Hispaniolan species and Andean sage lineages. However, those species represent only a fraction of the diversity found on the Antilles, and a more comprehensive sampling of West Indian taxa is still critical to reconstruct the history of colonization of the Caribbean mountains by mainland sages.

The recurrent instances of disjunctions in some lineages of *Calosphace* between the Antilles and eastern Brazil may represent artefacts derived from insufficient taxonomic sampling (i.e. species occupying intermediate areas have not been sampled) or, alternatively, result from extinction of widespread species or long-distance dispersal events. Regarding the latter possibility, a thorough review of fruit dispersal in *Salvia* by Zona (2017) suggests potential dispersal vectors that could have aided in the colonization of new territories by some species of sage. Fruits of *Salvia* are schizocarps, and the mericarps or nutlets can be dispersed either enclosed or not by the fruiting calyx; accrescent calyces containing fruits might act as diaspores that can be dispersed by wind (Zona 2017), and unusually strong wind blows could transport these diaspores across continents (Nathan et al. 2008) or even across oceans (Sanmartín et al. 2007). Another possible dispersal vector for sage diaspores is water; nutlets of *Salvia* are able to float for more than 24 hours, and they can be transported long distances by flowing water in streams or by rainfall (Zona 2017). This may explain why particular species, such as *S. pratensis* (Hegland et al. 2001) and *S. pichinchensis* (I. Fragoso-Martínez, personal observations), are distributed along riverbanks. On the other hand, the fruits of most species of *Salvia* secrete mucilage when moistened; mucilage can help the fruits to adhere to animal fur or feathers (Zona 2017). If, for instance, the nutlets were adhered to birds, epizoochory could account for dispersal of sage species between mainland and islands (Vargas et al. 2012; Vazačová and Münzbergová 2014).

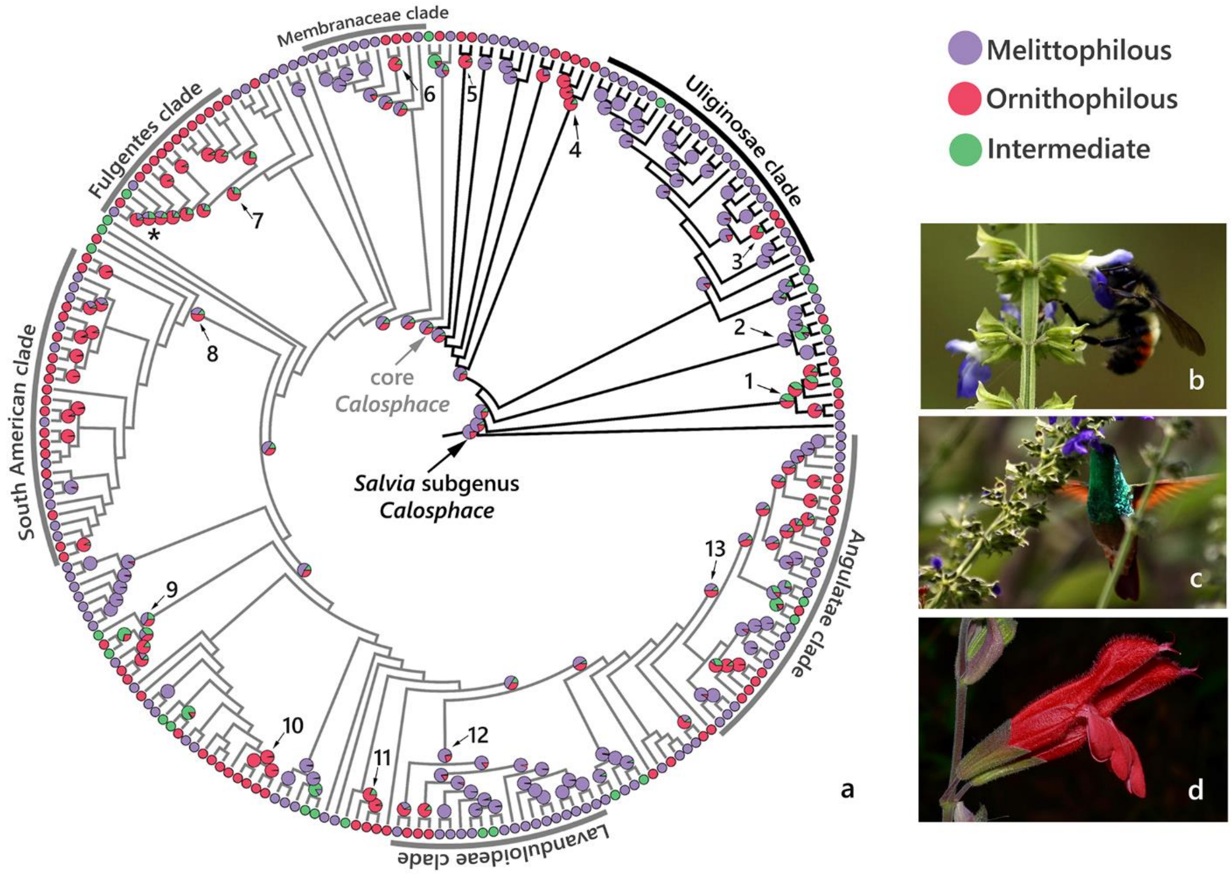

Fig. 6 a) Ancestral Reconstruction of pollination syndrome in *Salvia* subgenus *Calosphace,* using stochastic character mapping. Pies indicate the proportion of each pollination syndrome estimated for each node from 1,000 simulations on the ML phylogeny (only reconstructions for nodes with support >0.85 are shown). b) *Salvia longispicata*, a melittophilous species. c) *Salvia mexicana*, a species with intermediate characters and pollinated by both bees and birds d) *Salvia gesneriiflora*, an ornithophilous species. Arrows indicate clades with shifts to ornithophily: 1) Hastatae, 2) Tomentellae II, 3) section *Incarnatae* inside Uliginosae, 4) Erythorstachys, 5) *S. divinorum* + *S. venulosa*, 6) Membranaceae, 7) Fulgentes, 8) South American clade, 9) Rubescentes, 10) Biflorae, 11) Siphonantha, 12) Lavanduloideae; and 13) Angulatae; the asterisk indicates the reversal to melittophily from ornithophily in the Fulgentes clade. Photographic credits: Pablo Leautaud-Valenzuela (b, c), Gerardo Salazar (d).

Evolution of pollination syndromes

In agreement with the findings of Wester and Claßen-Bockhoff (2011), our character state reconstruction of the pollination syndromes indicates that the ornithophilous syndrome evolved independently several times from melittophily within subgenus *Calosphace* (e.g. in clades such as Hastatae, Tomentellae II, Uliginosae, Erythrostachys, Membranaceae, Fulgentes, Rubescentes and Angulatae) (Fig. 6; Online Resource 6). As in many other angiosperm groups, pollinator-driven selection appears to have fostered reproductive isolation that subsequently resulted in increased speciation and radiation (Schemske and Bradshaw 1999; Linder 2003; Whittall and Hodges 2007; Smith 2010). Although ornithophily has arisen in multiple occasions in *Calosphace*, overall floral morphology and function of the staminal lever in bird-pollinated species show differences among clades. According to Wester and Claßen-Bockhoff (2007), bird-pollination or the exclusion of bees by a flower is achieved by the increase in the length of the distance between nectar and pollen, essentially by increasing the length of the corolla tube, the anterior connective arm, or a combination of both. When there is elongation of the anterior portion of the connective arm, the staminal lever mechanism is usually lost since there is not enough room in the narrow tubular corolla for it to operate, and the pollen sacs make contact with the pollinator's body because they are exserted (Wester and Claßen-Bockhoff 2007). This is the morphology in clades such as Siphonantha, sections *Curtiflorae* and *Incarnatae*, and in the Hispaniolan species of the Angulatae clade. On the other hand, there are also clades like Erythrostachys, Fulgentes and Membranaceae that seem to have adapted to bird pollination by the elongation of the corolla tube, maintaining the staminal lever functional. The complex floral evolution in *Calosphace* is an interesting topic deserving more attention than the focus of this paper permits.

Our analysis also identified at least one reversal to melittophily within an ornitophilous clade (i.e. Fulgentes clade; Fig. 6), which suggests that reversals from specialized pollination types are possible in *Calosphace*, as has been reported for other plant groups (Tripp and Manos 2008; Marten-Rodríguez et al 2010; Serrano-Serrano et al. 2017), showing that the evolution of pollination syndromes is not always unidirectional, but labile (Wilson et al. 2007). Our results also show that melittophilous flowers derive from ornithophilous species only through an intermediate stage, since the probability associated to an ancestral intermediate state is higher than the one associated to a melittophilous state. The species of the Fulgentes subclade, where the reversal from ornithophily occurs, are characterized by stamens concealed by the upper lip of the corolla, a functional staminal lever and an extended lower corolla lip (Fig. 6c); these features are usually associated with bee-pollinated flowers (Wester and Claßen-Bockhoff 2007). However, the corolla tubes of most of the species are relatively long (2.5–3 cm) to be melittophilous. Hence, most floral characters associated with melittophily were already present and a reduction of corolla length could have led to the pollinator shift. Furthermore, studies on *Salvia arborescens*, a Hispaniolan species that was not assigned to a pollination syndrome by Wester and Claßen-Bockhoff (2011) due to lack of information, indicate that phalaenophily is present in *Calosphace* (Reith and Zona 2016). This syndrome represents another sort of specialization that deserves further investigation by expanding taxon sampling and conducting fieldwork focusing on the poorly known West Indian taxa.

Concluding remarks

The sectional classification proposed in the various contributions by Epling represented a colossal effort to systematize the vast diversity of *Salvia*. Twelve of his proposed sections are confirmed here as monophyletic (Table 1), eight of which were previously supported in the study of Jenks et al. (2013). Monophyly of Epling's sections *Biflorae*, *Curtiflorae*, *Lavanduloideae* and *Membranaceae* is substantiated here for the first time. However, sectional monophyly is the exception rather than the rule, as most of the non-monotypic sections were recovered in our phylogenetic trees as para- or polyphyletic. This may be a result of defining sectional limits using combinations of homoplastic characters, such as the presence of branching trichomes or the insertion of stamens in the upper lip of the corolla (e.g. section *Tomentellae*). Notably, several clades detected in our analysis show geographical structure. However, disjunctly distributed, non-monophyletic sections imply that the morphological traits shared by these groups likely evolved several times independently. An example of this is polyphyletic section *Tomentellae*, which includes species nested in three clades, two of them distributed in Mexico and one in the Andean region (Figs. 1, 2; Jenks et al. 2013).

In spite of the increased phylogenetic resolution and clade support achieved with our enlarged sample of taxa and molecular characters, there are still portions of the tree that are unresolved (mainly along core *Calosphace*), and groups that are weakly supported. Additional sampling of the Caribbean and Brazilian members of *Calosphace* is critical to improve our understanding of the relationships of these lineages. Moreover, use of additional, more informative loci such as single-copy nuclear genes in future phylogenetic analyses of *Salvia*, as well as highly efficient phylogenomic strategies such as anchored hybrid enrichment (Lemmon et al. 2012), which has recently showed promising results in different groups of flowering plants (angiosperms: Buddenhagen et al. 2016; *Protea*: Mitchell et al. 2017), including *Salvia* subgenus *Calosphace* (Fragoso-Martínez et al. 2017), will help to clarify such relationships and provide a firm ground for future macroevolutionary and biogeographic studies in the vast genus *Salvia*.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Legend to Electronic Supplementary Material:

Online Resource 1. List of currently recognized sections of *Salvia* subgenus *Calosphace.*

Online Resource 2. Voucher information and GenBank accession codes for the sampled species in this study.

Online Resource 3. Maximum likelihood tree from the nuclear dataset (nrITS).

Online Resource 4. Maximum likelihood tree from the plastid dataset (*trn*L-*trn*F*, trn*H-*psb*A).

Online Resource 5. Reconstruction of ancestral areas of *Calosphace*.

Online Resource 6. Simulated ancestral reconstruction of pollination syndrome.

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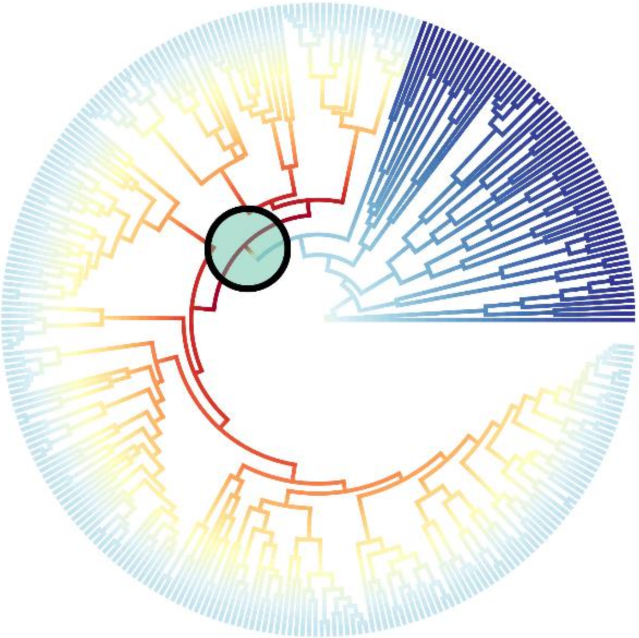
CAPÍTULO III. DIVERSIFICACIÓN DE LAS SALVIAS NEOTROPICALES ASOCIADA A LOS EVENTOS OROGÉNICOS DEL NEOGENO



III.I. Fragoso-Martínez, I.*, Salazar, G. A., Magallón, S., Martínez-Gordillo, M., Clase, T., Granados Mendoza, C. (*in prep*.) Orogenic processes as primary divers of the diversification of the Neotropical sages.

Formato para International Journal of Plant Sciences

Abiotic processes as primary drivers of the diversification of the Neotropical sages (*Salvia* subgenus *Calosphace*)

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RUNNING HEAD: Fragoso-Martínez et al.—Diversification of *Calosphace*

Keywords: Diversification, Lamiaceae, Neotropics, orogeny, radiation

Abstract

The Neotropical sages constitute one of the most iconic elements in the Mexican montane forests and constitute the most diverse genera among its flowering plant flora. The more than 600 species of this group are distributed in four diversity centers: Mesoamerica, the Andes, eastern South America and the Antilles, and it has been suggested that this lineage is result of a radiation. The drivers of this radiation could be the repeated shifts from melittophily to ornithophily or the niche abundance product of the orogenic processes during Neogene, but these hypotheses have not been tested yet. In this study we try to uncover the factors associated to the increases in diversification in *Salvia* subgenus *Calosphace*. By using the most-comprehensive taxonomic sample to date of the group, we estimate the ages of the Neotropical sages and we employ the calibrated phylogeny to conduct time-dependent and state-dependent diversification analyses, to: 1) Identify shifts in diversification rates in the evolutionary history of *Calosphace*; 2) Determine if there are differences in diversification rates associated to an specific pollination syndrome and if transition from a syndrome to another are ordered; 3) Determine if there are difference in diversification and dispersal rates among geographic diversity centers. The Neotropical sages originated during the Oligocene in the context of global temperature cooling. However, the radiation of the group occurred until middle Miocene, when a confluence of abiotic factors such as the closure of the Isthmus of Panama, the rise of temperature and the uplift of the Andes and the Trans-Mexican Volcanic Belt. Additionally, the radiation of hummingbirds in the Andes, happened. Contrary to expected, ornithophily has the same net diversification rates than melittophily. However, species pollinated by both birds and bees have higher diversification rates than specialist species, possibly due to their ability of secure pollinators. All the diversity centers showed higher diversification rates than Mesoamerica, but lower dispersal rates. Under this context, Mesoamerica behaves as a "species pump" providing the remaining areas of lineages, while the other diversity centers receive them constantly and disperse them less frequently. There could be nested radiations that were not uncovered by the limitations of our sampling or data, to further investigate the radiation of the Neotropical sages is necessary to implements NGS strategies.

Introduction

Biological diversity is distributed unevenly throughout the tree of life, while some clades hold large portions of species, other encompass just a few. The patterns associated with clades that are characterized by great morphological, ecological and/or lineage diversity are known as radiations (Sanderson 1998), and usually involve an increase in speciation rates during its evolutionary history (Donoghue and Sanderson 2015). Radiations can be triggered by intrinsic factors, i.e., key innovations, that allows them to interact with pollinators or physiological adaptations like carnivory that allow them to exploit a new adaptive zone (Donoghue and Sanderson 2015; Givnish 2015). However, extrinsic factors like new areas to disperse or niche availability can also trigger radiations (Donoghue and Sanderson 2015; Givnish 2015), and biotic and abiotic in synergy can influence radiations creating complex scenarios. The timing of the radiations where these factors appear can also affect the pattern differently; for instance, these factors can be classified as triggers if they cause the increase in diversification; background if they set the conditions for this shift to occur and modulator if it occurs once the radiation had started and can help regulate certain aspects of it and even trigger nested radiations (Bouchenak-Khelladi et al. 2015).

Salvia subgenus *Calosphace* is the most diverse lineage of *Salvia* (Walker et al. 2004; Walker and Sytsma 2007; Drew et al. 2017), with ca. 600 species endemic to the Neotropics (Santos 1995), distributed mainly in four diversity centers: Mexico and Central America (350 spp.), the Andean region (155 sp.), eastern Brazil (60 spp.) and the Antilles (45 spp.) (Jenks et al. 2013). The New World sages are pollinated mainly by bees (58% of the species); however, hummingbirds play an important role as pollinators of the group, especially in South America and the Antilles, being 30% of the species of *Calosphace* ornithophilous (Wester and Claßen-Bockhoff 2011). The shift to bird pollination from melittophily has been linked to an increase in speciation rates in different flowering plant lineages from the Neotropics (Martén-Rodríguez et al. 2010; Givnish et al. 2014; Roalson and Roberts 2016; Serrano-Serrano et al. 2017), and in *Calosphace* this relation has also been hypothesized as one of the factors responsible for the current species diversity of the group (Wester and Claßen-Bockhoff 2011; Jenks et al. 2013).

Another factor that has been hypothesized as responsible for the current diversity of the Neotropical sages are the Mesoamerican and Andean orogenic processes (Jenks et al., 2013; Ramamoorthy & Elliot, 1998), since most of the species are distributed mainly along the mountain chains of Mesoamerica and South America, being especially diverse in montane tropical forests (Espejo & Ramamoorthy, 1993). Montane habitats are considered “key landscapes”, i.e., landscapes that promote diversification through the occupation of new adaptive zones (Givnish 2015), geographically isolating lineages (e.g., sky islands, Bataineh et al. 2007; Pérez-Alquicira et al. 2018) or by providing new areas i.e., “key opportunities” (Moore and Donoghue 2007), for a lineage to colonize and diversify. Montane radiations have been documented for a number of angiosperm lineages such as *Lupinus* (Hughes and Eastwood 2006; Drummond et al. 2012), the Ericaceae (Schwery et al. 2015), *Viburnum* (Spriggs et al. 2015), etc.

In this study we use the most-comprehensive phylogeny of *Calosphace* to date, to estimate divergence ages of the main lineages, to put into a temporal framework the origin of the Neotropical sages. We use the calibrated tree to test for significative differences in diversification rates in the phylogeny. Finally, we conduct two state-dependent speciation extinction analyses to evaluate if there are different diversification rates associated to the pollination syndromes of *Calosphace* and/or the geographic regions where the species inhabit. The aim of this paper is to test if there are differences in the diversification rates in *Calosphace*, and if so, whether they are influenced by abiotic (i.e., orogenic processes, geographic regions) or biotic factors (i.e., pollinator shifts).

Material and Methods

Taxon sampling

To have a more accurate picture of the diversity of *Calosphace*, we tried to have a phylogeny as densely sampled as possible, achieving ca. 50% (304 spp.) of its diversity. The sequences of these species came from previous studies (Fragoso-Martínez et al. 2018; González-Gallegos et al. 2018), but also included data for 42 species that had not been sequenced previously. The sampling was expanded to include taxa from poorly represented diversity centers such as the Antilles, the Ecuadorian Andes and the Sierra Madre Occidental from Mexico. Additionally, GenBank sequences were downloaded to increase outgroup sampling and include all the identified lineages of *Salvia s.l.* (Drew et al. 2017). Following Fragoso-Martinez et al. (2018), *Melissa* and *Lepechinia* were used as functional outgroups. A list of species sampled, including GenBank accession numbers and voucher information is provided in Appendix 2 (S2). Nomenclature follows Govaerts & García Peña (2007) and (Martínez-Gordillo et al. 2017).

Molecular methods, sequence edition and alignment

Molecular markers were selected in order to achieve a more comprehensive sampling of the most complete existing dataset assembled for *Calosphace* (Fragoso-Martínez et al. 2018), which includes the nuclear ribosomal ITS region and two non-coding plastid regions: the trn*H*-psb*A* IGS and the trn*L*-trn*F* region. DNA was extracted from silica-dried leaf tissue and herbarium specimens. Extraction protocol followed (Fragoso-Martínez et al. 2017), primers used, and PCR profiles were the same as Fragoso-Martínez et al. (2018). However, the PCR kit was substituted by the GoTaq PCR Core Kit (Promega).

Sequences were edited and assembled with Geneious v. 10 (Kearse et al. 2012). Individual alignments were made with the online version of MAFFT (Katoh and Standley 2013), visual inspection and manual adjustment (if necessary) were performed in PhyDe (Müller et al., 2005). Matrices were concatenated with Geneious.

Fossil and secondary calibrations

The Lamiaceae family has a scarce fossil record, the oldest known fossils are either pollen grains or permineralized fruits (Martínez-Millán, 2010; Harley et al. 2004); from them, only the latter have been assigned with confidence to genera. To constrain the age of the root, we applied a secondary calibration from an angiosperm wide dating study (Ramírez-Barahona *et al*., in prep), that corresponds to the estimated age for the stem Nepetoideae clade (48.37-69.25 Ma). We were confident in assigning this secondary calibration to the root, because its confidence interval (CI) includes the age of the hexacolpate Nepetoid pollen grain from the early Eocene (49 Ma; Kar 1996), that is the earliest register of the Nepetoideae subfamily (Harley et al. 2004). The values of this confidence interval were used as the upper and lower limits of a uniform prior distribution.

Fossil calibrations were implemented as minimum ages with a log-normal prior. The former distribution has a single tail, assigning higher probability to ages older than the fossil, which declines as the tail extends further away from the node (Ho and Phillips 2009). The log-normal prior has three parameters whose values need to be set by the researchers: the mean, standard deviation and a hard minimum bound or offset value (Ho and Phillips 2009). The latter value is usually taken from the age of the fossil (Barido-Sottani et al. 2018) or the age of the fossil + 10% to place higher density of the distribution in ages older than the fossil (Magallón et al. 2015). To set the mean value of the log-normal density it is necessary to consider the expected age difference between the fossil and the most recent common ancestor (MRCA) of the clade (expected age) and use this value to solve the following equation: $\mu = ln(\text{expected age}) - \sigma^2/2$ (Barido-Sottani et al. 2018). Finally, the standard deviation controls the length of the tail of the distribution (Ho and Phillips 2009)

As previous Lamiaceae studies (Drew and Sytsma 2012), we employed the fossil nutlets of *Melissa parva* from the middle Oligocene (Reid and Chandler 1926) to calibrate the sister lineage of *Salvia s.l.* (i.e., *Melissa* + *Lepechinia*). The oldest age that has been estimated for the clade formed by *Melissa* + *Lepechinia* is ~32.7 Ma (Deng et al. 2015) and the age of the fossil nutlets assigned by Martínez-Millán (2010) is 28.4 Ma. Taking into account these dates, we considered the expected age difference to be 5 Ma and used it to calculate the mean of the log-normal distribution solving the above-mentioned equation. The log-normal prior applied had the following parameters: μ=1.4844; standard deviation= 0.5; offset = 28.4 Ma.

Although Will & Claßen-Bockhoff (2017) provided a table with almost 20 fossils for *Salvia s.l.*, we reviewed carefully the literature cited and discarded all the records that did not meet the following criteria: 1) a diagnosis of the fossil; 2) an image of the fossil and/or 3) voucher and collection information were provided. After this revision only the “*Audibertia*-type” pollen grain from the upper Miocene described by Emboden (1964) and further validated by Emboden (1969) and Muller (1981), was selected as a calibration. Since Emboden (1964) considered the morphology of the pollen grain to be similar to subgenus *Audibertia*, we decided to follow a conservative approach and applied the calibration to the stem group of the subgenus, which also includes

Calosphace. We used the criteria of Martínez-Millán (2010) and assigned the age of the upper bound of the period of the sediments where the fossil was found (Miocene, 5.33 Ma), based on Cohen et al. (2013). The oldest age estimate for stem *Calosphace* is 15.2 Ma (Walker et al. 2015), so we considered the expected age difference to be 10 Ma. The parameters of the log-normal density distribution were the following: μ= 2.1775; standard deviation= 0.5; offset = 5.33 Ma.

Divergence time estimation

Bayesian age estimation and phylogenetic inference was carried out in BEAST v.2.4.8 (Bouckaert et al. 2014), implemented in the CIPRES Science Gateway (Miller et al. 2010). The molecular matrix was partitioned by markers, the parameters of the three partitions were estimated independently, under the GTR+G nucleotide substitution model. A relaxed clock lognormal model for the substitution rates and the birth-death model for the tree were implemented. The calibration priors were implemented as described in the previous section. Two independent runs of 150 million of generations of MCMC were carried out. Samples from each run were taken every 30,000 steps, and from the 10,000 total trees, 30% were discarded as burn-in. The remaining 7,000 were combined into a single file in LogCombiner v.1.8.4 and the Maximum clade credibility tree was obtained in TreeAnnotator v.1.8.4, both through the CIPRES science gateway (Miller et al. 2010). Effective sample size (ESS) and chain convergence was evaluated in Tracer v.1.5 (Rambaut et al. 2018). ESS was higher than 200 for all the parameters. The chronogram figure was made in R (R Core Team), using the packages strap (Bell and Lloyd 2015), phyloch (Heibl, 2008), and phytools (Revell 2012), based on the code provided by Barido-Sottani et al. (2018).

Diversification analyses

Heterogeneity in speciation, extinction and diversification rates in the phylogeny was detected and quantified using the Bayesian Analysis of Macroevolutionary Mixtures v.2.5.0 program (BAMM; Rabosky 2014). BAMM employs reversible jump Markov Chain Monte Carlo (rjMCMC) to explore a diversification model universe of lineage diversification (Rabosky 2014). Recently, Moore et al. (2016) detected two problems that could render BAMM unable to provide reliable estimates on diversification rate shift models. According to the authors the main problems are that: 1) the likelihood function of BAMM does not account for unobserved rate shifts (i.e., in extinct lineages) and, 2) the calculation of the posterior distribution on the number of shifts is highly sensitive to the prior number of shifts. After evaluating these statements, Rabosky et al. (2017) determined that the effects of unobserved shifts are not likely to impact diversification estimation in empirical cases. Regarding prior sensitivity, BAMM proved to perform well in simulated datasets. However, as a cautionary measure we used different prior values in independent runs and compared the results.

The analysis was performed in a reduced tree that included *Calosphace* and its sister clade subgenus *Audibertia*, this subtree was pruned from the calibrated phylogeny using the “phythools” package (Revell 2012) in R. A correction for incomplete species sampling was implemented, setting the fraction sampled to 0.58 for *Audibertia*, and 0.48 or almost half of the species of *Calosphace*. Using the function setBAMMpriors, the priors on rate parameters were scaled to the dated phylogeny. The rate parameters of the exponential priors for the initial speciation (lambdaInitPrior) and extinction (muInitPrior) values were both: 1.1631514441834. The prior for the standard deviation of the normal distribution (lambdaShiftPrior; with mean=0) of the lambda shift parameter for rate regimes was set to: 0.0391271506704979. Rates were allowed to vary through time setting the prior lambdaIsTimeVariablePrior= 1. Due to possible non-independency of the prior from the posterior distribution, four independent BAMM runs were made using different prior values on the expected number of shifts (expectedNumberOfShifts): 0.1, 1, 2 and 3. MCMC ran for 100 million generations, sampling every 10,000 steps, from which 25% were discarded as burn-in. To assess convergence, the ESS was calculated and values higher than 200 were verified (ESS= 7501). Output files were processed in the R package BAMMtools (Rabosky et al. 2014).

Multiple-state Speciation and Extinction analyses

To test if pollinators were driving differentially speciation or extinction rates, we conducted a state dependent speciation and extinction analyses. The pollination syndromes of *Calosphace* were coded following (Wester and Claßen-Bockhoff 2011) or personal/collaborator field observations. To include the moth-pollinated species (Reith and Zona 2016), *Salvia arborescens* in the analysis, a category of invertebrate pollinated species was established. In the former state 158 melittophilous and one phalaenophilous species were pooled together. 120 Bird-pollinated species were coded into a separate state. Intermediate species, i.e., pollinated by both birds and bees were coded as an additional state. To test for ordered transitions among states, the states were coded as follows: (1) invertebrate pollinated; (2) intermediate; (3) bird-pollinated. We are aware that the intermediate state is in fact a polymorphism; however, the only SSE model that allows for simultaneously having two character states is GeoSSE (Goldberg et al. 2011). But this model was developed to explore the role of geographic expansion in diversification and its assumptions are not compatible with the morphological traits we are focusing on. We conducted a MuSSE analysis, as implemented in the “diversitree” package (Fitzjohn 2012). We accounted for incomplete sampling for each state, using the estimated proportions of pollination syndromes for New World sages provided by Wester and Claßen-Bockhoff (2011). Although this proportions include some species from subgenera outside of *Calosphace*, they're less than 30 species, so we decided to use the proportions as a rough estimate to account for species that were described after Wester's and Claßen-Bockhoff's publication (e.g. *Salvia madrigalii*, Zamudio and Bedolla-García (2017); *Salvia tetramerioides*, Martínez-Gordillo et al. (2016); *Salvia ozolotepecensis* González-Gallegos et al. (2018); *Salvia vazquezii* (Iltis et al. 2012), etc.). A pollination syndrome was assigned to these recently published species by information in the protologue (*Salvia vargas-llosae*; Sagástegui and Rodríguez 2012), by morphological comparison with their sister species or by the absence/presence of characters mentioned by Wester and Claßen-Bockhoff (2011). Considering a total 634 species of *Calosphace* (data non-published), our phylogeny had 43% of the invertebrate pollinated species, 36% of the intermediate species and 62% of the ornithophilous sages. Eight models were tested to see if they fitted out data (**Table 1**). These models differed in how the parameters for speciation (lambda: λ_1, λ_2, λ_3), extinction (mu: μ_1, μ_2, μ_3) and/or transition rates (q_{12}, q_{21}, q_{13}, q_{31}, q_{23}, q_{32}) were allowed to vary or remain equal, depending the character states. Models were compared through a likelihood ratio test (LRT) and the AIC criteria. The model that fitted the data the best was used to estimate parameters confidence intervals through a Bayesian approach in the "diversitree" package (Fitzjohn 2012). The MCMC ran for 50,000 generations and 25% were discarded as burn-in.

Geographic-state Speciation and Extinction analyses

To test if any of the diversity centers of *Calosphace* had associated an increment or decrement in diversification rates, we performed a series of geographic dependent speciation and extinction analyses using GeoSSE (Goldberg et al. 2011). Since the GeoSSE model only allows for the comparison of two areas at a time, we followed a strategy similar to Roalson and Roberts (2016) and analyzed each of the four diversity centers (Mesoamerica, Andes, Antilles and eastern South America; state 1 or A) against a category where all the remaining areas were pooled together (state 2 or B). We accounted for incomplete sampling and considering 634 species of *Calosphace*, our phylogeny had 52% of the Mesoamerican sages, 47% of the Andean species, 28% of the eastern South American sages, 38% of the Antillean taxa and 50% of the widespread species (that depending the analysis, changed from widespread to restricted to a "pooled" area). The epsilon value employed (ε= 0.19) was an average of the values provided by Magallón and Sanderson (2001) for the Lamiales in the absence of extinction (ε=0; 0.2111) and under a high extinction rate (ε=0.9; 0.1741). Eight models were tested for each combination of data, i.e., Mesoamerica vs. pooled areas, Andes vs. pooled areas, etc. The models (**Table 2**) varied in the constrains that were implemented to the speciation (sA, sB, sAB), extinction (xA, xB) and dispersal parameters (dA, dB). We used the likelihood ratio test (LRT) and the AIC criteria to compare and select the models that fitted the best each data partition. Selected models were used to estimate parameters confidence intervals through a Bayesian approach with a MCMC that ran for 25,000 generations, discarding 25% as burn-in.

Results

Relationships and estimated ages for Calosphace

The concatenated matrix consisted in 335 taxa (304 species of *Calosphace*), with a length of 2,235 bp: 721 bp from ITS region, 520 bp from the trn*H*-psb*A* IGS and 994 bp from the trn*L*-trn*F* region. In general, deep relationships were well supported (Posterior Probability, PP≥0.95), while shallow relationships were moderately (0.95 >PP>0.75) to poorly supported (PP≤0.75; **Figure 1**), especially in the core Calosphace clade, which is characterized by short internal branches and poor resolution. Upon an increased taxonomic sampling, some of the known clades in *Calosphace* were expanded, such as the *Salvia chamaedryoides* + *S. thymoides* clade (PP 0.95), that now encompasses 12 species from xeric scrublands, and the Angulatae clade (PP 0.98) that added 13 more Caribbean species to the Dominican subclade (PP 0.99; **Figure 2, S3**).

The whole lineage of *Salvia* seems to have originated during the Eocene-Paleocene limit, not long after the origin of the Nepetoideae subfamily (**Table 3**; **Fig. 1**). The age of the stem group of *Calosphace* is only three Ma older than the estimated age for *Calosphace*. However, the age estimated for subgenus *Audibertia*, the sister clade of *Calosphace*, places its origin during the Miocene compared to an Oligocene age estimate for the latter. Nevertheless, the CI for the age of *Audibertia* is broader. Divergence time estimation suggests that the crown group of *Calosphace* originated ca. 27.5 Ma ago (95% HPD: 20.6-35.1) ma ago, while the core Calosphace clade is almost ten Ma younger (**Table 3**). Within core Calosphace, three clades that might represent nested radiations in the different *Calosphace* diversity centers share similar ages 11.21-11.85 Ma in average (95% HPD: 7.61-16.22; **Table 3**; **Fig. 2**). Finally the Dominican clade is one of the youngest, most-diverse lineage and represents an independent event of colonization of the Antilles, probably previous to that of the Flocculosae clade (**Table 3**; **Fig. 2**).

Diversification analyses

Diversification of the Neotropical sages through time

The BAMM analysis detected heterogeneity in the speciation rates of subgenus *Calosphace*, identifying a single rate increase towards the origin of the core Calosphace clade, a lineage that includes ca. 70% of the species currently sampled. The pattern observed is an increase in speciation rates during the early stages of the radiation, followed by a slowdown of the rates towards the present (**Fig. 3a**). This behavior can be appreciated in the rate through time density plot of the core Calosphace clade, starting with high values of net diversification rates and decreasing towards the present and in the curve of the subgenus as an almost vertical line during the Miocene (**Fig. 3c**). The dynamics of diversification of *Calosphace* are completely different from those of its sister clade *Audibertia*, where there seem to be no increases in diversification rates and the plot is almost a horizontal line with a stable rate with a subtle decrease towards the present (**Fig. 3c**).

To verify posterior independency from prior distribution on the number of expected shifts, we ran four analyses employing different prior magnitudes (0.1, 1, 2 and 3). In all cases the configurations in the credible shift set (i.e., the configurations that account for 95% of the probability of the data) are consistent in recovering a single shift for *Calosphace*. For all the prior values, the configuration with the highest posterior probability (0.94-0.95 PP) places the increase in diversification rates in the branch that leads to the core Calosphace clade; while the second configuration with the highest posterior probability (0.05-0.06 PP) includes the stem core Calosphace clade (**Fig. 3b, Appendix**).

Pollination syndromes and diversification rates in Calosphace

The MuSSE model that fitted best the data with the maximum likelihood approach was the "Intermediate free2" model (Ln likelihood -1075.1; AIC: 2166.3), which assumes ordered transitions among states, and equal speciation and extinction rates between bird- and bee-pollinated species. Also, this model assumes that the only state that has an impact in diversification rates is the state 2 or intermediate (**Table 1**). Higher extinction rates were inferred with both the maximum likelihood (ML) and Bayesian inference (BI) criteria for the species with intermediate states, compared to melittophily and ornithophily. However, speciation rates are also higher in intermediate species, which makes net diversification (r) rates (lambda-mu) higher (**Table 4; Fig. 4a**) too. There are no direct transitions from melittophily to ornithophily and reversals are only possible through the intermediate state. Transition rates are higher going from intermediate to melittophilous syndrome (q_{21}=0.282), followed by intermediate syndrome to ornithophily (q_{23}=0.224). Once the species acquire a specialist syndrome (i.e., ornithophily or melittophily), transitioning out of it is less frequent, in comparison (**Table 4; Fig. 4b**).

Diversity centers and differential speciation rates in the Neotropical sages

The best GeoSSE model was selected for each diversity center compared against the remaining pooled areas. For Mesoamerica and the Andes, the best model was “Equal lambda within regions” (Ln likelihood -936.64, AIC: 1885.3; Ln likelihood -962.00, AIC: 1936.0, respectively), which assumed that the geographic area where the species are distributed does not have an impact on diversification, acquiring the same values for the speciation rates in both areas (**Table 4**). Comparing Mesoamerica with the remaining areas, the net diversification rate (r) was lower, but the dispersal rate was higher (**Table 4, Figure 4c-d**), which suggests that lineages are migrating from this area to colonize the others. Regarding the Andes, the r value was higher to that of the pooled areas (**Table 4, Figure 4e**), but the dispersal rate is lower (**Figure 4f**). Comparing the Antilles with the all the other areas pooled into another category, the best model was “free lambda”, which assumed that the geographic area where the species inhabit has a direct impact on the speciation rates only (Ln likelihood -877.65, AIC: 1763.3). In the Antilles the net diversification rate value was higher than in the other areas, but the dispersal rate was the same (**Table 4, Figures 4g-h**). When eastern South America (Brazil) was compared to the pooled areas the model that fitted the data was “Equal mu within regions”, which assumes that the geographic area has no influence on the extinction rates of the lineages (Ln likelihood -884.03, AIC: 1780.1). The net diversification rate and dispersal rate were higher than in the pooled areas (**Table 4, Figures 4i-j**).

Discussion

The origin of the Neotropical sages during the Paleogene-Neogene limit

The estimated age for the Nepetoideae subfamily is younger than estimates from previous studies (Drew and Sytsma 2012; Yu et al. 2014; Deng et al. 2015; Li et al. 2017), but the CI’s of both estimates overlap (**Table 5**). However, since it was not the aim of this study to date the subfamily, the taxon sample was skewed to *Calosphace* and other lineages of Nepetoideae were not included, which could account for the younger age obtained. Age estimate for the sister genera of the *Salvia* lineage was similar to those reported by Drew and Sytsma (2013) and (Deng et al. 2015), but younger ages have been estimated (**Table 5**).

The crown of the *Salvia s.l.* clade was estimated to have originated during the Eocene (47 Ma; 95% HPD: 36.5-58.2 Ma), this is the oldest age that has been obtained for the clade yet. Although the outgroup sampling was reduced compared to Drew et al. (2017), we employed one more fossil calibration (*Audibertia*-type pollen), which could be drawing back the estimated ages, as it has been observed that incorporating less fossils into node dating in the Mentheae provides younger estimates (Drew and Sytsma 2012). Regardless of the age, all the estimates coincide on a Paleogene origin of the lineage, which most likely took place in the Mediterranean or Europe, according to Drew and Sytsma (2012). The estimated ages for the *S. officinalis* and *S. glutinosa* (=subgen. *Glutinaria* sensu Hu et al. 2018) clades are older than those of previous studies (**Table 5**); however, the mean value falls into the 95% HPD CI of the ages provided by Hu et al. (2018). The age of the *S. aegyptiaca* clade is in agreement with that estimated by Drew et al. (2017), but the remaining ages are ~10-15 Ma older than other studies (**Table 5**). Since the divergence ages of *Calosphace* and core Calosphace have only been obtained in the context of studies of more inclusive clades (e.g., Drew and Sytsma 2012; Drew et al. 2017) or as the ages of the sister lineages of the focal clade (Hu et al. 2018). The older estimates obtained here could be result of a denser sampling of the Neotropical sages (~50% spp.), representing all known lineages of the subgenus, which has not been done before.

The stem group of *Calosphace* originated in North America (Drew and Sytsma 2012). The subgenus diverged from its sister clade *Audibertia* 30.3 Ma (95% HPD: 22.2-39.3; **Figs. 1-2**) ago, during the Eocene-Oligocene limit, at the onset of a global climate cooling due to atmospheric CO levels declining (Pound and Salzmann 2017). In this scenario of global temperature decreasing (as much as ~4-5°C) and temperate vegetation types expanding (Graham 2011), the crown group of *Calosphace* started to diversify 27.49 Ma ago (**Figs. 1-2**). The core Calosphace clade originated around the time the global temperature increased, during the middle Miocene climatic optimum (MMCO). On this period arid vegetation types (e.g., shrublands, caatingas, cerrados, etc.), which were later colonized by *Calosphace*, began to appear (Graham 2011).

Diversification of Calosphace *through the Cenozoic*

Although the Neotropical sages originated in the Oligocene, the increase in diversification rates was detected ca. 9 Ma later, in the Miocene, at the onset of the core Calosphace clade (**Fig. 3a**). The fact that the results were consistent among multiple runs with different prior values on the number of expected shifts, recovering a single significative shift in *Calosphace* (**Appendix**), suggests that the posterior is independent from the prior distribution. In all the analyses the same single shift was recovered with high probability at the core Calosphace clade, which gives us confidence in our results.

The pattern observed is that of an explosive radiation (Rabosky and Lovette 2008), characterized by high speciation rates during a certain period of time from 19-13 Ma before present, followed by their gradual slow-down (**Figs. 3c**). During the former window of time a combination of several abiotic factors could have triggered the radiation of *Calosphace*. For instance, during the middle Miocene the global temperature increased 4-5°C in the MMCO, dry vegetation types started to appear (Graham 2011) and the Isthmus of Panama closed definitively, establishing a complete connection between North and South America (Montes et al. 2015). Although there had been previous colonizations of South America from the Mesoamerican lineages of *Calosphace* (e.g., Uliginosae clade; **Fig. 2**), in the core Calosphace clade, these events happened repeatedly (~eight times).

Subsequently, factors that could have acted as modulators of the radiation are the radiation of the hummingbirds (~10-5 Ma) first in South America, then in North America and the Antilles (~15-5 Ma) (McGuire et al. 2014). For example, the Fulgentes clade (10.7 Ma; 6.4-15.4), a mainly ornithophilous Mesoamerican sage clade could have radiated during the diversification of the North American clade of hummingbirds in the mid-Miocene. Niche availability derived from the uplift of the Andes during the Miocene (Gregory-Wodzicki 2000) and the rise of the Trans-Mexican Volcanic Belt (Ferrari et al. 1999; Ferrari et al. 2000) could have played important modulator roles and triggered nested radiations (e.g., Lavanduloideae clade, South American clade, Angulatae clade). However, resolution of the phylogenetic framework and taxon sampling could be obscuring these patterns, increased taxonomical and character sampling are needed to test these hypotheses.

Differential diversification rates associated to the distinct pollination syndromes in Calosphace

Although bird pollination has evolved repeatedly in *Calosphace*, particularly in the core Calosphace clade nine times alone (Fragoso-Martínez et al. 2018), the state-dependent diversification analyses did not show significant difference between the speciation rate associated to this state and melittophily (**Fig. 4a**) and shifts to vertebrate pollination have played important roles in radiations in other angiosperm groups (e.g., Andean bell flowers; Lagomarsino et al. 2016; Lagomarsino et al. 2017). However, species pollinated by both birds and bees have higher diversification rates, possibly due to their ability of secure pollinators all the yearlong. The result obtained is unexpected because the species in the intermediate state are the smallest fraction of all (~10% of the species; **Fig. 6**), and include some widespread species like *Salvia mexicana*, *S. purpurea* and *S. scutellarioides* but also endemics such as *S. discolor* or *S. pexa* or *S. macellaria*. The intermediate state could be an “unstable” one, since a species with it could have difficulty isolating and speciating. Thus, this state might only promote speciation through transitioning to any of the other specialist syndromes, which is why its frequency could be low at the tips, but high as the reconstructed ancestral state (**Fig. 6**).

Transition rates from one state to another are ordered, in agreement with Fragoso-Martínez et al. (2018), shifts from melittophily to ornithophily and vice versa have to go through an intermediate state first. Transitions from the intermediate state to melittophily seem to be more frequent than to ornithophily. Passing from either specialized pollination syndrome to the intermediate state is the less frequent transition. However, this result could be influenced by the sampling frequency of ornithophilous species in the phylogeny, since the number of transitions observed in the phylogenies suggests otherwise (**Fig. 6**) and reversals to melittophily have only been documented in the Fulgentes, Membranaceae and Angulatae clades.

Mesoamerica as the “species pump” feeding the Calosphace *diversity centers*

The origin of *Calosphace* has been inferred to have occurred in North America (Drew and Sytsma 2012; Jenks et al. 2013), particularly in Mesoamerica (Fragoso-Martínez et al. 2018). The latter region showed the lowest diversification rates compared to the remaining areas, but the highest dispersal rates (**Figs. 5c-j**), excepting for eastern South America (although these results need reviewing). The increased dispersal rates of Mesoamerica, its current *Calosphace* diversity (almost half of the species) and the repeated colonizations of South America from Mesoamerican lineages (**Fig. 2**), suggest that the center of origin of the subgenus also constitutes a “species pump” that export lineages to the surrounding diversity centers, that diversify elsewhere. Once the lineages arrive to the remaining areas, they start diversifying due to a confluence of biotic and or abiotic local factors (e.g., topography, availability of hummingbirds as pollinators, etc.) and disperse among similar areas (in terms of diversification and dispersal rates), but rarely make their way back to Mesoamerica. Probably the Uliginosae clade is the only example where this could have happened (**Fig. 2**).

Concluding remarks

South America and the Antilles hold the highest diversity of bird pollinated sages, while Mesoamerica has the highest amount of melittophilous species (Wester and Claßen-Bockhoff 2011). The fact that the two areas in South America and the Antilles had higher diversification rates could be indicative of nested radiations that were modulated by the radiation of the hummingbirds in those areas, while in Mesoamerica the main pollinators remained the bees, especially in the northern region. Despite the increased understanding of macroevolutionary patterns of *Calosphace* provided by this study, it is possible that there are obscured nested radiations due to incomplete taxon sampling or the employed markers. To dissect the radiation of the Neotropical sages, it is imperative to employ large-scale phylogenomic data and expand the taxonomic sample to better represent the *Calosphace* diversity centers, as well as the pollination syndromes.

The State-dependent Speciation Extinction (SSE) methods employed here have been the object of concern due to a main issue: their susceptibility to type I error (Rabosky and Goldberg 2015), i.e., even if a trait studied does not cause increases in diversification rates, the SSE methods could still recover statistical support for this association. The latter is a consequence of employing a null hypothesis which assumes that all the diversification rates between states are equal and does not allow to discern if the diversification process is independent from the focal trait (FitzJohn 2010; Beaulieu and O'Meara 2016). To overcome this caveat, new extensions of the SSE models that account for unmeasured factors that could be driving diversification or "hidden states" have been developed (Beaulieu and O'Meara 2016; Herrera-Alsina et al. 2018; Caetano et al. 2018). These hidden states are as many as the ones in the focal trait, but they belong to an unobserved factor and potentially exhibit different diversification dynamics and transition rates than the focal trait. By incorporating these hidden states, testing for character independent diversification is possible (Beaulieu and O'Meara 2016). Since the diversification of the Neotropical sages seems to be influenced by a combination of factors, most of them abiotic, it would be advisable for future studies to test pollination syndromes under the HiSSE model (Beaulieu and O'Meara 2016), and the distribution data with the GeoHiSSE model (Caetano et al. 2018). Finally, it is important to evaluate more traits that could potentially be related to the diversification of the core Calosphace clade, such as ploidy level or edaphic adaptations.

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Tables

Table 1. Models tested in MuSSE for pollination syndrome evolution in *Calosphace*

Model name	**No. parameters**	**Free parameters**	**Constrained parameters**	**Ordered transitions (q_{13}=0, q_{31}=0)**	**Assumption**
full MuSSE model	12	all	0	No	Character state can affect each rate individually
null model	3	0	$\lambda=\mu=q$	Yes	Character state has the same effect on all rates
Free lambda	5	$\lambda_1, \lambda_2, \lambda_3$	$\mu=q$	Yes	Character state has the same effect on extinction and transition rates
Free mu	5	μ_1, μ_2, μ_3	$\lambda=q$	Yes	Character state has the same effect on speciation and transition rates
Free lambda-mu	7	$\lambda_1, \lambda_2, \lambda_3, \mu_1, \mu_2, \mu_3$	$q_{12}=q_{21}$, $q_{23}=q_{32}$	Yes	Character state has the same effect on transition rates
Unordered	8	$q_{12}, q_{21}, q_{13}, q_{31}, q_{23}, q_{32}$	$\lambda=\mu$	No	Character state has the same effect on speciation and extinction rates
Intermediate free	8	$\lambda_2, \mu_2, q_{12}, q_{21}, q_{23}, q_{32}$	$\lambda_1=\lambda_3, \mu_1=\mu_3$	Yes	Only the character state intermediate (2) has effect on all rates
Intermediate free2	8	$\lambda_2, \mu_2, q_{21}, q_{23}$	$\lambda_1=\lambda_3, \mu_1=\mu_3, q_{12}= q_{32}$	Yes	Only the character state intermediate (2) has effect on all rates. Transitions to state 2 have the same rate

*Rates: λ=speciation; μ= extinction; q= transition; r= net diversification

Table 2. Models tested in GeoSSE to identify differences in diversification rates among *Calosphace* diversity centers.

Model name	No. parameters	Free parameters	Constrained parameters	Assumption
full GeoSSE model	7	all	0	Distribution in area A or B can affect each rate individually
No between-region speciation	6	sA, sB, xA, xB, dA, dB	sAB=0	Speciation rates in widespread taxa inexistent
Equal lambda within regions	6	sAB, xA, xB, dA, dB	sA=sB	Distribution in area A or B has the same effect on speciation rates
Equal mu within regions	6	sA, sB, sAB, dA, dB	xA=xB	Distribution in area A or B has the same effect on extinction rates
Equal dispersal	6	sA, sB, sAB, xA, xB	dA=dB	Distribution in area A or B has the same effect on dispersal rates
Free lambda	4	sA, sB	sAB=0, xA=xB, dA=dB	Distribution in area A or B has the same effect on extinction and dispersal rates
Free dispersal and between region speciation	4	sAB, dA, dB	sA=sB, xA=xB	Distribution in area A or B has the same effect on speciation and extinction rates
Equal lambda within regions, no dispersal from B	5	sAB, xA, xB, dA	sA=sB, dB=0	Distribution in area A or B has the same effect on speciation rates. There is no dispersal from area B to A.

*Rates: s=speciation; x= extinction; d=dispersal; r= net diversification

Table 3. Estimated age (Ma) for relevant *Calosphace* clades and outgroups.

		95% HPD			
Clade	**Mean**	**Minimum**	**Maximum**	**Epochs**	**Period**
Dominican subclade	7.24	4.82	9.76	Miocene-Pliocene	Neogene
Angulatae clade	11.21	7.89	14.55	Miocene	
Lavanduloideae clade	11.33	7.61	15.09		
South American clade	11.85	7.66	16.22		
Core *Calosphace*	18.85	13.45	24.03	Oligocene-Miocene	Paleogene-Neogene
Calosphace	27.49	20.59	35.17	Eocene-Miocene	
Audibertia	20.82	10.02	31.46	Oligocene-Miocene	
Stem *Calosphace*	30.38	22.28	39.30	Eocene-Oligocene	
Melissa + *Lepechinia*	34.14	32.05	37.22		Paleogene
Salvia s.l.	47.12	36.51	58.25	Paleocene-Eocene	
Nepetoideae	51.65	47.80	59.66		

Table 4. Mean values of the parameter rates from the selected model in the MuSSE analysis.

	Criterion	$\lambda_{1,3}$	λ_2	μ_1	μ_2	μ_3	q_{12}, q_{32}	q_{21}	q_{23}	r_1	r_2	r_3
	ML	0.183	0.470	0.013	0.000	0.048	0.032	0.258	0.206	0.17	0.47	0.135
	BI	0.203	0.547	0.043	0.098	0.075	0.040	0.282	0.224	0.16	0.50	0.128

Analysis	Model	sA	sB	SAB	xA	xB	dA	dB	rA	rB
Mesoamerica vs pooled	Equal lambda within regions	0.268	0.268	0.256	0.105	0.017	0.013	0.002	0.163	0.251
Andes vs pooled	Equal lambda within regions	0.245	0.245	0.516	0.035	0.051	0.010	0.016	0.21	0.194
Brazil vs pooled	Equal mu within regions	0.298	0.232	0.582	0.021	0.021	0.075	0.003	0.277	0.211
Antilles vs pooled	Free lambda	0.454	0.231	0	0.018	0.018	0.003	0.003	0.436	0.228

*Rates: λ, s=speciation; μ, x= extinction; q= transition; d=dispersal; r= net diversification

Table 5. Comparison of estimated ages for *Calosphace* and outgroups in the present and previous studies

		Mean (95% HPD)			
Clade	**This study**				
Nepetoideae	51.6 (47.8-59.6)	57.6 (52.4-63.7); Drew and Sytsma (2012)	61.36 (52.4-74.7); (Yu et al. 2014)	~63 (~55-71); Deng et al. (2015)	63.4 (53.8-74.4); Li et al. (2017)
Melissa + *Lepechinia*	34.1 (32-37.2)	~14.5 (~9-20); Drew and Sytsma (2012)	29.6 (31.9-35), 29.6 (32.5-36.1); (Drew and Sytsma (2013)	~32.7 (~29.5-36); Deng et al. (2015)	20.51 (~12-29.7); Hu et al. (2018)
Salvia s.l.	47.1 (36.5-58.2)	~31 (~27-35); Drew and Sytsma (2012)	~30.5 (~25-36); Deng et al. (2015)	~34 (Drew et al. 2017)	27.9 (23.4-37.22); Hu et al. (2018)
Salvia officinalis clade + *Rosmarinus* + *Perovskia*	30.99 (18.3-44.3)	~19 (~14-24); Drew and Sytsma (2012)	~16 (~9-23); Deng et al. (2015)	~26 (Drew et al. 2017)	23.5 (16.6-30.6); Hu et al. (2018)
Salvia officinalis clade	20.2 (11.6-30.1)	~20 (Drew et al. 2017)	16 (~11-22); Hu et al. (2018)		
Salvia glutinosa clade	22 (12-33.7)	~12 (Drew et al. 2017)	17.4 (12.3-23.1); Hu et al. (2018)		
Zhumeria + *S. aegyptiaca* clade	22.4 (12.3-32.6)	~21.5 (Drew et al. 2017)	17 (11.4-23); Hu et al. (2018)		
Salvia aegyptiaca clade	17.6 (9.5-27.7)	~17 (Drew et al. 2017)	~7.1 (~11.2-3); Hu et al. (2018)		
Calosphace + *Audibertia* + *Dorystaechas* + *Meriandra*	33.6 (25.2-42.8)	~21.2 (Drew et al. 2017)	20.2 (14.9-25.7); Hu et al. (2018)		
Calosphace stem	30.38 (22.2-39.3)	15.2 (11.9-19.1); Walker et al. (2015)	~20 (Drew et al. 2017)	18.5 (~13-24); Hu et al. (2018)	
Audibertia	20.8 (10-31.4)	11.2 (6.6-15.6); Walker et al. (2015)	~15 (Drew et al. 2017)		
Calosphace	27.49 (20.5-35.1)	~17.4 (Drew et al. 2017)	~15 (~11-20); Hu et al. (2018)		
Core Calosphace	18.8 (13.4-24)	~5 (Drew et al. 2017)	~9.2 (~6-12.5); Hu et al. (2018)		

*~publications that lack estimates and only present HPD bars on trees

Figure legends

Figure 1. Calibrated phylogeny for *Calosphace* and its outgroups. Black circles in nodes denote posterior probability (PP) ≥0.95; gray circles 0.95 >PP>0.75; white circles PP≤0.75. The 95% credible intervals for estimated node ages are shown with the bars, green for the outgroup lineages and pink for the clades within *Calosphace*. The nodes in which calibrations were placed are marked with arrows, blue for fossil calibrations with lognormal priors and brown for the secondary calibration with uniform prior.

Figure 2. Dated phylogeny for *Calosphace* with tips displaying distribution of species. Color codes for the areas are shown in the map for endemic species or in the circles for widespread species.

Figure 3. BAMM analysis results for prior on the number of shifts expected= 1. A) Configuration with the maximum a posteriori (MAP) probability of the overall best rate set of rate shifts. Warm colors mark increases in speciation rates, cool colors, decreases. A single shift (increase) in speciation rates is detected near the origin of the core Calosphace clade. B) 95% credible set of shift configurations with their associated posterior probability (f). Only two different configurations account for 95% of the probability of the data, all of them detect an increase in speciation rates near the origin of the core Calosphace clade. However, the placement varies, including or excluding its sister lineage. Pink circles denote increases in diversification rates and its diameter is proportional to the marginal probability that the shift occurs in that particular branch. C) Rate through time (RTT) density plots, the central line represents the average across all samples from the posterior distribution and the shaded area denotes the 95% HPD. Left: RTT density plot for subgenus *Audibertia* where the net diversification rate is stable and shows a subtle decrease from 10 ma towards the present. Middle: RTT density plot for *Calosphace* as a whole, there is a notable increase in the diversification rate around 20 Ma before present that appears as an almost vertical line, which marks the origin of the core Calosphace clade. Left: RTT density plot for the core Calosphace clade showing a marked decrease in diversification rate towards the present.

Figure 4. Posterior probability distributions of the state-dependent speciation extinction analyses. Results from the MuSSE analysis: A) Net diversification rate is higher in species with state 2, pollinated by both birds and bees; but similar in species pollinated by either birds or bees. B) Transition rates from the ordered states: transitions from the intermediate state to melittophily or ornithophily are almost as probable, but transitions from melittophily to the state 2 are less frequent. Results from the GeoSSE analysis: C) Net diversification rate is lower in Mesoamerica than in the pooled areas, but dispersal rate (D) is higher. In the Andes the r value is similar to the pooled areas

(E), but the dispersal rate is lower (F). In the Antilles the net diversification rate is higher than in the other areas (G) and the dispersal rate is the same (H). Eastern South America has higher r (I) and d (J) values than the remaining areas. The vertical lines mark the mean values of the parameters.

Figure 5. Phylogeny of *Calosphace* with ancestral state reconstruction of the pollination syndromes under the maximum likelihood function of the “Intermediate free2” MuSSE model. Color-coded pie charts at each node indicate the proportion of the maximum likelihood supporting alternative reconstructed character states.

Figures

Figure 1

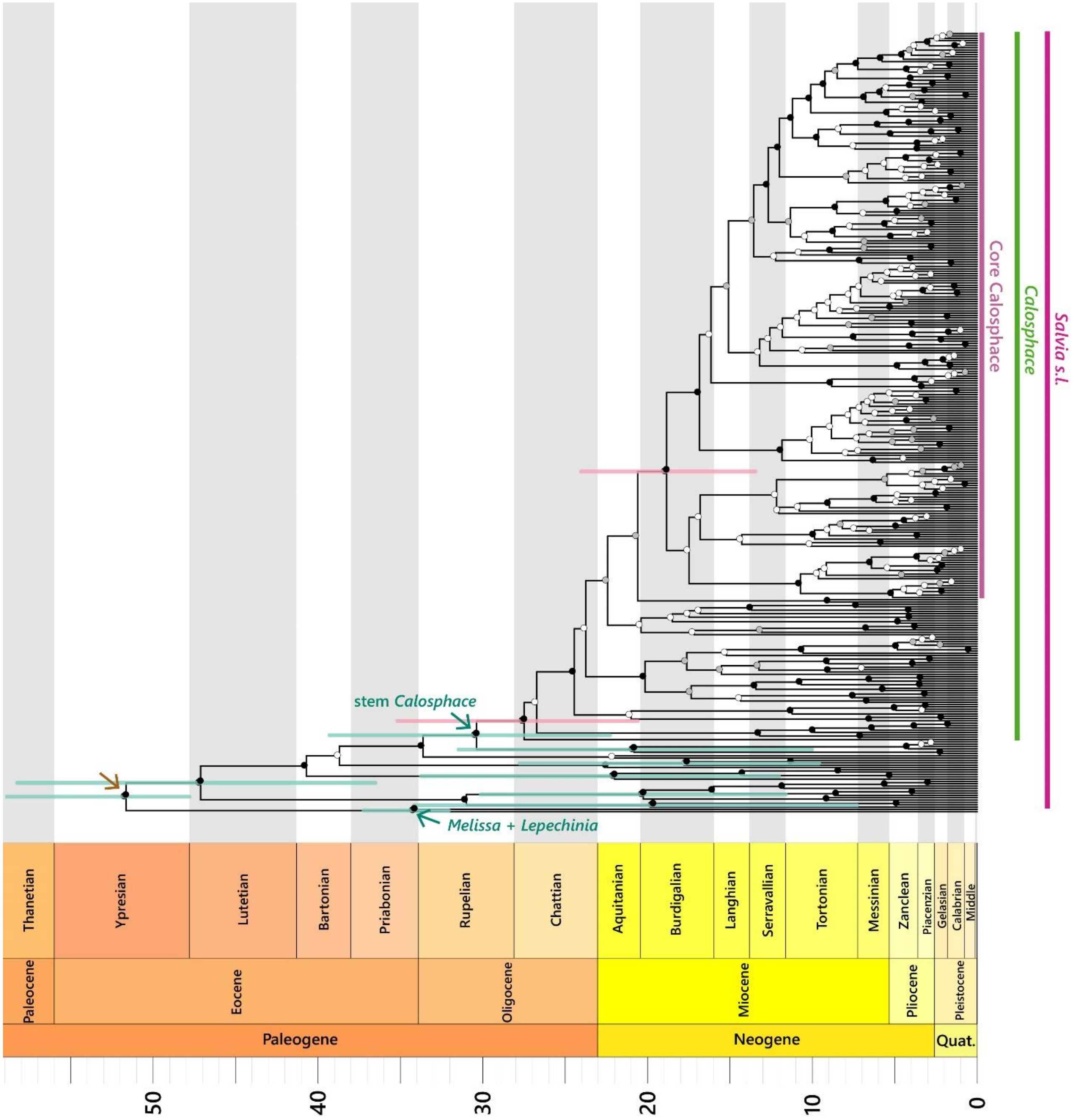


Figure 2

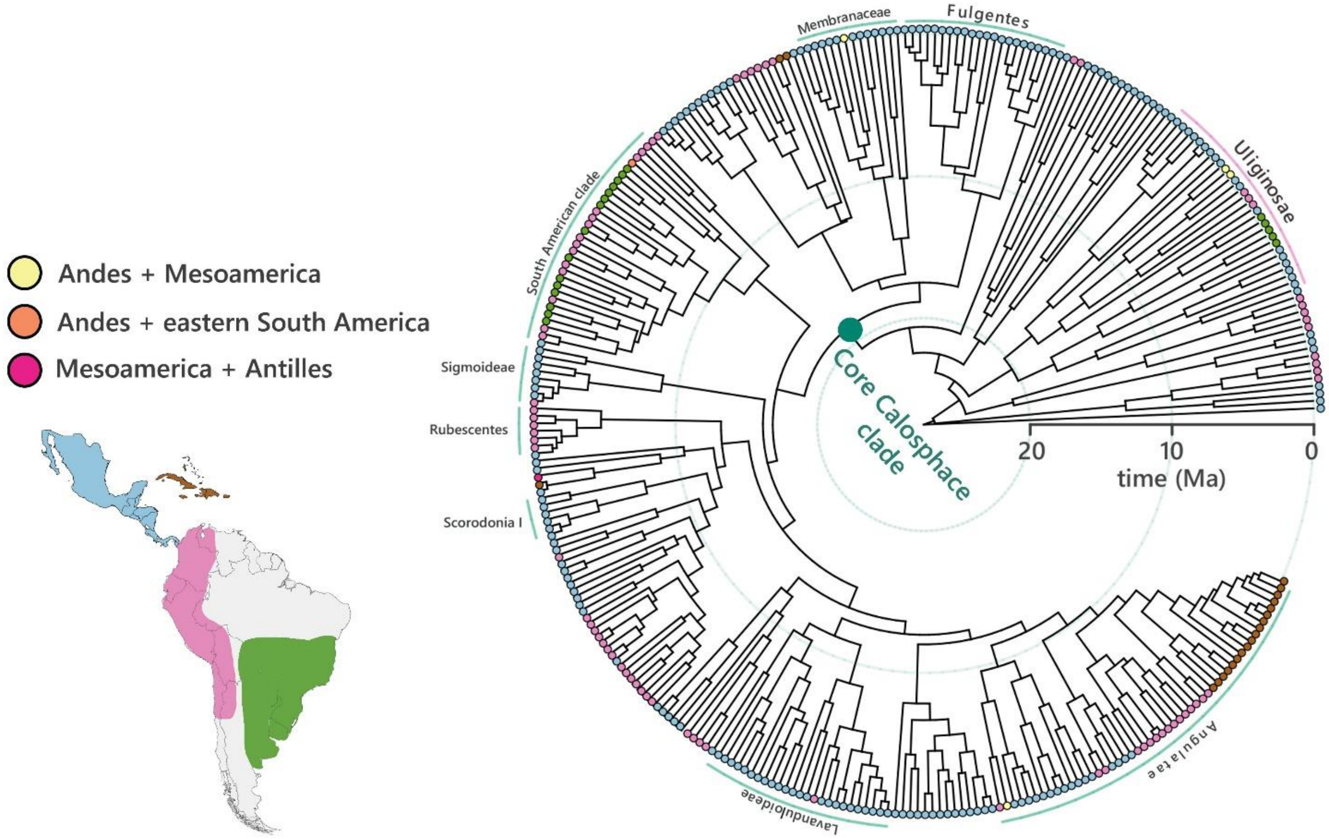

Andes + Mesoamerica
Andes + eastern South America
Mesoamerica + Antilles
Membranaceae
Fulgentes
Uliginosae
South American clade
Sigmoideae
Rubescentes
Scorodonia I
Core Calosphace clade
20
10
0
time (Ma)
Angulatae
Lavanduloideae

Figure 3

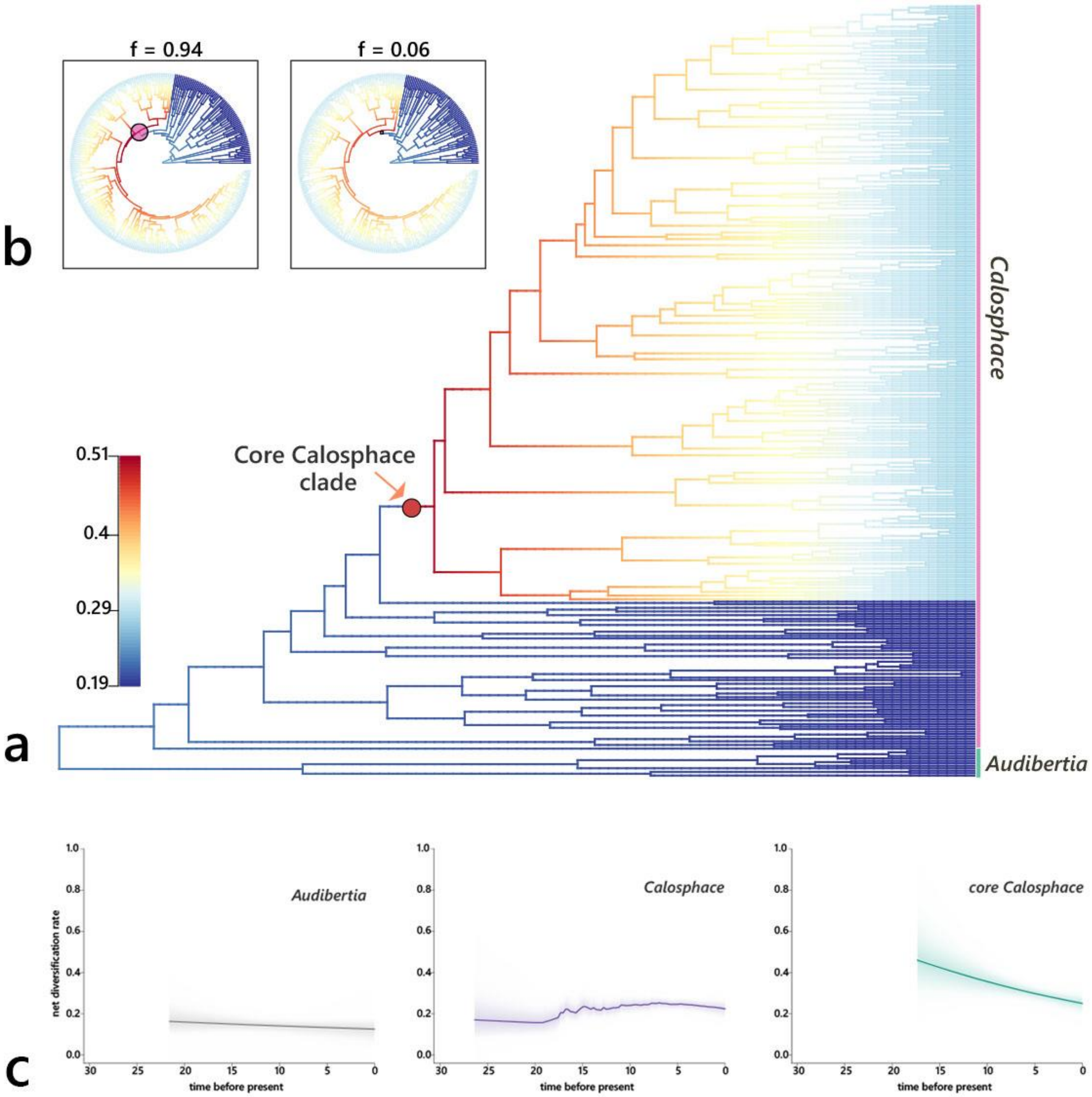

f = 0.94
f = 0.06
b
0.51
0.4
0.29
0.19
Core Calosphace clade
Calosphace
Audibertia
a
1.0
0.8
0.6
0.4
0.2
0.0
net diversification rate
Audibertia
Calosphace
core Calosphace
30
25
20
15
10
5
0
time before present
c

Figure 4

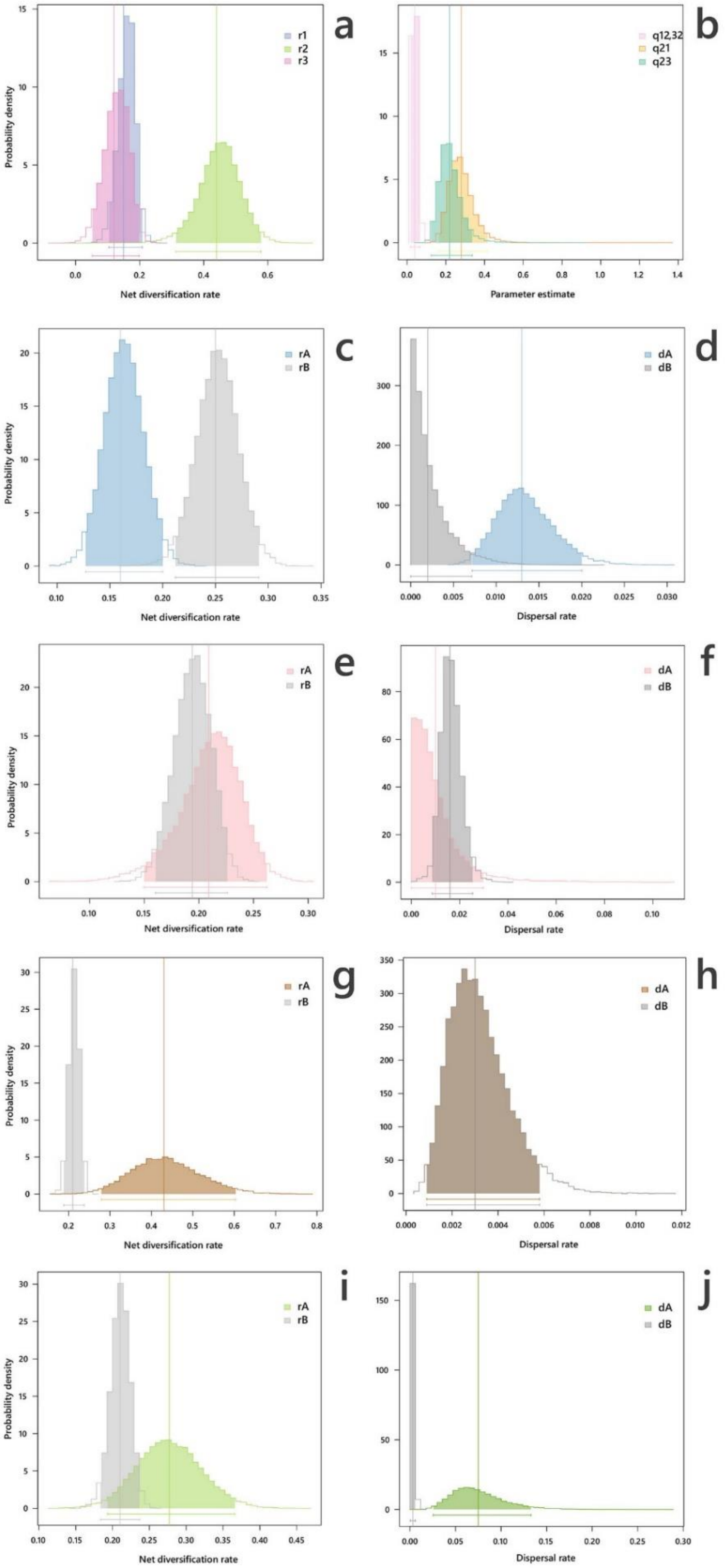
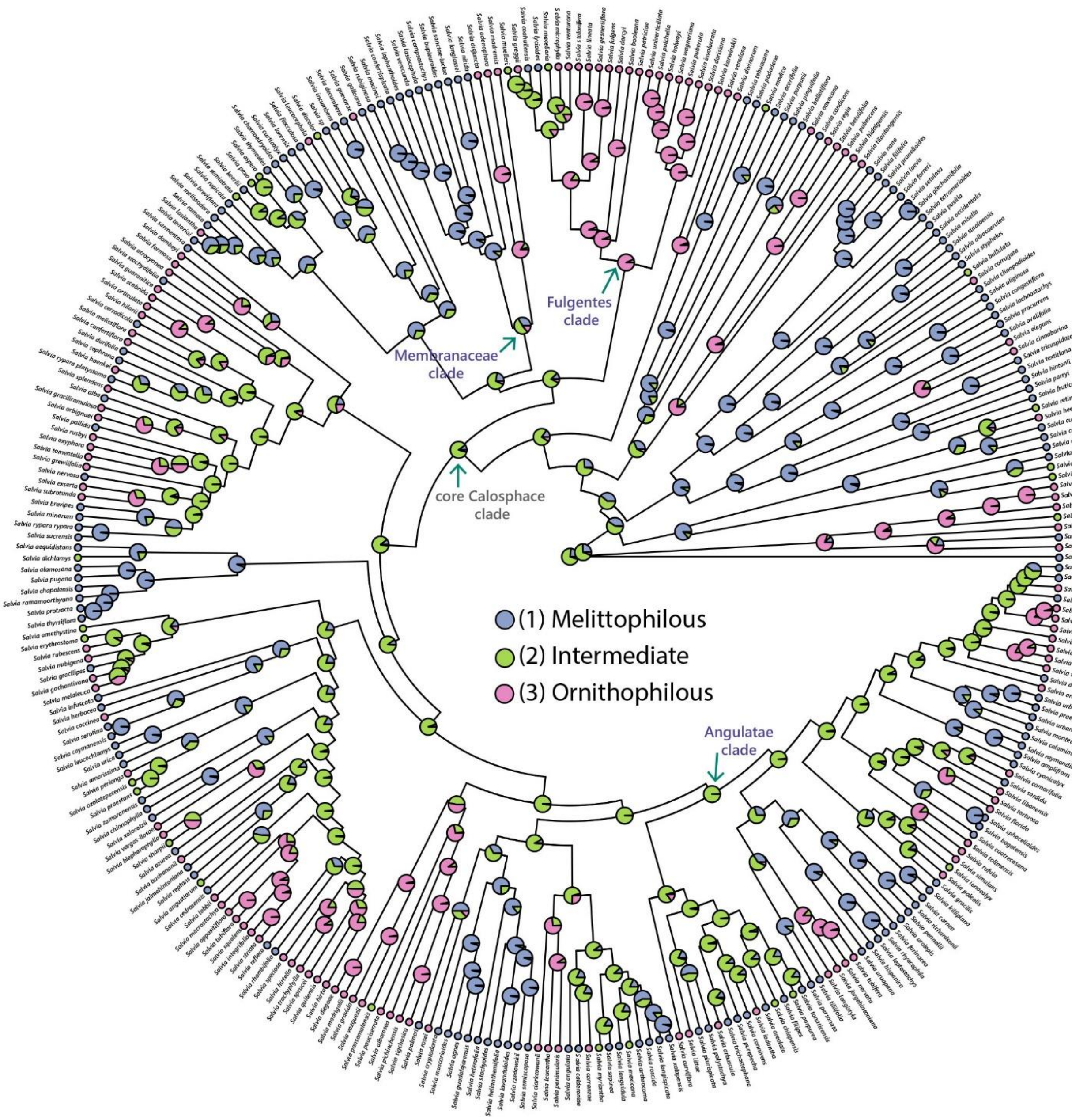
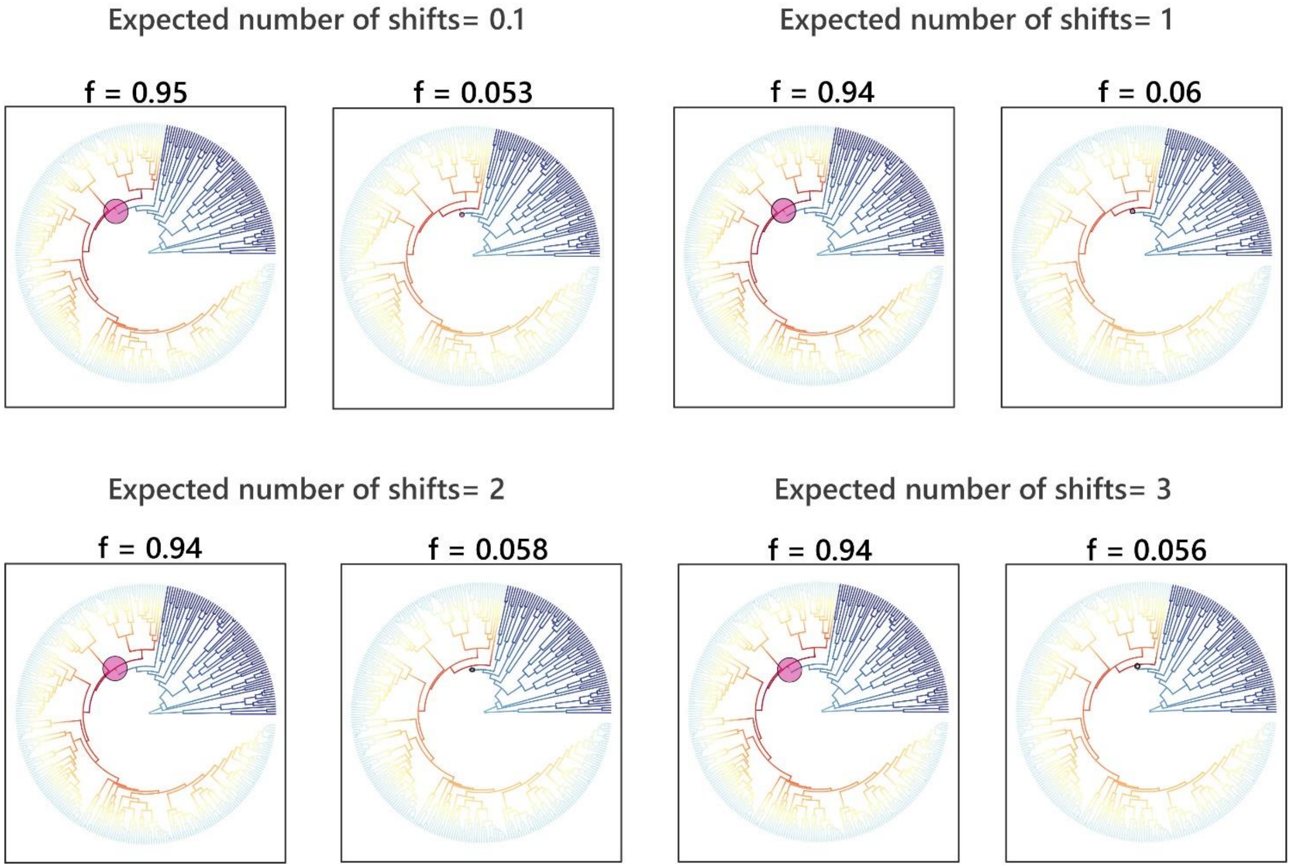

a
r1
r2
r3
Probability density
Net diversification rate
b
q12,32
q21
q23
Parameter estimate
c
rA
rB
Probability density
Net diversification rate
d
dA
dB
Dispersal rate
e
rA
rB
Probability density
Net diversification rate
f
dA
dB
Dispersal rate
g
rA
rB
Probability density
Net diversification rate
h
dA
dB
Dispersal rate
i
rA
rB
Probability density
Net diversification rate
j
dA
dB
Dispersal rate

Figure 5

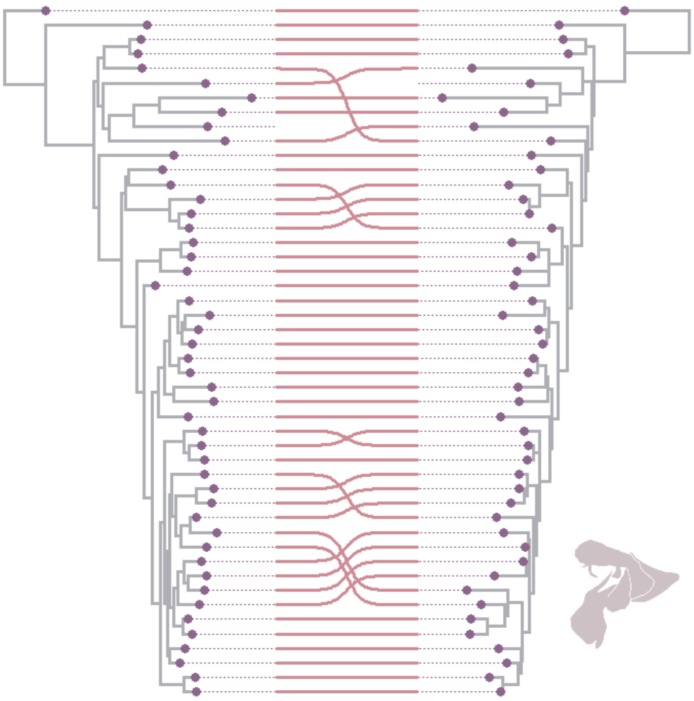

Membranaceae clade
Fulgentes clade
core Calosphace clade
Angulatae clade
● (1) Melittophilous
● (2) Intermediate
● (3) Ornithophilous

Appendix. Only two different configurations account for 95% of the probability of the data in the BAMM runs made with different prior magnitudes on the expected number of shifts (expectedNumberOfShifts), all of them detect an increase in speciation rates near the origin of the core Calosphace clade with the highest posterior probability (0.94-0.95 PP). The second configuration that holds the remaining probability (0.05-0.06 PP) shows a shift with a lower magnitude, located in the stem core Calosphace clade, which also includes the *Salvia divinorum* +*Salvia venulosa* clade.

Pink circles denote increases in diversification rates and its diameter is proportional to the marginal probability that the shift occurs in that particular branch.



CAPÍTULO IV. FILOGENÓMICA DE *Salvia* SUBGÉNERO *Calosphace* (LAMIACEAE): HACIA UNA FILOGENIA SÓLIDA DE UNA MEGA-RADIACIÓN NEOTROPICAL



IV.I. Fragoso-Martínez, I.*, Salazar, G. A., Martínez-Gordillo, M., Magallón, S., Sánchez-Reyes, L., Moriarty Lemmon, E., Lemmon, A.R., Sazatornil, F., Granados Mendoza, C.* 2017. A pilot study applying the Plant Anchored Hybrid Enrichment method to New World sages (*Salvia* subgenus *Calosphace*; Lamiaceae). *Molecular Phylogenetics and Evolution* 117: 124-134. DOI: https://doi.org/10.1016/j.ympev.2017.02.006

IV.II. PAMP—Plant Anchored MetaPrep: a high-throughput method for generating hundreds of orthologous loci for densely sampled phylogenomic studies: report on the performance in the Neotropical sages (*Salvia* subgenus *Calosphace*; Lamiaceae)

*autores de correspondencia

A pilot study applying the Plant Anchored Hybrid Enrichment method to New World sages (*Salvia* subgenus *Calosphace*; Lamiaceae)

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Abstract

We conducted a pilot study using Anchored Hybrid Enrichment to resolve relationships among a mostly Neotropical sage lineage that may have undergone a recent evolutionary radiation. Conventional markers (ITS, *trnL-trnF* and *trnH-psbA*) have not been able to resolve the relationships among species nor within portions of the backbone of the lineage. We sampled 12 representative species of subgenus *Calosphace* and included one species of *Salvia*'s s.l. closest relative, *Lepechinia*, as outgroup. Hybrid enrichment and sequencing were successful, yielding 448 alignments of individual loci with an average length of 704 bp. The performance of the phylogenomic data in phylogenetic reconstruction was superior to that of conventional markers, increasing both support and resolution. Because the captured loci vary in the amount of net phylogenetic informativeness at different phylogenetic depths, these data are promising in phylogenetic reconstruction of this group and likely other lineages within Lamiales. However, special attention should be placed on the amount of phylogenetic noise that the data could potentially contain. A prior exploration step using phylogenetic informativeness profiles to detect loci with sites with disproportionate high substitution rates (showing "phantom" spikes) and, if required, the ensuing filtering of the problematic data is recommended. In our dataset, filtering resulted in increased support and resolution for the shallow nodes in maximum likelihood phylogenetic trees resulting from concatenated analyses of all the loci. Additionally, it is expected that an increase in sampling (loci and taxa) will aid in resolving weakly supported, short deep internal branches.

Key words

anchored phylogenomics; Lamiales, maximum likelihood; phylogenetic noise; target enrichment

1. Introduction

1.1 Genomic partitioning strategies and hybrid enrichment methods

Genomic partitioning strategies incorporate selectively large-scale genomic data that can be obtained and analysed at lower costs compared to sequencing whole genomes. The genomic partitioning strategies are methods for enriching sequence libraries for specific regions of the genome (Turner et al., 2009). These methods include, among others, transcriptome sequencing, multiplex PCR, reduced representation methods (RAD-seq, RRL) and hybrid enrichment (Lemmon and Lemmon, 2013). These strategies can recover large amounts of homologous loci from multiple individuals in shorter periods of time and with less effort compared to Sanger sequencing (Blaimer et al., 2015). Data derived from different genomic partitioning strategies have been incorporated into phylogenetic analyses, increasing the amount of informative characters and improving the resolution and support of relationships in some groups (e.g. pines: Parks et al., 2009; birds: Prum et al., 2015; grape family: Wen et al., 2013).

Hybrid Enrichment is a group of genomic partitioning strategies that use probes which hybridize with selected genome regions to capture them before high-throughput sequencing. This combination of methods yields hundreds or thousands of loci from nuclear and/or organellar compartments that are potentially informative at different phylogenetic scales (Faircloth et al., 2012; Jones and Good, 2015; Lemmon et al., 2012). These methods are starting to play an important role in large-scale data generation for phylogenetic and ecological studies. Another appealing property of these methods is their potential to use highly degraded DNA from tissue recovered from museum specimens (Cronn et al., 2012; Faircloth et al., 2015; Mamanova et al., 2010).

All hybrid enrichment methods involve as a first step a phase of bioinformatic probe design, using as references genomic resources of species from the group of interest or related groups, generated *de novo* or available in public databases (Blaimer et al., 2015; Faircloth et al., 2012; Lemmon et al., 2012). The probe design phase allows the researchers to tailor probes to answer biological questions at deep or shallow evolutionary scales (Barrett et al., 2016). Moreover, the flexibility of hybrid enrichment methods allows to use previously available markers, that have been traditionally obtained through Sanger sequencing, into the target enrichment probe design (Grover et al., 2012). Probes, or "baits," are usually RNA oligonucleotides (~60–120 bp long) complementary to conserved regions of the genome such as exons (Lemmon et al., 2012; Portik et al., 2016; Sass et al., 2016), conserved orthologous sequences (COS; Mandel et al., 2014) or ultraconserved elements (UCEs; Faircloth et al., 2012; McCormack et al., 2012; Sun et al., 2014), flanked by highly-variable regions. Capturing both conserved and variable regions makes the sequences recovered potentially informative at a wide range of phylogenetic levels (Faircloth et al., 2012; Lemmon et al., 2012). Probes are designed such that captured DNA fragments overlap, allowing the targeted regions to be assembled into larger contigs, thus increasing the coverage when sequenced using next-generation sequencing (NGS) platforms (McCormack et al., 2013).

The next step in hybrid enrichment methods is library preparation, which involves DNA fragmentation, usually by sonication, and a subsequent ligation of adapters specific for particular high-throughput sequencing platforms. These adapters can be indexed to help identify samples from different individuals when they are pooled for sequencing (Lemmon and Lemmon, 2013; Prum et al., 2015; Young et al., 2016). Libraries are enriched with the designed probes and hybridization can be done either in solution (e.g. Gnirke et al., 2009; Faircloth et al., 2012; Lemmon et al., 2012) or in a solid structure such as microarrays (e.g. Bi et al., 2012). After hybridization, the captured DNA fragments are separated from the non-targeted DNA, usually by means of streptavidin-coated beads that attract biotinylated probes, or by washing away the background DNA from the microarrays (Mamanova et al., 2010). Before sequencing, targeted DNA is subjected to amplification using adapter primers to increase copy number (Prum et al., 2015; Young et al., 2016; Buddenhagen et al., 2016).

In land plants, different hybrid enrichment tools have been used to obtain phylogenomic data, including plastome sequencing through organelle capture in gymnosperms and angiosperms (Cronn et al., 2012; Heyduk et al., 2015; Parks et al., 2012; Stull et al., 2013), nuclear loci using exon capture

(Grover et al., 2015; Heyduk et al., 2015; Mandel et al., 2014; Sass et al., 2016; Sousa et al., 2014; Stephens et al., 2015a; Stephens et al., 2015b), Anchored Hybrid Enrichment (Buddenhagen et al., 2016; Mitchell et al., 2017), and a combination of both plastid and nuclear data through Hyb-Seq (Folk et al., 2015; Schmickl et al., 2016; Weitemier et al., 2014).

1.2 The Anchored Hybrid Enrichment method

The Anchored Hybrid Enrichment (AHE) method is a genomic partitioning strategy originally designed for vertebrates, that uses probes to capture hundreds of loci (~500), that are potentially useful in recovering relationships at different phylogenetic depths. The universality of these probes results from designing them using genomic resources from representative species across the lineage of interest (Lemmon et al., 2012). The AHE method has been applied to different groups of animals, improving the resolution and support of their phylogenetic trees (Brandley et al., 2015; Eytan et al., 2015; Prum et al., 2015; Ruane et al., 2015; Hamilton et al., 2016; Stout et al., 2016; Tucker et al., 2016; Young et al., 2016). Recently, the AHE method has been applied successfully to flowering plants by Buddenhagen et al. (2016) and Mitchell et al. (2017), using a probe kit designed from publicly available and newly generated genomic resources for 25 species distributed across the phylogeny of flowering plants. The Angiosperm v. 1 kit (Buddenhagen et al., 2016) targets 517 nuclear loci that are potentially shared as a single copy among various lineages of angiosperms. This capture kit was tested in 53 species belonging to 10 angiosperm families distributed in six different orders, resulting in a high enrichment success (Buddenhagen et al., 2016) that makes it a promising tool for phylogenetic reconstruction in angiosperms (e.g. to resolve the rapid radiation in the genus *Protea*; Mitchell et al. 2017).

1.3 Phylogenetic noise reduction

Phylogenetic noise as defined by Straub et al. (2014) consists of some features of DNA sequences that contradict or obscure the phylogenetic signal of a true gene genealogy. This noise can have an impact in phylogenetic reconstruction, causing some nodes to have low support or providing high support for wrong relationships (Rokas and Carroll, 2006; Townsend et al., 2012; Wenzel and Siddall, 1999). In some cases, phylogenetic noise can be due to substitution saturation or alignment errors, and exclusion of unusually fast-evolving sites has been recommended as a strategy of noise reduction (Buddenhagen et al., 2016; Goremykin et al., 2015, 2010; Granados Mendoza et al., 2013; López-Giráldez and Townsend, 2011; Straub et al., 2014; Zhong et al., 2011).

1.4 Salvia *subgenus* Calosphace *(Lamiaceae; Lamiales)*

With ca. 900 species, *Salvia* L. (sages) is the most diverse genus of Lamiaceae, the mint family (Harley et al., 2004). *Salvia* is not monophyletic, and it contains three main lineages with other five genera embedded (Walker et al., 2004, 2015). Nevertheless, *Salvia* clade II (Walker et al., 2004) constitutes a lineage endemic to the New World that includes the only two monophyletic subgenera of *Salvia*: *Calosphace* (Benth.) Epling and its sister subgenus, *Audibertia* J. B. Walker, B. T. Drew & K. J. Sytsma (Walker et al., 2015). Subgenus *Calosphace* (in the following referred to simply as *Calosphace*), includes ca. 600 species and is the most species-rich lineage of *Salvia* (Epling, 1939; Santos, 1995). This subgenus attains its greater species richness in the Neotropics, and most of its diversity is found in Mexico (ca. 307 spp.; Martínez-Gordillo et al., 2013). Other important diversity centers are the Andes (155 spp.), eastern South America (60 spp.) and the Greater Antilles (45 spp.) (Jenks et al., 2013). The phylogenetic relationships among the species of *Calosphace* have been explored with DNA sequences of the nuclear-ribosomal internal transcribed spacers (ITS) and the non-coding chloroplast regions *psbA-trnH* and *trnL-trnF*. The phylogenies resulting from these combined "conventional" markers were poorly resolved, and many clades lacked support due to low sequence variation among closely related species. However, those markers permitted the identification of six main *Calosphace* lineages (Jenks et al., 2013). One such lineage is "core *Calosphace*," which contains most of the species that have been sampled in prior phylogenetic studies and is believed to represent a recent radiation, given its short internal branches and the lack of resolution along its backbone (Jenks et al., 2013, 2011; Walker, 2006).

Core *Calosphace* includes species from all four main diversity centers of the subgenus, and exhibits a wide variety of floral attributes related to pollination syndromes. Mexico and Central America have been inferred as the ancestral distribution area of the subgenus, and the ancestral pollination syndrome for the lineage is melittophily (Jenks et al., 2013; Wester and Claßen-Bockhoff, 2011). However, numerous events of migration seem to have occurred leading to the current distribution of the subgenus, and multiple origins of ornithophily have likewise been suggested. To explore further the evolutionary history of this lineage of Neotropical sages, a better resolved and supported phylogeny is required.

The present study uses 12 species of *Salvia* subgenus *Calosphace*, including 10 representatives core *Calosphace*, and a species of the closely related genus *Lepechinia*, to test the performance of phylogenomic data obtained through Anchored Hybrid Enrichment coupled with next generation sequencing, in resolving phylogenetic relationships within this plant group. The aims of this study are to: 1) evaluate the effectiveness of gene capture and high-throughput sequencing for *Calosphace*; 2) test the performance of the captured loci in resolving and supporting internal relationships in the group; and 3) compare the effect of different data-filtering schemes on phylogenetic reconstruction.

2. Material and methods

2.1 Sampling

2.1.1 Loci sampled

The nuclear loci sequenced correspond to those selected by Buddenhagen et al. (2016) for their Angiosperm v. 1 enrichment kit. As starting point, these authors used the 959 APVO genes (Duarte et al., 2010), which are single-copy nuclear genes shared by *Arabidopsis* Heynh., *Populus* L., *Vitis* L. and *Oryza* L. The corresponding exons of *A. thaliana* (L.) Heynh. were mined and only those long enough for probe design (≥150 bp) were selected, yielding 3,050 exons. Homologous regions from *Oryza* were extracted and compared to those of *A. thaliana* in order to filter exons with less than 55% similarity between these two species. Using the resulting 1,721 exons, orthologous regions were mined among 42 angiosperm reference genomes. The final 499 regions were selected based on their low average copy number (≤1.2) and their presence in at least 85% of the genomes used. Additionally, the authors included 18 selenium-tolerance loci, yielding a total of 517 regions. Finally, to make a universal flowering plant capture kit, the probe set was designed based on a selection of 25 angiosperm reference species, which are distributed across the angiosperm tree (Buddenhagen et al., 2016). Probes were designed to be ~120 bp long and tiled across the complete sequence of all the loci, overlapping by ~43 bp (2.8× tiling density).

2.1.2 Taxon sampling

The sample of taxa consisted of 12 species belonging to different clades of subgenus *Calosphace* and one species of *Lepechinia* Willd., the sister genus of *Salvia* s.l., as outgroup. Clade representation was based on a previous phylogenetic study (Jenks et al., 2013), with emphasis on species of core *Calosphace*. To evaluate the power of the AHE loci to resolve relationships at different time scales, the sister species of the remaining *Calosphace* species (*Salvia axillaris* Moc. & Sessé ex Benth.) and one species of the genus *Lepechinia* were included, to represent ancient divergence events. Two species closely related to each other (*Salvia protracta* Benth. and *S. ramamoorthyana* Espejo Serna *emend.* J.G.González) were included to represent recent divergences. Sampled species and voucher information are provided in Table 1.

Table 1. Voucher specimens of the sampled species. Taxa marked with an asterisk (*) belong to the core Calosphace clade.

Species	Country, collector (Herbarium)
Lepechinia caulescens (Ortega) Epling	Mexico, *M. Castañeda-Zarate 841* (MEXU)
Salvia atrocyanea Epling*	Argentina, *A. Cocucci 5516* (CORD)
S. axillaris Moc. & Sessé *ex* Benth.	Mexico, *M. Castañeda-Zarate 1084* (MEXU)
S. connivens Epling*	Mexico*, F. Sazatornil 18* (MEXU)
S. fulgens Cav.*	Mexico*, F. Sazatornil 5* (MEXU)
S. helianthemifolia Benth.*	Mexico*, F. Sazatornil 9* (MEXU)
S. hispanica L.*	Cultivated, *I. Fragoso-Martínez 4* (FCME)
S. laevis Benth.	Mexico, *I. Fragoso-Martínez 284* (MEXU)
S. melissodora Lag.*	Mexico, *D. Sandoval 1264* (MEXU)
S. mexicana L.*	Mexico, *M. Castañeda-Zarate 840* (MEXU)
S. mocinoi Benth.*	Mexico, *G. Salazar-Chávez 9231* (MEXU)
S. protracta Benth.*	Mexico, *G. Salazar-Chávez 9463* (MEXU)
S. ramamoorthyana Espejo Serna *emend*. J.G.González*	Mexico, *G. Salazar-Chávez 9237* (MEXU)

2.2 Molecular methods

2.2.1 DNA extraction

DNA was extracted from silica gel-dried leaf tissue using a modification of the 2× CTAB method (Doyle and Doyle, 1987) described in Salazar et al. (2003), adding incubation with RNase A (Qiagen) and Proteinase K (Promega). In some cases, DNA was precipitated using a 3M sodium acetate solution to increase purity. DNA concentration and purity ratios were measured using a Nanodrop 2000 spectrophotometer (Thermo Scientific).

2.2.2 Library preparation, enrichment and sequencing

The following steps were carried out at the Center for Anchored Phylogenomics at Florida State University (http://anchoredphylogeny.com/) and followed Buddenhagen et al. (2016). Genomic DNA was fragmented using a Covaris E220 Focused-ultrasonicator, to a fragment size of ~300–800 bp. The adapters and indexes were linked to the fragmented DNA using a Beckman-Coulter Biomek FXp liquid-handling robot, using a modification of the protocol of Meyer and Kircher (2010). Once indexed, samples were pooled and one solution-based enrichment reaction was carried out using the Angiosperm v. 1 kit (Agilent Technologies Custom SureSelect XT kit). Enriched DNA was separated from non-hybridized DNA using streptavidin magnetic beads. The enrichment reaction was sequenced in one PE150 Illumina HiSeq 2500 lane at the Translational Science Laboratory in the College of Medicine at Florida State University, Tallahassee, Florida, USA.

2.3 Bioinformatic data processing

2.3.1 Raw data processing

Raw reads were processed with the CASAVA v. 1.8 pipeline with the high-chastity setting to filter low-quality reads. Reads were demultiplexed using 13 in-house developed indexes which are 8 bp long and with at least two different base pairs. Reads that failed to match any of the expected indexes were discarded.

2.3.2 Pair-read merging and assembly

Reads were merged based on the method proposed by Rokyta et al. (2012), to increase their accuracy and length. Unmerged reads were also used for assembly. Read assembly followed Prum et al. (2015) and Buddenhagen et al. (2016). The read assembler employs a two-level strategy to achieve sequence assembly. The first level is the divergent reference assembly, which uses a selection of the species (*Arabidopsis thaliana*, *Billbergia nutans* H.Wendl. and *Carex lurida* Wahlenb.) used during probe design. These species are distantly related to the study group and are employed as references for the assembly to map the reads to the conserved regions. The second level is a quasi-de novo assembly that uses a preliminary consensus sequence from the study group, generated in the previous step, to extend the mapping to less conserved flanking regions (Prum et al., 2015). Both levels of assembly were used to traverse repeatedly the read files until no additional reads were mapped. Once assembly clusters were formed, consensus bases were called. If a polymorphism could not be explained as a sequencing error, ambiguity bases were called, and if the coverage was lower than ten, the bases called were marked as N. Consensus sequences with less than 30 reads were discarded to prevent cross contamination or sequencing errors in index reads.

2.3.3 Orthology assessment

Each locus should produce a single cluster in the absence of contamination, low coverage or gene duplication. To identify loci with duplications and to discern between potential loci copies, orthologous sequences were determined following Prum et al. (2015) and Buddenhagen et al. (2016). The sequences were grouped by locus and a pairwise distance between two sequences was calculated as the percent of 20-mers found in both sequences. A distance matrix was constructed with this information and, using the neighbour-joining algorithm (Saitou and Nei, 1987), sequences were clustered, allowing a single sequence per species per locus. When more than one cluster was detected within a region, clusters of orthologs were separated and considered as different loci. When gene duplication occurs within a clade a subset of taxa will lack the locus, resulting in missing data. To reduce the effect of missing data, clusters including less than 50% of the species were discarded.

2.3.4 Alignment and alignment trimming

Each locus was aligned using MAFFT v. 7.023b (Katoh and Standley, 2013) with "-genafpair" and "maxiterate 1000" flags. Alignments were trimmed/masked in three steps: 1) Each column of the alignment was considered "conserved" if >40% of the sequences presented the most commonly observed character. 2) Regions of 20 bp in each sequence were scanned to identify those that lacked at least 10 characters matching the common base at a conserved site; these regions were not well aligned and were masked. 3) Sites were removed from the alignments when they presented fewer than 12 unmasked bases. Alignments for all loci were retrieved, including those missing the outgroup.

2.4 Evaluation of locus performance in phylogenetic resolution and support

The performance of captured loci in resolving internal relationships in *Calosphace* was evaluated with two different criteria: 1) phylogenetic reconstruction (concatenated and coalescent-based analyses); and 2) phylogenetic informativeness (Townsend, 2007). The results yielded by the original dataset were compared to the ones obtained from the filtered datasets under four different scenarios (see Data screening and matrix filtering, below).

2.4.1 Concatenated analyses

The trimmed matrices of the captured loci were concatenated and analysed with maximum likelihood (ML) using the programme RAxML-HPC2 on XSEDE v. 8.2.8 (Stamatakis, 2014), implemented in the CIPRES Science Gateway (Miller et al., 2010). The phylogenetic trees were inferred under the GTRGAMMA model, using the "rapid bootstrapping and search for the best-scoring ML tree" algorithm (Stamatakis et al., 2008), with 1,000 bootstrap replicates. Two partition schemes were employed for the concatenated matrix: 1) partitions per locus (448 partitions) and 2) partitions selected by PartitionFinder v. 1.1.1 (Lanfear et al., 2012), which permitted to explore the effect of model assignment on tree resolution and support. A concatenated matrix of the conventional markers (ITS, *trnL-trnF* and *trnH-psbA* IGS) from Fragoso-Martínez et al. (in review) was analysed under the same parameters for comparison purposes.

2.4.2 Coalescent-based analyses

Unrooted gene trees were inferred using RAxML-VI-HPC (Stamatakis, 2006) through a workflow in Geneious v.8.1.8 (http://www.geneious.com, Kearse et al., 2012) using the same model and algorithm used in the concatenated analyses, but with 100 bootstrap perturbations. To infer the species tree we employed ASTRAL (Mirarab et al., 2014) and support was calculated using local posterior probabilities, which have shown to be more precise than multi-locus bootstrapping (Sayyari and Mirarab, 2016). The quartet approach implemented in the ASTRAL algorithm makes it time-efficient and has proven to provide more accurate results than other coalescent-based and concatenated methods (Mirarab et al., 2014). Additionally, due to the small number of taxa sampled, we were able to find the globally optimal species trees using the exact version of ASTRAL (Mirarab et al., 2014) and, because ASTRAL does not require rooted gene trees, we could use all captured loci in the species tree inference, instead of only alignments that contained the outgroup.

2.4.3 Phylogenetic informativeness analyses

To evaluate the performance of the captured loci in the phylogenetic reconstruction of *Calosphace*, we performed a Phylogenetic Informativeness (PI) analysis (Townsend, 2007). For this analysis, the ML tree from the concatenated analysis was made ultrametric with a relative time scale of 0 at the tips and 1 at the root, using the non-parametric rate smoothing method (Sanderson, 1997), implemented in TreeEdit v 1.0a10 (Rambaut, 2002). The partitioned concatenated matrices and the reference ultrametric tree were uploaded to PhyDesign, an online application for profiling phylogenetic informativeness (López-Giráldez and Townsend, 2011; http://phydesign.townsend.yale.edu/). Substitution rates were estimated in HyPhy (Pond et al., 2005) using the GTR model with empirical base frequencies. A first PI analysis was performed using the alignments obtained after the bioinformatic data processing and the ML tree from those aligments, and a subsequent PI analyses were performed over filtered concatenated matrices (see next section), and the resulting ML trees.

2.5 Data screening and matrix filtering

Sites with unusually high substitution rates were visualized as “phantom” spikes on the individual PI profiles (Granados Mendoza et al., 2013; López-Giráldez and Townsend, 2011; http://phydesign.townsend.yale.edu/). To identify the specific positions in the alignments that could be introducing phylogenetic noise due to their unusually high substitution rate, we developed a R (R Core Team, 2014) script. This script (Appendix A) takes as input the spreadsheets with the estimated substitution rate per locus from HyPhy (Pond et al., 2005; obtained from the PhyDesign tool (López-Giráldez and Townsend, 2011; http://phydesign.townsend.yale.edu/) and constructs a new data frame including the name of the locus, position of the sites and their substitution rate. These data frames were filtered by removing sites with substitution rates lower than a given value. In this study, we tested four different substitution rate thresholds (i.e. deleting sites with rate values higher than 5, 10, 15 and 20), but these values can be modified by the user after observing the behaviour of the dataset in question. The output of the script is a spreadsheet of positions with high substitution rates and scatterplot files per locus that can be used to explore the behaviour of each alignment. Sites included in the spreadsheets were manually removed from the alignments from the AHE bioinformatic process using Geneious v.8.1.8 (http://www.geneious.com, Kearse et al., 2012). The scatterplots of the substitution rates of each site per locus and the list of the removed sites from each scenario are provided in Appendix A. The concatenated matrices, including the original matrix and the four filtered matrices are available in Mendeley data (doi: 10.17632/6f4pmh7ds8.1).

3. Results

3.1 Gene capture and high-throughput sequencing for Calosphace

From the 517 loci targeted by the plant capture probe set, 399 were captured. From these, 38 genes had 2 to 7 copies that were separated into different matrices, yielding 448 alignments, 370 of which included the outgroup. *Salvia ramamoorthyana* had the best capture (441 loci), whereas *S. axillaris* was the ingroup species for which the fewest loci were captured (378). The length of the alignments ranged from 158 to 1891 bp, with an average length of 704 bp. Sixty four percent of the alignments included all 13 taxa and only in 21% of the alignments there were more than one missing species. The concatenated matrix comprised 316,701 bp.

3.2 Performance of the AHE data

3.2.1 Phylogeny estimation within subgenus Calosphace

The phylogenetic hypothesis derived from the concatenated AHE data (Fig. 1a) showed increased support and resolution compared to the one obtained with conventional markers (Appendix A, Fig. A.1). In the tree resulting from the AHE dataset, most of the internal branches were supported by the highest bootstrap value (100%), including the core *Calosphace* clade. Three short branches had lower support and only one had <85% bootstrap support (BS). These branches are: branch 1 (B1; 92% BS) and branch 2a (B2a; 58% BS), which include *S. helianthemifolia* and *S. hispanica*, and these two species plus *S. connivens*, respectively. Branch 3 (B3; 86% BS) is a short deep branch within core

Calosphace, which includes *S. melissodora* plus the eight remaining sampled species of core *Calosphace* (Fig. 1a). The BS values of the concatenated analysis using the partition schemes estimated by PartitionFinder (Lanfear et al., 2012) were similar to those using the 448 partitions, albeit slightly lower (Appendix A, Figs. A.7–A.9). In the tree resulting from conventional markers (Appendix A, Fig. A.1) most of the internal branches were supported by >85% BS and only the clade formed by *S. protracta* and *S. ramamoorthyana* had 100% BS. Four branches had <85% BS, two of them along the backbone of the core *Calosphace* clade, one of them equivalent to B3 of the concatenated AHE analysis. The other two branches with low support included the same species than B1 and B2a. There are topological incongruences between the phylogenetic trees from the analyses of both concatenated AHE and conventional markers, all involving the species from the above-mentioned branches (Appendix A, Fig. A.1).

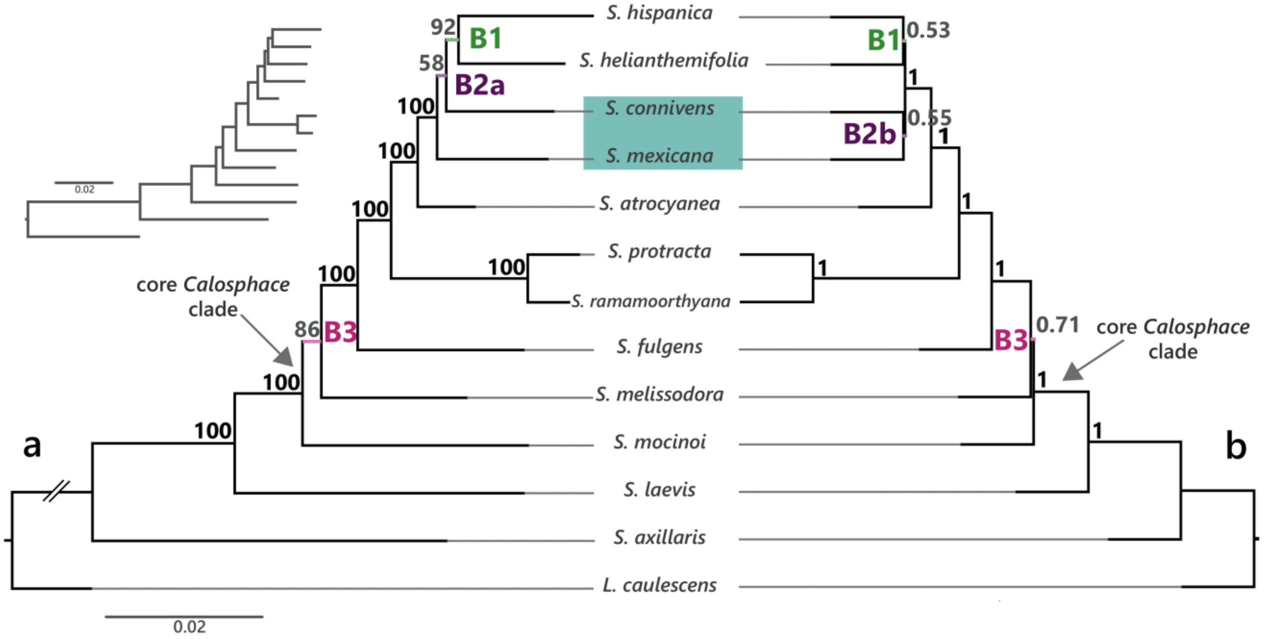

Fig. 1. Trees inferred from the AHE data: (a) maximum likelihood tree from the analysis of the concatenated matrix, (b) species tree from the coalescent-based method. Bootstrap support (BS) or local posterior probability (LPP) values are shown over the branches. Branches with low support are labeled as B1–B3, and only the branches B1 and B3 contain the same clades in both trees. The topological incongruence between the trees from different reconstruction methods is highlighted. In the concatenated analysis, only B2a lacked significant BS values, while in the coalescent-based analysis B1–B3 have low LPP values.

The exact ASTRAL search of the 448 gene trees resulted in a species tree with most of its internal branches showing high local posterior probabilities (1 LPP) and three short branches with low LPP values (Fig. 1b). The branches that did not receive the highest support were the same in both the concatenated and the coalescent-based analyses from the AHE data. The branches B1 and B3 contain clades consisting of the same species than in the concatenated analyses, and B2b, in addition to *S. connivens* (B2a), included as well *S. mexicana*. This node was the only topological incongruence between both methods; however, this relationship lacked support in the coalescent-based tree (Fig. 1a–b).

3.2.2 Phylogenetic informativeness

The amount of net phylogenetic informativeness varied among the captured loci, ranging from 2.8 to 303.5, with an average of 84.9. Most loci (65%) reached their maximum value at the time interval of 0.11–0.23, which includes B2a and the branch below B3 in Fig. 2a. All loci peaked at time 0.36, prior to the divergence of the core *Calosphace* clade. Most of the PI curves showed a steady increase in phylogenetic informativeness until the maximum value was reached. However, the PI curves of several of loci showed "phantom" spikes towards the present, as a result of a disproportionate increase in the substitution rate of a few characters relative to the remaining sites (Fig. 2a).

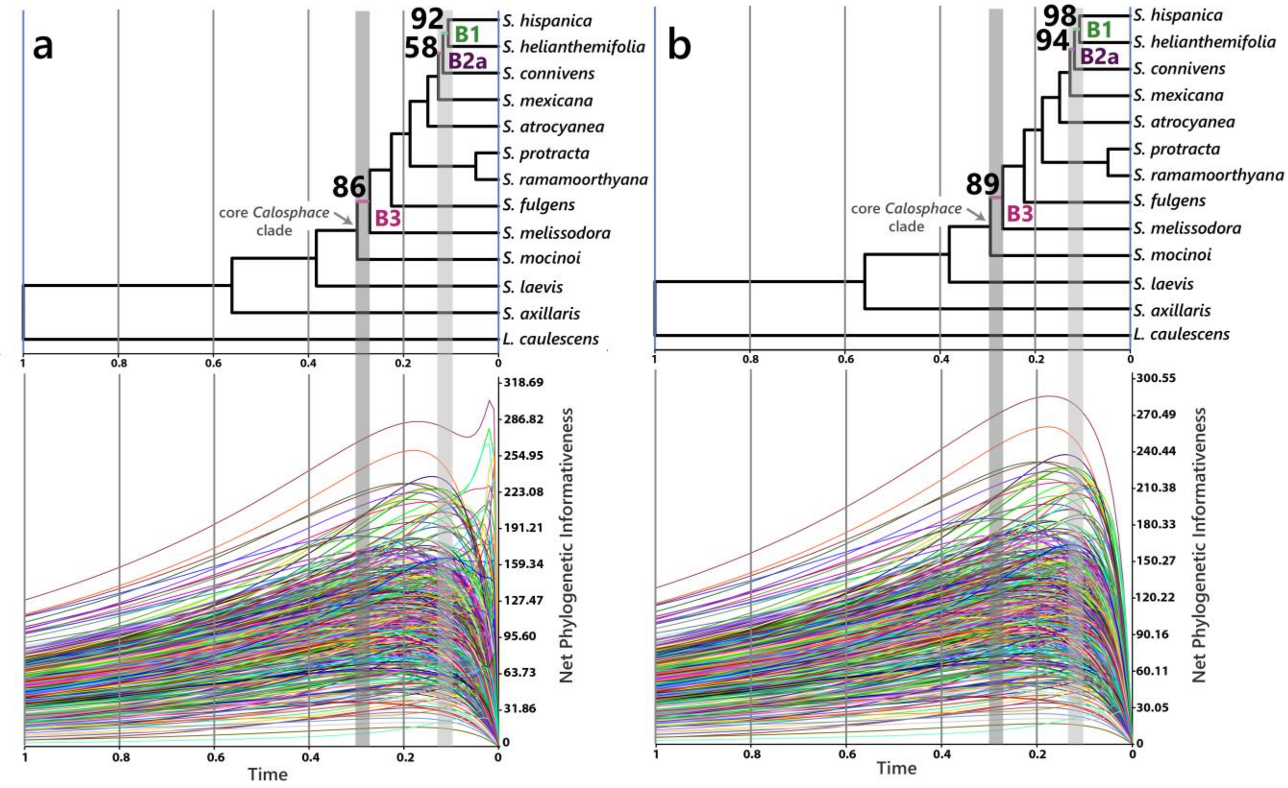

Fig. 2. Net phylogenetic informativeness of the AHE data through an arbitrary time scale: (a) profiles from the original, unfiltered dataset, (b) profiles from one filtered scenario where the sites with substitution rates >10 were removed. Branches that lacked 100% BS in the ML analysis are labeled as B1–B3. Most loci peak at/or deeper than B1 and B2a, and the slopes of most loci have decreased by B3, which is related to the decrease in PI for this branch. The curves from a number of loci of (a) show "phantom" spikes that suggest the presence of phylogenetic noise, whereas the curves from the loci of the filtered matrix (b) lack such spikes, which is related to the increased support values at B1–B3.

Regarding the conventional markers, ITS had higher net phylogenetic informativeness values than the two chloroplast markers. However, ITS reached its maximum value (168.2) before time 0.06, after the divergence of the core *Calosphace* clade. Both plastid markers had a similar behaviour, with lower PI values, that decreased slowly through time, and showed "phantom" spikes towards the present (Appendix A, Fig. A.2)

3.3 Comparison of the impact of different filtering schemes

3.3.1 Phylogenetic reconstruction

After the inspection of the PI curves (Fig. 2a) and the substitution rate scatterplots of each locus (Appendix A), the presence of a few loci with "outlier" sites was evident. Due to the great variation in the substitution rate values across loci, we decided to exclude sites with rates higher than an arbitrary threshold value. We evaluated the impact on phylogenetic reconstruction of four different filtering scenarios (Table 2), i.e. removing sites with substitution rates higher than 5, 10, 15 and 20.

Table 2. Characteristics of the concatenated matrices from each filtering scheme evaluated.

	Matrix	Total number of characters (% removed from the original matrix)	Loci filtered (% from total)
	Original	316,701 (0%)	0 (0%)
Less restrictive	**SR>20**	316,432 (0.08%)	160 (35.7%)
	SR> 15	316,332 (0.11%)	198 (44.1%)
	SR> 10	316,169 (0.16%)	251 (56%)
Strictest	**SR> 5**	315,626 (0.33%)	349 (77.9%)

SR= substitution rate

In most filtering scenarios, the trees obtained from the concatenated and coalescent-based analyses had some topological incongruences, albeit without high support. Trees derived from the same type of analysis but subjected to a different filtering scenario varied only in branch support. In the analyses of the concatenated matrices the gradual removal of sites with high substitution rates resulted in an increase of bootstrap support for the shallow branches (B1 and B2a, Fig. 2). The BS values showed a tendency to increase as more sites were removed. In the branch B3, while support values increased with site removal, this amount was not substantial and the BS values remained between 83–89%, decreasing below 85% in the strictest scheme, i.e. removal of substitution rates >5 (Fig. 3a–b; Appendix A, Figs. A.3–A.9). Support values for those three branches were higher in the scheme where sites with substitution rates higher than 10 were removed (Fig. 3a–b), than in the unfiltered dataset.

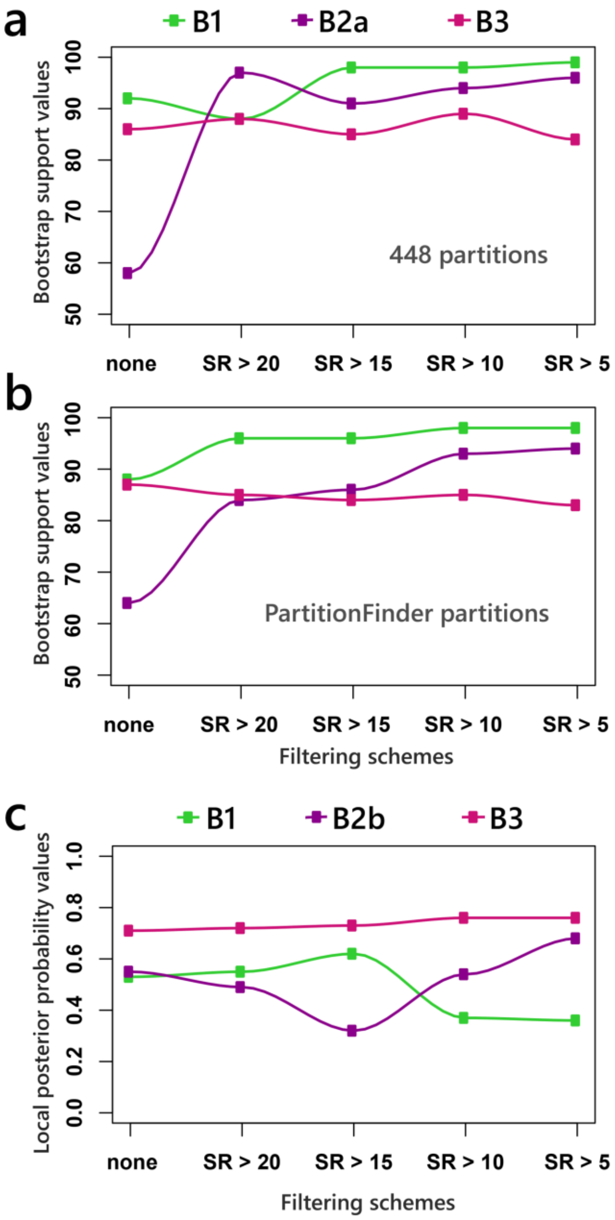

Fig. 3. Changes in support values in the different filtering schemes: bootstrap values on the analysis of the concatenated matrices (a) using 448 partitions, (b) using PartitionFinder schemes (7–17 partitions), (c) local posterior probability values on the coalescent-based analyses. Substitution rate removal scenarios depicted from less restrictive to the strictest: none = original dataset, sites removed with substitution rates higher than (SR>): 20, 15, 10 and 5. In general, for the concatenated analyses, in B1 and B2a the removal of sites with high substitution rates resulted in increased BS values, whereas for B3, removal of the sites increased slightly support values. In the coalescent-based analyses although LPP values increased in some scenarios, a significant value was never reached, and support values for B1 and B2b fluctuated between different scenarios, while the LPP values for B3 remained almost constant.

In the coalescent-based analyses, although the Local Posterior Probability (LPP) values increased in some scenarios, none of the changes were significant, unlike the analyses of the concatenated matrices. The LPP values of B3 were the highest among the branches of the three problematic nodes, and these values were stable across different schemes, behaving similarly to the concatenated analyses. In turn, the LPP values of B1 and B2b fluctuated strongly. The support value of B2b showed a tendency to increase, while that of B1 tended to decrease towards the strictest scheme (Fig. 3c; Appendix A, Figs. A.3–A.6).

3.3.2 Phylogenetic informativeness profiles

In general, the gradual removal of sites with high substitution rates (SR) resulted in smoother PI curves than those from the original matrix (Fig. 2; Appendix A, Figs. A.10–A.12). However, in the less strict scheme (SR> 20 removed) “phantom” spikes can still be distinguished in the profiles, although with lower peaks than those exhibited by the original dataset (Appendix A, Fig. A.10). In the remaining scenarios, no “phantom” spikes are visible and all the curves are smooth (Fig. 2b; Appendix A, Figs. A.11–A.12).

4. Discussion

4.1 Efficiency of AHE data for resolving relationships in *Salvia* subgenus *Calosphace*

As noted earlier, the probe design of the Angiosperm v. 1 kit (Buddenhagen et al., 2016) was based on the genomes of 25 angiosperms distributed along the flowering plant phylogeny to make the probes useful at a broad scale. Among those 25 species, the closest relative to *Salvia* is *Mimulus guttatus* DC. (Phrymaceae), also within order Lamiales. The fact that we were able to recover 399 loci (plus 49 additional orthologs) for our species of Lamiaceae, even though probe design was not based on a species of this family, confirms the universality of the Angiosperm v. 1 kit and suggests that this method could work for other families of the Lamiales. Our capture success suggests that the Angiosperm v. 1 kit, in combination with the AHE method, may allow to standardize loci sampling across major angiosperm lineages to produce large-scale phylogenomic trees.

4.2 Performance of captured phylogenomic data

4.2.1 Phylogenetic relationships

The phylogenetic tree from the concatenated matrix of the AHE data was better, in terms of support and resolution, than the tree obtained from the analysis of the concatenated matrix of conventional markers. In the tree derived from phylogenomic data all of the deep internal branches and most of the shallow ones were highly supported. The tree obtained represents a substantial advance in our understanding of phylogenetic relationships within core *Calosphace*, as phylogenetic resolution along the backbone of the clade had not been achieved previously. These results suggest that AHE data are a promising resource for phylogenetic reconstruction of this, and potentially other recently derived groups, even in the presence of short branches.

It is worth noting that the branches that did not achieve the highest levels of support using the AHE data involve the same clades that exhibited weak support with conventional markers. The branches B1, B2 and B3 received low support, even when using different datasets and inference methods (Fig. 1a–b; Appendix A, Fig. A.1, A.7). In the ML tree from the concatenated analysis of AHE data (Fig. 1a), the branches above B1–B3 are all 7–11 times longer than the extremely short problematic branches. These short branches are particularly difficult to resolve due to the combination of a low probability of synapomorphies to arise in such short time window involved, and the higher probability of homoplastic characters (e.g. by saturation resulting from multiple, independent changes to the same base) in the long branches above them, which could mislead phylogenetic reconstruction (Rokas and Carroll, 2006; Townsend et al., 2012).

Usually, short branches with low support below long branches have been interpreted as a rapid radiation (Rokas and Carroll, 2006). This could be a plausible explanation for the pattern observed here, since all the "problematic" branches of the tree are within the core *Calosphace* clade. Moreover, the lack of support on B1–B3 in the coalescent-based analysis suggests that there are incongruences among the gene trees at those nodes, which could be due to the small taxon sample analyzed or to incomplete lineage sorting, or reflect hybridization events. To resolve these problematic short branches, additional sampling will be needed, not only in characters, but also including more taxa, as suggested by Townsend and Leuenberger (2011). The latter could have a greater impact since many of the autapomorphies in the matrix could turn into synapomorphies with an expanded taxon sampling.

4.2.1 Phylogenetic informativeness

The combination of the difference in the amount of phylogenetic informativeness and the difference in the time at which each locus peaks led to a robust phylogenetic hypothesis for *Calosphace*, with most of the branches having strong support. When similar PI values are obtained, loci that peak at deeper phylogenetic levels than a time of interest are preferred for phylogenetic reconstruction; this choice minimizes the probability of including characters that could have evolved to convergent states (Townsend and Leuenberger, 2011). In this case, the fact that 21 loci peaked deeper than the core *Calosphace* crown node further supports the potential of the AHE loci for resolving the backbone of this clade.

During substitution rate estimation, the rates of certain sites in the alignments (usually indels or ambiguous base calls) cannot be accurately estimated by ML. This lack of accuracy causes the substitution rate values for these sites to be estimated as fast evolving sites (i.e. with high substitution rates), which are visualized in the PI profiles as "phantom" spikes towards the tips of the tree (López-Giráldez and Townsend, 2011; http://phydesign.townsend.yale.edu/). The presence of these spikes in the curves of some loci suggests that certain sites of those loci might introduce phylogenetic noise (Granados Mendoza et al., 2013). Because these spikes occur shallower than B1 and B2a, they may be introducing phylogenetic in them, which could account for the low support values obtained, compared with the highly supported adjacent branches. Regarding B3, "phantom" spikes are not affecting its resolution and support, but the obtained low BS values could depend on a combination of factors. First, loci with high peaks in recent times are likely to lead to noise at deeper phylogenetic levels. As most of the loci peaked before B3, it is expected for the curves to decrease deeper in the phylogeny, with a concomitant signal decrease and noise increase (Townsend and Leuenberger, 2011). This effect is due to the decrease in the number of sites evolving at an optimal rate for PI after the maximum PI is reached, which in turn increases the probability of introducing phylogenetic noise (Townsend and Leuenberger, 2011). Second, the short length of B3 might result in greater sensitivity to phylogenetic noise, as the latter may outweigh the signal in the data (Townsend et al., 2012; Townsend and Leuenberger, 2011).

An effort to resolve these conflicts could involve increasing the number of loci sampled, in particular loci that peak deeper than conflicting nodes, which have greater potential to resolve polytomies while introducing minimum noise (Townsend et al., 2012; Townsend and Leuenberger, 2011). However, taxon sampling could have a greater impact on resolution and support (Townsend and Leuenberger, 2011). If taxon sampling were to be increased, it is recommended to base it on previous phylogenetic hypotheses such that added taxa represent branches deeper than the clade of interest, as sampling recently derived taxa will have little effect on phylogenetic informativeness (Townsend and Lopez-Giráldez, 2010). Furthermore, because many loci have unusually fast substitution rates in the sample of taxa analysed here, adding more taxa will be a more productive approach in using this kind of data for phylogenetic reconstruction (Townsend and Leuenberger, 2011). Increasing taxon sampling has been suggested as a strategy to reduce or eliminate “phantom” spikes, as the addition of new taxa can aid in the estimation of substitution rates of sites with otherwise poor estimations (López-Giráldez and Townsend, 2011; http://phydesign.townsend.yale.edu/). The latter suggests that some of the sites here classified as having high substitution rates could be classified differently in the context of an expanded taxonomic sample, as calculations of substitution rates depend on the alignments but also on the branch lengths in the reference phylogeny. The addition of taxa would modify the branch lengths, which in turn will result in different substitution rate estimates for the sites in the alignment.

Increased support for “problematic” branches found here will require two distinct sampling approaches. Branches 1 and 2 are shallow, and occur in a time interval characterized by high informativeness, where poor resolution could result from close divergences (Townsend, 2007), but they are also affected by the phylogenetic noise introduced by the “phantom” spikes. In turn, B3 is a deeper short branch, in a phylogenetic level where data has low informativeness. In these cases, the addition of more data (i.e. loci or taxa) is recommended (Townsend 2007). In particular, an increased taxon sampling would have a greater impact on both kinds of branches, potentially decreasing the noise and resolving the relationships. Therefore, a future study of phylogenetic relationships of

Calosphace should ideally include a denser sampling of the deepest lineages within the core *Calosphace* clade to better resolve B3, whereas a denser sampling within the clade with *S. atrocyanea* as its deepest-branching lineage could improve support for B1 and B2, or alternatively, would confirm that these nodes are the result of a rapid radiation.

4.3 Impact of character removal on phylogenetic hypotheses

As mentioned earlier, the "phantom" spikes that appear in some of the PI curves are the result of sites usually having either indels or ambiguous base calls. Although it is difficult to appropriately estimate substitution rates for these sites, they are estimated to evolve at very fast rates, for which their removal is advisable (López-Giráldez and Townsend, 2011; http://phydesign.townsend.yale.edu/). In the concatenated analyses, the impact of removing sites with high substitution rates was positive, resulting in an increase in clade support for recent divergences. The support values for B1 and B2 showed a tendency to increase when more data were removed, which is also consistent with the disappearance of "phantom" spikes on most of the filtering schemes. Particularly, support of B2a showed a dramatic increase (from 58% to 97% BS) when the less strict scheme (i.e. removal of substitution rates >20) was applied to the dataset (Fig. 3a–b; Appendix A, Fig. A.3–A.9). Such increases in BS values when the "phantom" spikes were removed confirm that those sites were introducing noise into the phylogenetic inference, affecting directly B1 and B2a (Fig. 2). The fact that BS values for these branches improved upon character removal suggests that, if the clades associated with B1 and B2a represent a rapid radiation as hypothesized before, processes such as sequence saturation or incomplete lineage sorting could be affecting phylogenetic reconstruction. Support values for B3 also increased with character removal, though slightly, and decreased with the strictest scheme, which involved the exclusion of most of the sites. The latter suggests that the removal of certain amount of data (from 0.08–0.16% of the data from the original matrix, Table 2, Fig. 3a–b) can improve resolution and support on this branch by reducing the slopes in the profiles, and causing the curves to decrease in a steadier fashion, resulting in a slight decrease in noise at B3 (Fig. 2b, Appendix A, Figs. A.10–A.12). However, the tendency of BS values to decrease in B3 when data removal increases (removal of 0.33% of the data from the original matrix, i.e. strictest scheme; Appendix A, Figs. A.12) agrees with the hypothesis discussed earlier, regarding the need to add more data to resolve this node (Townsend, 2007). Since there is already a lack of data in the dataset to resolve B3, the removal of significant amounts of data (as in the strictest scheme, Table 2) can result in removing also informative characters, i.e. synapomorphies, for B3. An additional interpretation could be that B3 is only supported by noise. If this were the case, removal of sites with faster rates would decrease its support.

In the coalescent-based analyses, although there was fluctuation among the LPP values across different removal schemes (especially in B1 and B2b), none of these changes were sufficient to make these probability values significant (Fig. 3c). These results agree with the fact that coalescent-based methods are more robust to high substitution rates than concatenated methods (Xi et al., 2014). Low LPP values suggest incongruences among individual gene trees that affect those branches, which could result from lack of data to support certain parts of the phylogeny, or from incomplete lineage sorting, which is expected given the recent history of *Calosphace* (~19.1–11.9 Ma since its estimated split from subgenus *Audibertia*; Walker et al., 2015).

Although all the exclusion schemes applied resulted in higher BS values on at least two of the problematic branches than the original dataset, none produced the highest BS values for all three branches. This means that there is not a single scheme that on itself will increase BS support on all branches, and it is highly recommended the exploration of several filtering schemes. Most of the sites with high substitution rates that were removed contained unresolved (N) or missing information for at least one taxon, which resulted in lack of information. However, sites involving transversions also had high substitution rates, sometimes indistinguishable from those caused by lack of information, suggesting that during character removal some informative sites could be excluded along with "noisy" characters. It would be desirable to develop an algorithm to distinguish between high substitution rates resulting from missing information or transversions, to test the effect of the removal of these two kinds of data.

5. Conclusion

Loci capture using the Angiosperm v. 1 kit (Buddenhagen et al., 2016) with the AHE method in a New World sage lineage (*Salvia* subgenus *Calosphace*), was successful. We were able to capture 78% of the 517 genes targeted by the kit, confirming its universality. We obtained 448 alignments, over one half of them including sequences for all the sampled taxa, and the remaining missing less than 30% of the taxa. The success of the AHE method in our study group is encouraging and suggests that other lineages from Lamiales could potentially recover similar amounts of data.

The AHE data allowed the recovery of strongly supported phylogenetic trees for *Calosphace*, with strong support for most of the internal branches. These phylogenetic trees were superior in terms of support and resolution over those based on conventional markers. However, problematic branches with low support were still found. In both the concatenated and coalescent-based phylogenetic reconstruction methods applied here, these extremely short branches with limited support subtended groups that included the species: *S. hispanica, S. helianthemifolia, S. connivens, S. mexicana* and *S. melissodora*. The phylogenetic informativeness analysis showed that the recovered loci varied greatly in their net phylogenetic informativeness and its temporal distribution, suggesting that these data are promising for the reconstruction of the relationships of our model group. Nevertheless, noise in some loci is suggested by "phantom" spikes in the recent portion of phylogenetic informativeness curves.

The removal of sites with high substitution rates to remove noise resulted in the elimination of the "phantom" spikes and an increase in bootstrap support in the shallower problematic branches. Although the support values increased for the problematic deep branch, progressive removal of greater amounts of data had a negative effect on its support values. We suggest that an exploration of the PI profiles to detect "phantom" spikes, and a subsequent filtering of the data testing different rate substitution removal scenarios should be a step prior to phylogenetic inference using this kind of phylogenomic data. Also, increased taxon sampling is likely to aid in the resolution of short branches and the reduction or elimination of "phantom" spikes.

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PAMP—Plant Anchored MetaPrep: a high-throughput method for generating hundreds of orthologous loci for densely sampled phylogenomic studies: report on the performance in the Neotropical sages (*Salvia* subgenus *Calosphace*; Lamiaceae)

Abstract: Targeted sequence capture followed by next-generation sequencing (NGS), allows researchers to obtain phylogenomic data in short amounts of time, boosting studies in several plant groups. However, to achieve taxon sampling as required for macroevolutionary studies, the cost of these methods becomes prohibitive; almost 40% of this cost correspond to library preparation. In this study, we applied a modification to this step that involves combining DNA from six different lineages of angiosperms: *Aristolochia* (Magnoliidae), *Epidendrum* and *Tillandsia* (Monocotyledonae), Crassulaceae and Malvales (Superrosidae) and *Salvia* subgenus *Calosphace* (Superasteridae). These samples are indexed as a project and not individually or by lineage as commonly done. In order for the Plant Anchored MetaPrep (PAMP) to function, the lineages included need to be at least 100 ma apart, so that genetic distance will be enough to separate the loci sequences bioinformatically. The results presented here correspond to the Neotropical sages. We evaluated the effectiveness of gene capture and high-throughput sequencing for *Calosphace,* tested the performance of the captured loci in resolving and supporting internal relationships in the group and identified possible conflictive taxa by comparing allele trees. 315 alignments corresponding to 290 of the loci from the capture kit were recovered, the remaining 25 alignments belonged to paralogs of some loci that were separated successfully. Upon visual inspection of the matrices we found a high amount of N's, ambiguities of two and three nucleotides which seem to increase in species with poor capture or polyploidy. Phylogenetic relationships recovered using this phylogenomic data were more robust than with conventional markers; however, topological incongruence among several clades was identified. We attribute this incongruence to polyploidy, since some of the taxa involved were not monophyletic in allele trees. Furthermore, some of these species were confirmed to be polyploids in literature, suggesting that knowledge of ploidy levels is key to process phylogenomic data, to stablish accurate contamination filters and parameters during allele phasing.

1. Introduction

Genomic partition strategies are alternatives to whole-genome sequencing, that incorporate selectively targeted regions of the genome, yielding large-scale phylogenomic data (Lemmon and

Lemmon, 2013; Turner et al., 2009). These data are composed of a large amount of potentially homologous loci from multiple individual in less time and with less effort that Sanger-based sequencing (Blaimer et al., 2015). As the amount of data increases, so does the quantity of informative characters for phylogenetic reconstruction, allowing for the recovery of better resolved and supported phylogenetic frameworks at different phylogenetic scales (e.g., Zingiberales: Carlsen et al., 2018; Apocynaceae: Fishbein et al., 2018; *Heuchera*: Folk et al., 2015; Bignoniae: Fonseca and Lohmann, 2018).

One of such strategies is the Anchored Hybrid Enrichment (AHE; Lemmon et al. 2012), that targets over 500 highly-conserved regions of the genome, flanked by less-conserved regions, in order to obtain a set of loci useful to resolve relationships at different phylogenetic depths. This method was originally developed for vertebrates, but after its implementation in flowering plants (Buddenhagen et al., 2016), it has been used to investigate the relationships of a number of groups at different scales, including the whole angiosperm clade (Buddenhagen et al., 2016), the Cyperaceae family (Léveillé-Bourret et al., 2018), the genera *Aristolochia* (Wanke et al., 2017), *Protea* (Mitchell et al., 2017), *Salvia* (Fragoso-Martínez et al., 2017), and even a species complex (*Lepanthes horrida* complex; Bogarín et al., 2018).

As other genomic partitioning strategies, the AHE has become a powerful tool to untangle recent radiations, something that researchers were unable to do before using sanger-based strategies, due to the time and effort needed to accumulate large amounts of data. However, when considering a large number of taxa or individuals/samples, as commonly occurs in macroevolutionary studies, the cost of the method becomes prohibitive. Library preparation is a procedure that involves the ligation of adapters, specific for particular high- throughput sequencing platform to fragmented DNA. These adapters can be indexed to help identify samples from different individuals when they are pooled for sequencing (Lemmon and Lemmon, 2013). In the AHE method, library preparation is the most expensive step of the whole procedure, representing ~40% of the total cost per sample (200USD per sample= 80USD library preparation).

To overcome the elevated cost per sample of library preparation, Lemmon (2015) developed the Anchored MetaPrep method, that involves and additional step in the AHE methodology, before library preparation. In this step, samples from different taxa are pooled before libraries are prepared, and instead of indexing each individual as performed in the AHE, a group of samples are indexed per project. The success of the Anchored MetaPrep relies on the taxa pooled to be sufficiently phylogenetically distant, for the sequenced reads to be sorted out bioinformatically by means of genetic distances. By implementing the Anchored MetaPrep, the cost of library preparation per sample decreases by a factor of 10 (from ~80USD to ~8USD), enhancing the study of many angiosperm clades per project.

In this study the Plant Anchored MetaPrep (PAMP) is first implemented in an extended sample (48 samples per lineage) of six lineages distributed throughout the angiosperm phylogeny. All of these lineages are the focus of individual macroevolutionary investigations and were selected for this pilot PAMP study based on the evolutionary distances among them (greater than 100 ma). These six groups represent four major angiosperm lineages: Magnoliidae, Monocotyledonae, Superasteridae and Superrosidae. It is the aim of this report to present the results and discuss the performance of the method in only one of the lineages: the Neotropical sages (*Salvia* subgenus *Calosphace*; Lamiaceae).

Calosphace is the most species-rich subgenus of *Salvia* with ca. 600 species (Santos, 1995). These Neotropical sages are distributed mainly along the mountain chains of Mesoamerica and South America, being especially diverse in montane tropical forests (Espejo and Ramamoorthy, 1993). Most of its diversity is found in Mexico (295 spp.; Martínez-Gordillo et al., 2017), but there are other important diversity centers like the Andes (155 spp.) and the Antilles (45 spp; Jenks et al., 2013). The phylogenetic relationships between the species of *Calosphace* have been explored based on conventional markers. However, the backbone of the core Calosphace clade, a lineage characterized by short internal branches and likely product of a radiation, remains unresolved (Fragoso-Martínez et al., 2018; Jenks et al., 2013). The AHE method has already shown promising results in the phylogenetic reconstruction of *Calosphace*, using a reduced but representative taxon sampling (Fragoso-Martínez et al., 2017).

In the present study a taxon sampling of 47 members of *Calosphace* and a species from its sister clade (*Salvia* subgenus *Audibertia*) are used to test the performance of the phylogenomic data obtained from the PAMP, coupled with high-throughput sequencing. The aims of this preliminary report are to: 1) evaluate the effectiveness of gene capture and high-throughput sequencing for *Calosphace*; 2) test the performance of the captured loci in resolving and supporting internal relationships in the group using different phylogenetic inference methods (maximum likelihood vs. coalescence-based analyses); 3) compare allele trees to identify possible conflictive taxa.

2. Material and methods

2.1 Loci and taxon sampling

Loci sampled were the 517 nuclear regions selected for the Angiosperm v.1 enrichment kit (Buddenhagen et al., 2016). To evaluate the performance of the PAMP data to resolve the backbone of the core Calosphace clade, taxon sampling consisted of 37 species representing its subclades and ten species from the remaining lineages of *Calosphace.* A species from subgenus *Audibertia* was used as an outgroup (Appendix, Table S1).

2.2 Molecular and bioinformatic methods

DNA extraction and fragmentation followed Fragoso-Martínez et al. (2017). Library preparation and bioinformatic processing of NGS data followed Granados Mendoza et al. (in prep.). Allele phasing was performed with the Allele phaser program developed by Pyron et al. (2016), which determines the number of alleles that are supported statistically by the assembled reads. In each locus, polymorphic sites are identified from the read pairs, base calling is performed for each polymorphic site and alleles containing only these sites are constructed. Using a Bayesian statistical framework, the posterior distribution of allele sets is estimated, where changes in allelic states either by recombination (exchanging bases between two randomly selected alleles) or mutation (drawing from a uniform prior a new base for a random position of a randomly selected allele) are proposed. The likelihood of the newly proposed state is computed using a probability matrix that assumes 0.75% of sequencing error. The likelihood value is used to calculate the Hastings ratio, which is the difference between the likelihood scores of the initial state and the current state. The new state is accepted if the ratio is greater than the natural logarithm of a randomly chosen number between 0 and 1. After the chains are ran for 20,000 generations and the posterior distribution of the alleles is estimated, the polymorphic bases in the original sequence are replaced with the bases obtained from the posterior distribution. This method can consider uncertainty in two different ways: 1) retrieving fully-phased alleles, lacking ambiguities, randomly from the posterior distribution, or 2) generating partially-phased alleles that lack ambiguities in the largest region (with 95% of posterior confidence) and the remaining of the sequence with polymorphic sites.

2.3 Phylogenetic analyses

Maximum likelihood analyses from the concatenated and single locus matrices were performed in RAxML-HPC2 (Stamatakis, 2014). Phylogenetic trees were inferred using the GTRGAMMA model with 100 bootstrap replicates. The concatenated matrices were partitioned by locus: three loci in the conventional marker matrix and 315 loci in the phylogenomic matrix. The coalescent-based analyses were performed over the gene trees obtained in the previous step in ASTRAL (Mirarab et al., 2014).

2.4 Statistical analyses

Indexes (ambiguities, N's and gaps amount per species) from the concatenated alignment were drawn from Geneious (Kearse et al., 2012) and plotted into the phylogeny using the function "plotTree.barplot" from the phytools package (Revell, 2012) in R (R Core Team, 2017). Correlation tests and histograms were also made in R, and the latter plotted using the ggplot2 package (Wickham, 2016).

3. Results

3.1 Gene capture and high-throughput sequencing for Calosphace

3.1.1 Number of loci captured

From the 517 loci targeted in the angiosperm V.1 enrichment kit, 290 loci were captured; 23 of those loci had 2-4 copies that could be separated successfully into orthologous sets, yielding 315 alignments. The species that had the best capture was *Salvia libanensis* (306 loci), while the species with the poorest capture was *Salvia vazquezii* (79 loci). Although, the latter seems to have been related to a pipetting error during sample pooling, since capture was poor for all the lineages in the same plate well (B5) that sample was contained and pooled with (**Table 1**). The second species with lowest capture was *Salvia holwayii* (140 loci).

Table 1. Number of raw reads recovered for the samples contained in well B5, compared to those in the well with the worst and best capture.

	No. Raw reads		
Lineages	**B5 well**	**Worst capture**	**Best capture**
Crassulaceae	2,908	2,908	43,382
Epidendrum	4,160	4,160	67,662
Malvales	1,560	1,560	234,546
Salvia	18,872	18,872	185,194
Tillandsia	6,544	3,666	197,268

*Lineages with highlighted cells are those in which well B5 had the worst capture among all sequenced 48 wells.

3.1.2 Alignment features

The length of the alignments ranged from 168-1700 bp, with an average size of 495 bp (**Fig. 1a**). Sixty one percent of the matrices contained 75-100% of the species, from them only 27 had all the species (**Fig. 1b**). The length of the concatenated alignment was 156, 189 bp. In this alignment, the percentage of sites with ambiguities of two (**Table 2; Fig. 1c**) and three nucleotides (D, V, H, B; **Fig. 1d**) was low. However, the proportion of N's (**Table 2; Fig. 1e**) and gaps (**Fig. 1f**) was higher than the ambiguities in the concatenated alignment, most of the species with highest percentage of gaps, are the same species with the lowest capture (e.g., *S. vazquezii, S. holwayii, S. raymondii*).

Table 2. Features from the concatenated alignment of 48 taxa as an indirect quality indicator of the data.

Alignment feature	Range (mean value)
Two nucleotide ambiguities	0.19-4.1% (1.6%)
Three nucleotide ambiguities	0-0.9% (0.25%)
N's	0-8.6% (2.9%)
Insertions-deletions	4.9-83.8% (20.5%)

3.1.1 *Performance of the PAMP data in the phylogenetic relationships of the core Calosphace clade*

Compared to the phylogeny based on conventional markers, the one inferred with phylogenomic data has a higher number of supported nodes, with only eight nodes exhibiting low bootstrap support (BS≤85) vs. 13 nodes. However, there is topological incongruence between both topologies in at least five clades (**Fig. 2a**), involving shallow internal branches (e.g., Angulatae clade), as well as deep internal branches (e.g., Erythrostachys + Tomentellae II clade). Albeit, only one of these incongruences are strongly supported. When phylogenies based on the PAMP data, using maximum likelihood vs. coalescent methods are compared, discordance between topologies is lower, lack support and is localized only in subclades of core Calosphace (recent divergences; **Fig. 2b**). Despite of the large amount of data used for phylogenetic inference, there are 8 and10 (17-27.2%) short internal branches with low support values in the trees from the concatenated and coalescent analyses, respectively.

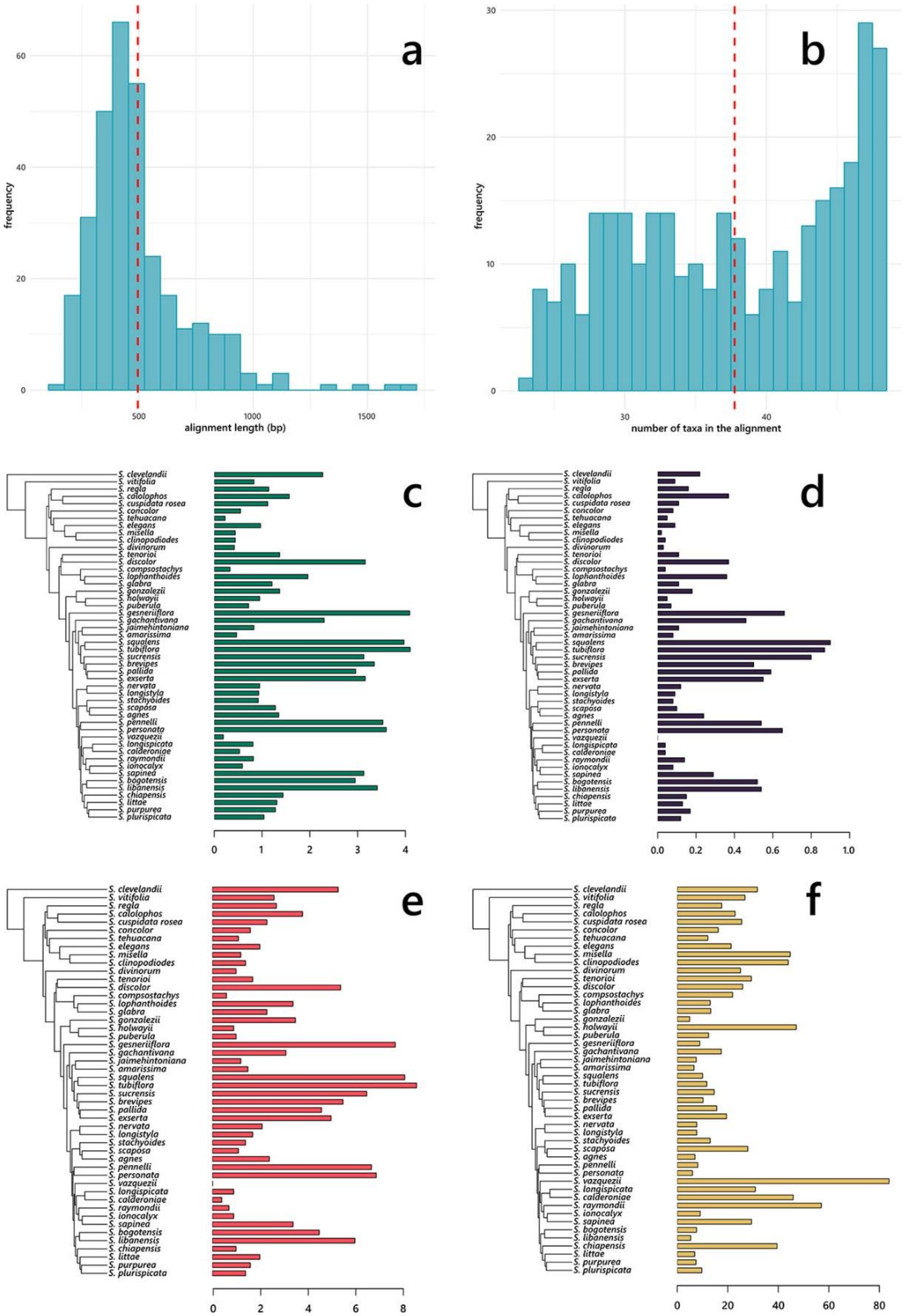

Fig 1. Histograms showing features of the individual alignments recovered: a) alignment length; b) number of taxa in the alignments. The pink dashed line marks the mean value. Phylogenetic trees with bar plots of the percent of certain attributes present in the concatenated matrix: c) ambiguities of two nucleotides; d) ambiguities of three nucleotides; e) N or any base; f) gaps representing only fragments of the loci were recovered.

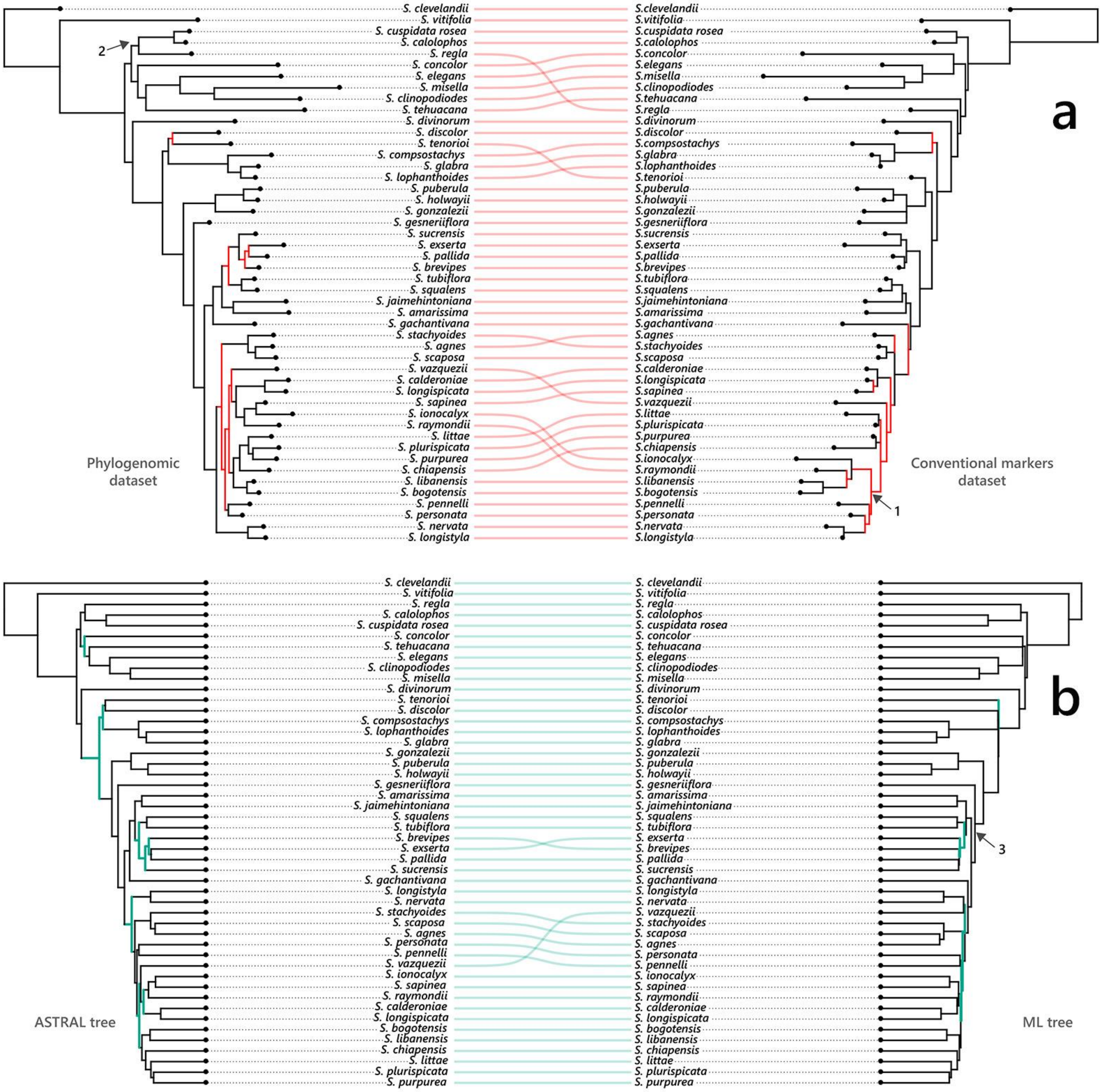

Fig 2. Phylogenetic hypotheses for representative species of *Calosphace*; a) Maximum likelihood trees from the concatenated matrices of the PAMP dataset (left) and the conventional markers (right); b) analyses performed on the phylogenomic dataset, coalescence (left) and ML (left). Central lines mark topological incongruences, coloured internodes denote low support (≤85 BS). Arrows and numbers mark the following clades: 1. Angulatae, 2. Tomentellae II, 3. South American clade.

3.2 Allele trees and monophyly of species

Using both alleles for the 48 taxa sampled, phylogenetic relationships were inferred to investigate monophyly of the species. The trees from the maximum likelihood method (concatenated matrix) and the coalescent based method were highly congruent and only the latter is shown in **Figure**

3. Nine species were not recovered as monophyletic, all included in the core Calosphace clade: *Salvia bogotensis, S. brevipes, S. gesneriiflora, S. libanensis, S. pallida, S. sapinea, S. sucrensis, S. squalens* and *S. tubiflora*. Some of these species are included in the clades with low support in the analyses of the consensus sequences (i.e., *S. pallida, S. brevipes* and *S. sucrensis*; **Fig. 2b**). Other species are sources of topological incongruence between datasets (e.g., *S. gesneriiflora* and *S. sapinea*; **Fig. 2a**) or methods (i.e., *S. brevipes*; **Fig. 2b**).

4. Discussion

4.1 *Loci capture and genome size*

Compared to the amount of loci captured using the regular AHE method for *Calosphace* in a previous study (Fragoso-Martínez et al., 2017), with the implementation of the PAMP method, loci capture decreased ca. 30%. During library preparation, samples from the different lineages were pooled in proportion to their DNA concentration to try to ensure an equal amount of DNA from every lineage. However, in an DNA extract from a polyploid species this concentration value should be corrected to account for the excess of genetic material. This step is particularly important, as recently a positive correlation between genome size and loci captured has been found (Bossert et al., 2018). Since most of the species that we included in the sampling have not been studied in terms of ploidy level or DNA content, we were unable to account for this variable. It is possible that the inclusion of a lineage like Crassulaceae, with a large genome size compared to the remaining lineages (**Table 3**) could have led to the poor enrichment of the latter groups. Nevertheless, other factors that have been related to poor enrichment during wet-lab procedures are: low DNA extract quality (Li et al., 2013), pooling variance during library preparation as seen here in plate well B5 and discussed by Gates et al. (2018) and sequencing platform (Quail et al., 2012).

Table 3. DNA C-values* (Bennet & Leitch, 2012) from the lineages included in the PAMP experiment.

Lineages	n counts	Range (sd)	Mean
Crassulaceae *(Graptopetalum)*	2	3.35-4.20 (0.43).	3.78
Epidendrum	1	1.45	-
Malvales	152	0.19-4.10 (0.88)	1.60
Salvia	12	0.43-1.18 (0.25)	0.71
Tillandsia	2	1.11-1.26 (0.08)	1.18

*DNA amount in the unreplicated gametic nucleus of an organism

4.2 *Quality of the phylogenomic data recovered from the PAMP method*

The individual locus alignments were in average shorter in length than those the regular AHE, and the concatenated matrix was half the size of the one recovered by Fragoso-Martínez et al. (2017). The outcome of the reduction in loci capture and matrix length is decrement of characters for phylogenetic inference. While this amount of missing data might not affect analyses performed on concatenated matrices (Jiang et al., 2014), the inference with coalescence-based methods can be affected. For instance, in an individual locus when matrix length decreases, so does the probability of having sufficient phylogenetic information to produce a resolved or reliable gene tree, affecting the inference of the species tree (Xi et al., 2015). It has been proposed that an alternative to outweigh the unresolved or unreliable gene trees is to sample more genes (Xi et al., 2015). Since our loci capture decreased, we are unable to follow that approach; however, taxon sampling was expanded compared to Fragoso-Martínez et al. (2017), which could increase resolution.

In addition to the size of the matrices, other three indexes were retrieved for the concatenated matrix. The ambiguities of two nucleotides (1.6% in average; **Fig. 1c**), which suggests heterozygosity for some loci as expected for nuclear genes. However, the high-values of three nucleotide ambiguities (0.25% in average; **Fig. 1d**) and N's (2.93% in average; **Fig. 1e**) recovered for some species could be pointing towards concerning issues that could have been overlooked during the bioinformatic process, such as: polyploidy (higher number of alleles) or inability in separating paralogs. These two indexes could be useful to identify putative polyploids or conflictive taxa, since among the 15 taxa with the highest values are the nine non-monophyletic species in the alleles tree (**Fig. 3**). Finally, the number of gaps (20.5% in average; **Fig. 1f**) can be interpreted as the result of the partial recovery of certain loci, probably due to poor capture, leading to low number of reads in certain regions; but can also be the result of insertion-deletion events in some species.

Further exploration of these data showed that the indexes are strongly correlated **(Fig. 4)**. Positive correlation among three nucleotide ambiguities and N's percent could point towards problems in the contamination filter of the pipeline or during allele phasing. Moreover, the correlation of these two variables with two nucleotides ambiguities might, in fact mean that the latter are an artifact of the same pipeline problems and not a result of heterozygosity. On the other hand, negative correlation among those three variables and gap percentage is expected since they tend to decrease when missing data increases. High percentage values of these indexes in the matrices could result in a phylogenetic reconstruction with erroneous relationships for *Calosphace*. Nevertheless, there is a bioinformatic strategy that can be followed in order to improve matrix quality, that will be discussed in the next section.

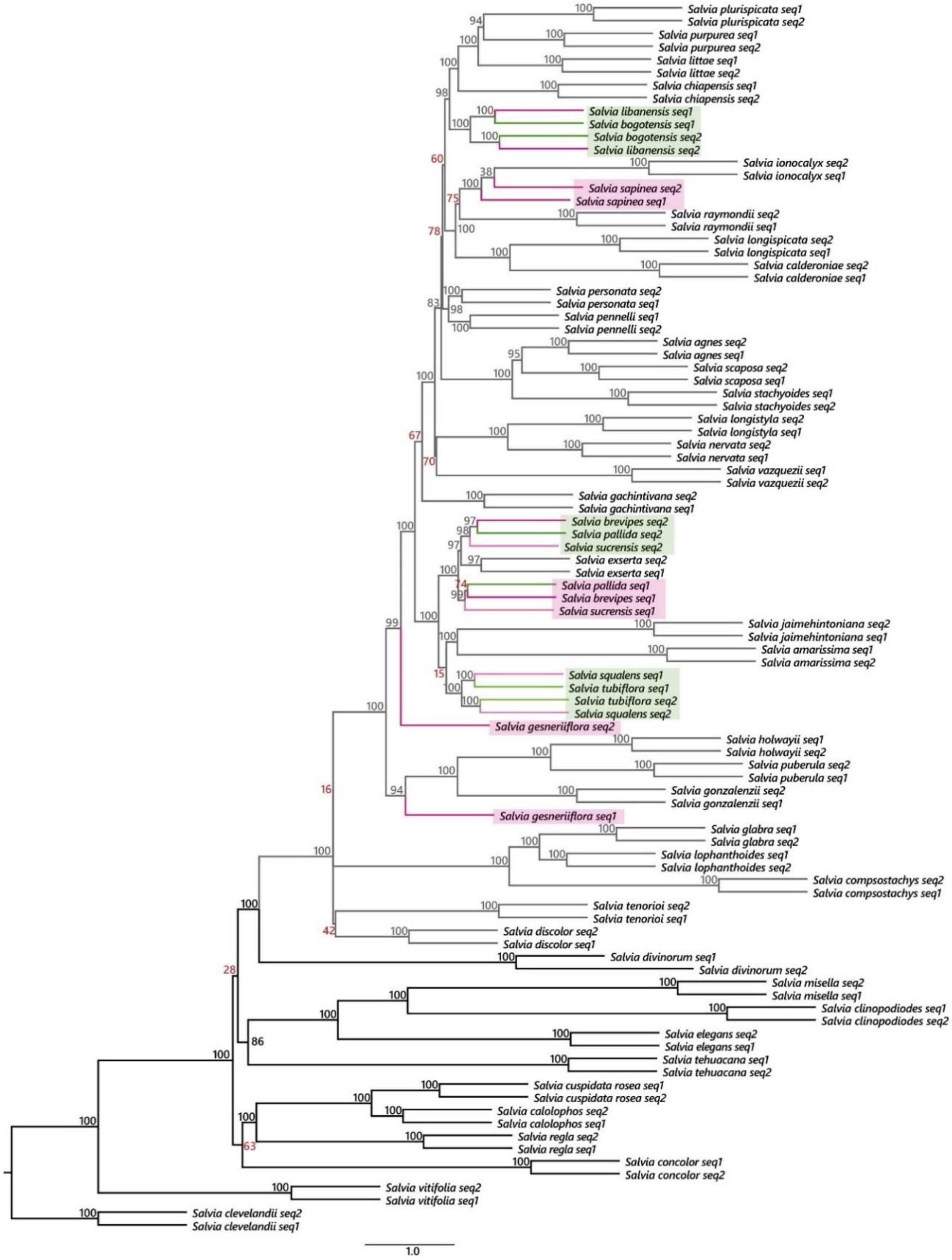

Fig 3. Tree from the coalescence-based analysis of the alleles. Non-monophyletic species are denoted by the coloured boxes.

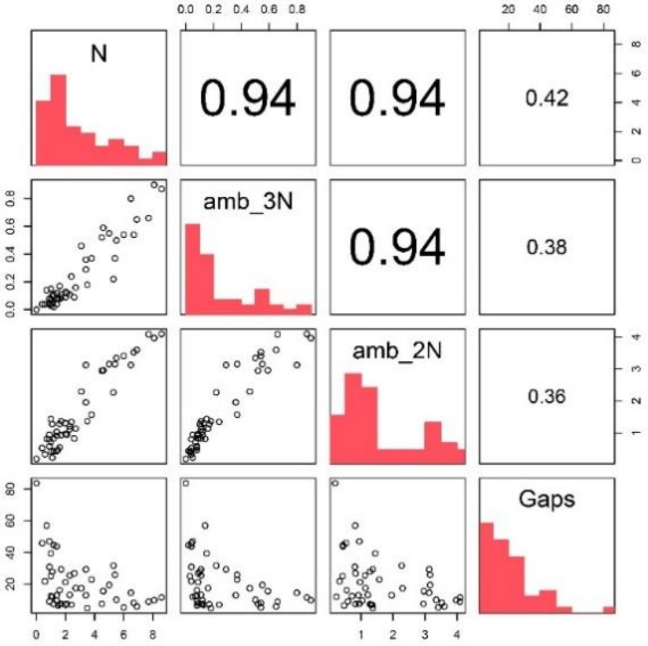

Figure 4. Relationships among the four indexes drawn from the concatenated matrix. Lower panel with scatterplots, diagonal showing histograms of each variable and upper panel with correlation coefficients. All correlations were significative under Pearson's test p<0.05.

4.3 Phylogeny of Calosphace, topological incongruence and non-monophyletic species

Although the phylogeny based on the phylogenomic dataset had increased support (**Fig. 2a**) and most recalcitrant nodes coincide in both topologies, the features discussed in the previous section make the phylogenomic trees less reliable hypotheses. This possibility is further supported by the high amount of topological incongruency found between those datasets, conversely to what had been previously reported using data from the regular AHE and conventional markers, where no topological incongruence was found (Fragoso-Martínez et al., 2017). It is expected that the probability of incongruence increases when taxon sampling is expanded compared to Fragoso-Martínez et al. (2017). Nevertheless, identifying high proportions of three nucleotide ambiguities and N's, as well as non-monophyletic species in the allele tree suggest that this incongruence is product of poliploidy in some of the sampled species.

Particularly, polyploidy in *Calosphace* has been well documented in the South American species (Alberto et al., 2003; Jenks et al., 2011) and only in a few North American species (Palomino et al., 1986). However, proportional to the diversity of the subgenus, only the chromosomic numbers of a few species are known. From the species included in the PAMP pilot study, the ones with known chromosome numbers are displayed in **Table 4.** From the species in the table, three are known polyploids and only two were non-monophyletic in **Fig. 3**.

Table 4. Known chromosome numbers of the species included in the PAMP sampling.

Species	Basic number (x)	Ploidy level	Reference
Salvia amarissima	10	2x	Mercado et al. (1989)
Salvia clevelandii	15	2x	Epling et al. (1962)
Salvia discolor	11	4x	Harley & Heywood (1992)
Salvia divinorum	11	2x	Reisfield (1989)
Salvia elegans	10	2x	Harley & Heywood (1992)
Salvia gesneriiflora	11	4x	Harley & Heywood (1992)
Salvia longispicata	11	2x	Mercado et al. (1989)
Salvia pallida	11	8x	Alberto et al. (2003)

*Highlighted cells correspond to polyploid species

Polyploidy is an important process of plant evolution and needs to be considered and dealt with during phylogenomic data processing. *Calosphace* is not the first lineage with polyploid representatives that has been found in the AHE datasets. A subset of genus *Aristolochia* had similar problems, the solution was to assume a tetraploid scheme for those species during the allele phasing step in the pipeline and four alleles instead of two were generated, resulting in monophyletic species (Wanke et al., 2017). The first approach that needs to be followed to curate the data from the *Calosphace* PAMP study is to assume a higher level of ploidy to the nine non-monophyletic species plus *Salvia discolor*, that although resulted monophyletic (**Fig. 3**) there is evidence that is a tetraploid species (**Table 4**) and should be treated as such.

If the phylogenies continue to show topological incongruences or high amounts of ambiguities, the next approach to follow add to the scheme the other species that ranked among the 15 with the highest amounts of N's and three nucleotide ambiguities, and thus could be under suspicion of polyploidy*: S. personata, S. exserta, S. pennelli, S. gachantivana, S. calolophos* and *S. lophanthoides* (**Fig. 2d-e**). If chromosome counts for some of these species could be obtained for some of these species and polyploidy is confirmed, the proportion of ambiguities in the alignments could be good indicators of a high level of ploidy in *Calosphace*. If after these two approaches are followed and no substantial improvement of the alignments is seen, data extraction could be tried through a different pipeline (e.g., HybPiper; Johnson et al., 2016) as has been done by Herrando-Moraira et al. (2018), or poorly captured taxa could be filtered to evaluate if phylogenetic reconstruction is benefited from it (Gates et al., 2018).

5. Conclusion

The PAMP method constitutes a powerful strategy that will enable collaboration among research groups and boost macroevolutionary studies. The results obtained in this pilot study are promising and although we encountered some challenges, we were able to recover more than 300 loci for *Calosphace* and over 150 for the remaining lineages (excepting Crassulaceace). We found that the amount of N's and ambiguities per species in an alignment might be an indirect indicator of polyploidy. With this information, along with known chromosome numbers of some species of the study group, parameter adjustment can be performed to the pipeline and improve data quality. There are some considerations that need to be taken into account when selecting the lineages to be included in the PAMP experiment. For instance, evolutionary divergence among lineages (>100ma), genome size, ploidy level, DNA quality. If capture or data quality is poor even when these points were considered, there are some strategies to follow, to enhance matrix features. The first approach is bioinformatic, since most of the problems outlined in this study can be alleviated by changing some parameters during the allele phasing of the pipeline. However, if the problem seems to be failure to separate paralogs, parameters could be adjusted to make a stricter contamination filter. Also, other authors have shown that removal of poorly captured individuals improve phylogenetic reconstruction. If the bioinformatic procedure fails, a wet laboratory strategy that can be followed is re-sequencing poorly captured samples through the conventional AHE method or running additional rounds of sequencing to try to increase coverage and decrease the amount of missing data in the matrix.

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Appendix

Table S1. Sampled species, voucher specimens and genbank accession codes.

Taxa	Author or Voucher	1) ITS	2) *trn*L-*trn*F	3) *psb*A-*trn*H
S. clevelandii (Audibertia)	*I. Fragoso-Martínez & R. Middleton 229* (cult.)	MF664560	MF663961	MF663819
Salvia agnes	Mexico. *M. Harker 2986* (IBUG)	MF664541	MF663941	MF663800
Salvia amarissima	Mexico. *M. Castañeda-Zarate 837* (MEXU)	MF664544	MF663945	MF663803
Salvia bogotensis	*I. Fragoso-Martínez & R. Middleton 221* (cult.)	MF664550	MF663951	MF663809
Salvia brevipes	Argentina. *S. Benitez 16* (CORD)	MF664552	MF663953	MF663811
Salvia calderoniae	Mexico. *S. Zamudio et al. 16545* (IEB)	MH574780	MH594469	
Salvia calolophos	Argentina. *S. Benitez-Vieyra 2* (CORD)	MF664556	MF663957	MF663815
Salvia chiapensis	Mexico. *G. Salazar-Chávez 9593* (MEXU)			
Salvia clinopodioides	Mexico. *F. Sazatornil 10* (MEXU)	MF664561	MF663962	MF663820
S. compsostachys	Mexico. *Hinton et al. 24799* (MEXU)	MF664562	MF663963	MF663821
Salvia concolor	Mexico. M. Martínez s.n. (FCME)	HQ418858	HQ418961	HQ418910
S. cuspidata var. *rosea*	Bolivia. *A. Cocucci 5547* (CORD)	MF664569	MF663971	MF663828
Salvia discolor	*I. Fragoso-Martínez & R. Middleton 224* (cult.)	MF664572	MF663974	MF663831
Salvia divinorum	Mexico. *I. Fragoso-Martínez & M. Martínez 176* (FCME)	MF664573	MF663976	MF663832
Salvia elegans	Mexico. G. Salazar-Chávez 9430 (MEXU)	MF622127	MF663978	MF663834
Salvia exserta	Bolivia. *A. Cocucci 5537* (CORD)	MF664575	MF663979	MF663835
S. gachantivana	*I. Fragoso-Martínez & R. Middleton 205* (cult.)	MF664580	MF663984	MF663840
Salvia gesneriiflora	Mexico. *G. Salazar-Chávez 9217* (MEXU)		MF663986	MF663842
Salvia glabra	Mexico. *I. Fragoso-Martínez 390* (FCME)			
Salvia gonzalezii	Mexico. *M. Castañeda-Zarate 1109* (MEXU)			
Salvia holwayi	Mexico. *G. Salazar-Chávez 9589* (MEXU)			
Salvia ionocalyx	*I. Fragoso-Martínez & R. Middleton 206* (cult.)	MF664595	MF664001	
S. jaimehintoniana	Mexico. *G. Cornejo 4352* (IEB)			
Salvia libanensis	*I. Fragoso & R. Middleton 306* (cult.)			
Salvia littae	Mexico. *I. Fragoso-Martínez 357* (FCME)			
Salvia longispicata	Mexico. *I. Fragoso-Martínez 273* (FCME)	MF664605	MF664015	MF663866
Salvia longistyla	Mexico. *J. Rojas et al. 3931*(FCME)	MF664606	MF664016	MF663867
S. lophanthoides	Mexico. *A. Nava 2240* (MEXU)	MF664607	MF664017	MF663868
Salvia misella	Mexico. *I. Fragoso-Martínez 292 (MEXU)*	MF664614	MF664024	KJ473958
Salvia nervata	Mexico. *G. Salazar-Chávez 9450* (MEXU)		MF664030	
Salvia pallida	Argentina. *S. Benitez-Vieyra 10* (CORD)	MF664623	MF664035	MF663884
Salvia pennellii	Mexico. *L. Hernández et al. 1606* (MEXU)	MF664626	MF664039	MF663888
Salvia personata	Argentina. *S. Benitez-Vieyra 5* (CORD)	MF664628	MF664041	MF663890
Salvia plurispicata	Mexico. *G. Cornejo 4298* (MEXU)	MF664629	MF664042	MF663891
Salvia purpurea	Mexico. *M. Martínez 5109* (FCME)	MF664636	MF664049	MF663898
Salvia raymondii	Bolivia. *A. Cocucci 5521* (CORD)	MF664639	MF664053	MF663901
Salvia regla	Mexico. *F. Sazatornil 15* (MEXU)	MF664641	MF664056	MF663903
Salvia sapinea	Mexico. *G. Salazar-Chávez 9441* (MEXU)	MF664652	MF664069	MF663915
Salvia scaposa	Mexico. *M. Martínez 55540* (FCME)			

Salvia squalens	*I. Fragoso-Martínez & R. Middleton 213* (cult.)	MF664659	MF664078	MF663922
Salvia sapinea	Mexico. *G. Salazar-Chávez 9441* (MEXU)	MF664652	MF664069	MF663915
Salvia squalens	*I. Fragoso-Martínez & R. Middleton 213* (cult.)	MF664659	MF664078	MF663922
Salvia stachyoides	Mexico. *G. Flores 5372 et al.* (FCME)	MF664661	MF664080	MF663924
Salvia sucrensis	Bolivia. *A. Cocucci 5543* (CORD)	MF664664	MF664084	MF663927
Salvia tehuacana	Mexico. *M. Castañeda-Zarate 801* (FCME)	MF664665	MF664085	MF663928
Salvia tubiflora	*I. Fragoso-Martínez & R. Middleton 263* (cult.)	MF664672	MF664092	MF624011
Salvia vazquezii	*I. Fragoso-Martínez & R. Middleton 305* (cult.)	MF622194		MF624013
Salvia vitifolia	Mexico. *C. Cervantes 44* (FCME)	MF664675	MF664096	MF663937

I. Muestreo representativo de *Calosphace*

El presente estudio se enfoca en el subgénero *Calosphace* del género *Salvia* desde un punto de vista macroevolutivo, tratando de responder ¿Por qué hay tantas especies de *Calosphace* en el Neotrópico? Para ello se requirió tener una filogenia con un muestreo lo más representativo posible. En un principio, una manera de representar la diversidad del grupo fue a través de los grupos infragenéricos (i.e., secciones), pues consideramos que de manera indirecta ayudaría a representar la diversidad morfológica del grupo (Fragoso-Martínez *et al*. 2018). Fue así como durante la primera etapa del proyecto, desarrollando trabajo de campo, muestreo en herbarios y colecciones privadas logramos cubrir 85% de las 99 secciones descritas por Epling (1939; Apéndice S1). Es importante reconocer la relevancia del trabajo sistemático básico, pues a partir de éste se detectaron seis taxa que no pudieron ser asignados a ninguna de las especies conocidas, por lo que se procedió a describirlas (capítulo I).

II. Filogenia base de las salvias Neotropicales

El esfuerzo de muestreo taxonómico mencionado en el apartado anterior permitió la actualización de la hipótesis filogenética previa para *Calosphace* (Jenks *et al*. 2013), mejorando la resolución y apoyo (capítulo II). Lo anterior se logró gracias a una combinación de incremento en el muestreo taxonómico (243 especies de *Calosphace vs.* 171) y de caracteres (ITS, *trn*L-*trn*F, *psb*A-*trn*H). A partir de la filogenia obtenida se detectó que la mayoría de las secciones representadas por dos o más especies resultaron no monofiléticas, revelando que caracteres de la morfología floral (e.g., estambres exsertos), en los que se basa la clasificación, son homoplásicos y pueden haber surgido por presiones ejercidas por polinizadores similares (Fragoso-Martínez *et al*. 2018).

Mediante la reconstrucción de áreas ancestrales se confirmó el origen de *Calosphace* en Mesoamérica (Jenks *et al*. 2013; Fragoso-Martínez *et al*. 2018). Los linajes mesoamericanos migraron de manera independiente al menos doce veces a Sudamérica y los linajes andinos colonizaron las Antillas. Con respecto a la reconstrucción de síndromes de polinización ancestrales, la melitofilia se recuperó como el estado ancestral del subgénero, mientras que la ornitofilia evolucionó a partir de dicho síndrome en más de 13 ocasiones, pasando primero por un estado ambófilo. Sólo se detectó una reversión a la melitofilia a partir de la ornitofilia en el clado Fulgentes.

III. Diversificación de *Calosphace*

En la segunda etapa del proyecto se continuó ampliando el muestreo del subgénero, enfocándonos ahora en el muestreo de centros de diversidad poco representados como Las Antillas (República Dominicana) y Sudamérica (Ecuador). Habiendo logrado un muestreo de 304 especies (~50%) y tres marcadores moleculares, se hicieron los análisis de fechamiento. La filogenia se calibró empleando una calibración secundaria para la raíz y dos fósiles, pues el registro fósil en Lamiaceae es escaso y generalmente se encuentra compuesto de frutos y granos de polen que son difíciles de asignar a grupos particulares con certeza (capítulo III).

La edad estimada para *Calosphace* en este trabajo, constituye la más antigua que se ha propuesto para el grupo: 27.49 Ma (95% HPD: 20.5-35.1 Ma). Sin embargo, este es el primer estudio que se enfoca en el subgénero, por lo que el muestreo es más denso que en estudios previos (Drew *et al*., 2017); un efecto similar se observó al incrementar el muestreo de los clados focales en las Restionaceae africanas (Linder *et al*., 2005). Por otro lado, la edad del linaje de *Salvia s.l.*, se calculó en 47 Ma (95% HPD: 36.5-58.2 Ma), mientras que estudios recientes, con base en datos filogenómicos la calculan 37 Ma (Sytsma, com. pers.). Aunque las edades estimadas en el presente trabajo son 10 Ma más viejas, la edad estimada por Sytsma y colaboradores se encuentra dentro de nuestro intervalo de confianza del 95%. Es posible que sea necesario emplear una edad más reciente para calibrar la raíz del árbol (e.g., Tribu Mentheae), en lugar de la del grupo troncal de la subfamilia Nepetioideae, como se hizo en este trabajo. También ampliar el muestreo de los linajes hermanos de *Calosphace* podría ayudar a una estimación más adecuada de las edades del subgénero.

Aunque la edad obtenida para las salvias del Neotrópico no ha sido reportada en estudios previos, las edades estimadas coinciden con la disminución de los niveles de CO_2 atmosférico y como consecuencia, el enfriamiento global (alrededor de 4-5°C) (Pound & Salzmann, 2017), el cierre del Istmo de Panamá (Montes *et al*., 2015) y la expansión de los tipos de vegetación templados (Graham, 2011), siendo estos últimos donde prosperan, actualmente, la mayoría de las especies de este grupo. Por otro lado, core Calosphace se originó cuando dos factores extrínsecos confluyeron (*sensu* Donoghue & Sanderson, 2015): el aumento relativo de la temperatura y el establecimiento de los ecosistemas áridos. La temperatura se incrementó durante el óptimo climático de mediados del Mioceno, permitiendo el establecimiento de los ecosistemas áridos como los matorrales xerófilos, caatingas y cerrados (Graham, 2011), que después serían colonizados por las salvias del clado core Calosphace. Adicionalmente, la radiación de los colibríes en Sudamérica, Norteamérica y las Antillas pudo fungir como un modulador de radiaciones anidadas (no descubiertas en este trabajo), hacia finales del Mioceno (McGuire *et al.*, 2014). Otros factores que pudieron modular radiaciones particulares, por ejemplo, en el caso de los clados Sudamericano o Lavanduloideae, son eventos orogénicos del Mioceno, como el surgimiento del Eje Neovolcánico Transversal (Ferrari et al. 1999; Ferrari et al. 2000) o Los Andes (Gregory-Wodzicki 2000).

A pesar de que la polinización por aves ha causado radiaciones en otros linajes Neotropicales (e.g., Lagomarsino *et al.*, 2016), en el caso de *Calosphace*, tanto la melitofilia, como ornitofilia mostraron las mismas tasas de diversificación, siendo las especies ambófilas las que presentaron tasas más elevadas. Este resultado es interesante, pues este último grupo se encuentra en menor proporción en *Calosphace* (ca. 10%; Wester & Claßen-Bockhoff, 2011), posiblemente porque es más difícil lograr el aislamiento reproductivo en especies generalistas. Como se había observado en estudios previos (Fragoso-Martínez *et al.*, 2018), se confirmó que la transición de un síndrome de polinización especialista a otro es a través del estado generalista y es más común pasar de especies ambófilas a ornitófilas o melitófilas, que de cualquier síndrome especialista a ambófilas. Las transiciones siempre son ordenadas, es decir no hay transiciones directas de melitofilia a ornitofilia y viceversa.

En un estudio anterior (Fragoso-Martínez *et al.*, 2018) se confirmó que el área ancestral de *Calosphace* era México y Centroamérica. En el presente estudio se encontraron tasas de diversificación más bajas para Mesoamérica, que para el resto de los centros de diversidad de *Calosphace*, pero tasas de dispersión más altas. Estos resultados, aunados a las continuas colonizaciones de Sudamérica y Las Antillas (por el linaje de *Salvia serotina* y *S. misella*), así como a la alta diversidad táxica encontrada en Mesoamérica (ca. 50% de las especies), permite proponer a Mesoamérica como una “bomba de linajes”, que provee al resto de los centros de diversidad de *Calosphace* con linajes que pueden diversificar en Los Andes, este de Sudamérica o Las Antillas, donde los colibríes son más diversos y la topografía de los Andes más diversa. Las tasas de dispersión de estas últimas áreas fueron menores a las de Mesoamérica. Sin embargo, existen migraciones entre estos centros de diversidad, por ejemplo, de los linajes Andinos a las Antillas (linaje del subclado dominicano y linaje de *S. decumbens*) y al este de Sudamérica (clados Uliginosae y Sudamericano).

Los métodos de diversificación asociada a estados de carácter (State-dependent Speciation Extinction; SSE) aquí empleados para llevar a cabo los análisis de síndromes de polinización

(MuSSE; Fitzjohn 2012) y para centros de diversidad (GeoSSE; Goldberg *et al*. 2011) han recibido críticas por ser susceptibles a errores estadísticos de tipo I (Rabosky & Goldberg 2015). Por lo anterior, incluso si un carácter no tiene impacto sobre las tasas de diversificación, los métodos SSE podrían recuperar soporte estadístico para esta asociación. Para evitar este tipo de errores y verificar los resultados obtenidos, es recomendable analizar los datos empleando modificaciones a estos modelos, como HiSSE (Beaulieu & O'Meara 2016) o GeoHiSSE (Caetano *et al*. 2018), las cuales incorporan estados ocultos (*hidden states*), que permiten considerar, además del carácter de interes, un factor que no está siendo observado, y que puede presentar dinámicas de diversificación y tasas de cambios distintas al carácter de interés (Beaulieu & O'Meara 2016).

IV. Perspectivas

Es posible que existan radiaciones anidadas dentro de core Calosphace, que no pudieron ser detectadas debido a las limitaciones de nuestros datos, i.e., resolución de la filogenia, variación de los marcadores, muestreo taxononómico. Se requiere representar de manera más adecuada a los linajes Centroamericanos y brasileños en la filogenia. Además, para poder desentrañar completamente la radiación de *Calosphace* no es suficiente el emplear marcadores convencionales, se requiere comenzar a aplicar técnicas que generen datos masivos de secuenciación, como el Enriquecimiento Hibrido Anclado (AHE por sus siglas en inglés; capítulo IV.I) o alguna otra estrategia de enriquecimiento híbrido. Sin embargo, es necesario implementar filtros bioinformáticos adecuados para procesar los datos crudos de secuenciación y nuevas estrategias para lidiar con los niveles elevados de ploidía, que causan incongruencias en la inferencia filogenética. Es decir, aunque incrementar la cantidad de caracteres de manera masiva no implica que dichos caracteres pueden ser analizados de manera inmediata, es necesario explorarlos y filtrarlos. Un marco filogenético más robusto permitirá mejorar la confianza en los estimados de edades de divergencia y aproximarnos un poco más a entender la radiación de las salvias del Neotrópico

Conclusiones generales

- El trabajo taxonómico es la base para cualquier investigación, en este caso macroevolutiva. En el presente estudio se describieron seis nuevas especies mexicanas de *Salvia*, en colaboración con investigadores de otras instituciones, al llevar cabo trabajo básico de colecta y revisión de material en diversos herbarios. Este punto también resalta la gran diversidad del género en México y la labor taxonómica que aún queda por desempeñar.
- Se avanzó en la exploración de las relaciones filogenéticas de *Calosphace*, ampliando el muestreo taxonómico y de caracteres. Se lograron detectar diez clados principales y 17 nuevos subclados dentro del clado core Calosphace.
- Solamente 12 de las 42 secciones muestreadas resultaron monofiléticas, lo que apunta a la inclusión de una gran cantidad de caracteres homoplásicos en la clasificación infragenérica. La mayoría de las secciones con distribución disyunta resultaron polifiléticas.
- Se propone que *Calosphace* se originó en Mesoamérica, posteriormente se dieron colonizaciones repetidas de Los Andes (al menos 12). A partir de la región andina, los linajes migraron al este de Sudamérica y a Las Antillas.
- Mesoamérica es el centro de diversidad de *Calosphace* con tasas más bajas de diversificación, pero más altas de dispersión. Dado que constituye el centro de origen del subgénero, la mayoría de la diversidad del grupo se concentra ahí (ca. 320 spp.) y han existido repetidas colonizaciones de Sudamérica por linajes mesoamericanos, este centro de diversidad podría constituir una "bomba de linajes" que exporta grupos a Sudamérica o las Antillas (a través de los Andes), donde encuentran las condiciones propicias para diversificar.
- Las salvias Neotropicales se originaron hace 27.4 Ma, en el Oligoceno, cuando las temperaturas globales comenzaron a disminuir. Sin embargo, su radiación no se dio hasta mediados del Mioceno, en el clado core Calosphace.
- Un conjunto de factores extrínsecos (e.g., cierre del Istmo de Panamá, incremento temporal de temperatura, el establecimiento de tipos de vegetación áridos) pudieron haber propiciado el aumento en las tasas de especiación de core Calosphace. Factores que pudieron haber influido de manera secundaria en la diversificación del clado son las radiaciones de los distintos linajes de

colibríes en Sudamérica, Norteamérica y Las Antillas, y los procesos orogénicos del Neogeno (e.g., levantamiento de los Andes y la Faja Volcánica Transmexicana).

- El síndrome de polinización ancestral de *Calosphace* es la melitofilia, la transición a la ornitofilia se dio al menos en 12 ocasiones a lo largo de la historia del grupo. Se registraron tres reversiones a melitofilia a partir de la ornitofilia.
- Las transiciones de melitofilia a ornitofilia y viceversa son ordenadas, i.e., se dieron a través de un estado ambófilo intermedio (polinizadas por aves y abejas). Las transiciones de ambofilia a melitofilia u ornitofilia son más comunes que el paso de cualquier síndrome especializado a ambofilia.
- Las especies melitófilas y ornitófilas presentaron tasas similares de diversificación, mientras que las especies ambófilas tuvieron las tasas más altas. Dado que varias de las especies ambófilas son de amplia distribución (e.g., *S. mexicana*, *S. purpurea*, *S. scutellarioides*), es posible que sea una estrategia que les permita asegurar la polinización a lo largo de todo el año, si es que dependen de colibríes migratorios.
- Es posible que existan radiaciones anidadas dentro de core Calosphace, que las limitaciones del muestreo, la variación de los datos o la resolución de la filogenia hayan impedido detectar. Para poder desentrañar de una manera más minuciosa la radiación de las salvias del Neotrópico es necesario emplear datos masivos para aumentar la probabilidad de obtener marcos filogenéticos robustos y resueltos.
- Al realizar un estudio piloto empleando una estrategia filogenómica, el Enriquecimiento Anclado Híbrido (AHE, por sus siglas en inglés). Se recuperaron 448 loci, que permitieron la reconstrucción de una filogenia altamente apoyada para *Calosphace*. Solamente tres ramas internas tuvieron valores bajos de apoyo, dos de los cuales mejoraron cuando se eliminó el ruido filogenético de la matriz de datos. Estrategias de partición genómica similares resultan promisorias en la reconstrucción filogenética de *Calosphace*. Para fomentar los estudios a nivel macroevolutivo, reduciendo costos, es posible implementar el AHE incrementando la cantidad de linajes secuenciados. El MetaPrep Anclado para plantas (PAMP) se basa en la distancia filogenética entre linajes para poder separar bioinformáticamente los resultados.

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S1. Lista de secciones de Epling y literatura relevante

Phylogeny of the Neotropical sages (*Salvia* subgenus *Calosphace*; Lamiaceae) and insights into pollinator and area shifts

Plant Systematics and Evolution

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Online Resource 1. List of currently recognized sections of *Salvia* subgenus *Calosphace* (*monotypic)

	Section	Sampled
1	*Albolanatae*	1
2	*Angulatae*	1
3	*Atratae**	1
4	*Axillares**	1
5	*Biflorae*	1
6	*Blakea*	1
7	*Bracteata**	1
8	*Brandegeia*	1
9	*Briquetia*	1
10	*Brittonia*	0
11	*Caducae*	1
12	*Carneae*	1
13	*Coeruleae*	1
14	*Conzattiana*	1
15	*Corrugatae*	1
16	*Cucullatae**	1
17	*Curtiflorae*	1
18	*Cylindriflorae*	1
19	*Discolores**	1
20	*Donnellsmithia*	1
21	*Dusenostachys*	1
22	*Ekmania*	1
23	*Erythrostachys*	1
24	*Exiles**	1
25	*Farinaceae*	1
26	*Fendlera**	0
27	*Fernaldia**	1
28	*Flavidae**	0
29	*Flexuosae**	1
30	*Flocculosae*	1
31	*Floridae*	1
32	*Fulgentes*	1
33	*Gardoquiiflorae*	1
34	*Gentryana**	1
35	*Glareosae*	1
36	*Hastatae*	1
37	*Hintoniana**	1
38	*Hoehneana*	1
39	*Holwaya*	1
40	*Incarnatae*	1
41	*Insigniflorae**	1
42	*Iodanthae*	1

	Section	Sampled		Section	Sampled		Section	
43	*Iodophyllae**	0	61	*Muricatae**	0	80	*Rudes*	1
44	*Killipiana*	1	62	*Nelsonia**	1	81	*Scorodonia*	1
45	*Lanatae**	1	63	*Nivalis*	1	82	*Secundae*	1
46	*Latentiflorae**	0	64	*Nobiles*	1	83	*Sigmoideae*	1
47	*Lavanduloideae*	1	65	*Palmerostachys**	1	84	*Siphonantha*	1
48	*Leonuroideae*	1	66	*Pavonia**	1	85	*Skeptostachys*	1
49	*Leucocephalae*	1	67	*Pedicellata**	1	86	*Sphacelioides*	1
50	*Longiflorae*	1	68	*Peninsulares**	1	87	*Standleyana*	1
51	*Longipes*	1	69	*Pennellia**	1	88	*Steyermarkia**	0
52	*Lopeziana**	1	70	*Phoeniceae**	1	89	*Subrotundae*	1
53	*Macrostachyae*	1	71	*Platycheilos**	0	90	*Sulcatae**	0
54	*Malacophyllae*	1	72	*Polystachyae*	1	91	*Tenuistachya*	0
55	*Maxonia*	1	73	*Potiles**	1	92	*Tomentellae*	1
56	*Membranaceae*	1	74	*Pruinosae**	1	93	*Tuberosae**	1
57	*Micranthae*	1	75	*Punctatae*	0	94	*Tubiflorae*	1
58	*Microsphace*	1	76	*Purpureae*	1	95	*Uliginosae*	1
59	*Mineatae**	1	77	*Purpusiana**	1	96	*Umbratiles*	0
60	*Mitratae**	1	78	*Rhombifoliae*	1	97	*Urbania*	0
			79	*Rubescentes*	1	98	*Weberbaueria**	0

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S2. Especies muestreadas para filogenia calibrada de *Calosphace*

Abiotic processes as primary drivers of the diversification of the Neotropical sages (*Salvia* subgenus *Calosphace*)

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Section	Taxa	Author or Voucher	1) ITS	2) *trn*L-*trn*F	3) *psb*A-*trn*H
outgroup	*Lepechinia caulescens*	1) Drew and Sytsma (2011) ;2), 3) Mexico, *M. Castañeda-Zarate 841* (MEXU)	JF301345	MF663938	MF663798
outgroup	*Melissa axillaris*	1,2) Drew and Sytsma (2012) ; 3) Tong (unpub.)	JQ669114	JQ669051	KY197901
outgroup	*Melissa officinalis*	Walker and Sytsma (2007)	DQ667291	DQ667477	DQ667387
	Salvia				
outgroup	*Salvia aegyptiaca*	Walker and Sytsma (2007)	DQ667285	DQ667470	DQ667380
outgroup	*Salvia aristata*	Walker & Sytsma (2007)	DQ667280	DQ667465	DQ667375
outgroup	*Salvia aucheri var. canescens*	Walker and Sytsma (2007)	DQ667286	DQ667471	DQ667381
outgroup	*Salvia austriaca*	Walker and Sytsma (2007)	DQ667323	DQ667512	DQ667408
outgroup	*Salvia cabulica*	Walker and Sytsma (2007)	DQ667287	DQ667472	DQ667382
outgroup	*Salvia canariensis*	Hu et al. (2018)	MG824183	MG824442	MG823916
outgroup	*Salvia chienii*	Hu et al. (2018)	MG824193	MG824452	MG823926
outgroup	*Salvia dianthera*	Walker and Sytsma (2007)	DQ667329	DQ667518	DQ667414
outgroup	*Salvia dorystaechas*	1)Walker and Sytsma (2007); 2) Walker et al. 2004; 3) Walker and Sytsma (2007)	DQ667252	AY570454	DQ667360

outgroup	*Salvia garipensis*	Walker and Sytsma (2007)	DQ667281	DQ667466	DQ667376
outgroup	*Salvia koyamae*	1) Takano & Okada (2011)	AB541114		
outgroup	*Salvia lyrata*	Hu et al. (2018)	MG824225	MG824485	MG823966
outgroup	*Salvia majde*	Walker & Sytsma (2007)	DQ667335	DQ667524	DQ667420
outgroup	*Salvia nemorosa*	*MOBOT2014-1401*			
outgroup	*Salvia officinalis*	1), 3) Walker and Sytsma (2007); 2) Walker et al. (2004)	DQ667241	AY570465	DQ667351
outgroup	*Salvia roborowskii*	1), 3) Walker and Sytsma (2007)	DQ667289	EU200178	DQ667384
outgroup	*Salvia roemeriana*	1)Walker & Sytsma (2007); 2) Walker et al. (2004); 3) Walker et al. (2015)	DQ667211	AY570491	KP852654
outgroup	*Salvia rosmarinus*	1,3) Walker & Sytsma (2007); 2) Walker et al (2004)	DQ667241	AY570465	DQ667351
outgroup	*Salvia scrophulariifolia*	Walker and Sytsma (2007)	DQ667330	DQ667519	DQ667415
outgroup	*Salvia sinica*	Hu et al. (2018)	MG824271	MG824530	MG824018
outgroup	*Salvia sonchifolia*	Hu et al. (2018)	MG824273	MG824532	MG824020
outgroup	*Salvia transsylvanica*	MOBOT1998-0854			
outgroup	*Salvia trichocalycina*	Walker and Sytsma (2007)	DQ667283	DQ667468	DQ667378
outgroup	*Salvia yangii*	1)Walker and Sytsma (2007); 2) Walker et al. 2004; 3) Walker and Sytsma (2007)	DQ667223	AY570464	DQ667341
outgroup *(Audibertia)*	*Salvia apiana*	Walker and Sytsma (2007)	DQ667214	DQ667425	DQ667338
outgroup *(Audibertia)*	*Salvia californica*	1) Walker and Sytsma (2007); 2), 3) Jenks et al. (2011)	DQ667213	HQ418955	HQ418904
outgroup *(Audibertia)*	*Salvia carducea*	Walker et al. (2015)	KP852786	KP852899	KP852623
outgroup *(Audibertia)*	*Salvia clevelandii*	I. Fragoso-Martínez & R. Middleton 229 (cult.)	MF664560	MF663961	MF663819
outgroup *(Audibertia)*	*Salvia columbariae*	Walker et al. (2015)	KP852793	KP852905	KP852631
outgroup *(Audibertia)*	*Salvia greatae*	1), 3) Walker and Sytsma (2007) ; 2) Walker et al. (2004)	DQ667215	AY570481	DQ667339
outgroup *(Audibertia)*	*Salvia leucophylla*	Walker et al. (2015)	KP852813	KP852913	KP852639
outgroup *(Audibertia)*	*Salvia mellifera*	1) Walker and Sytsma (2007); 2), 3) Jenks et al. (2011)	DQ667220	HQ418981	HQ418931
outgroup *(Audibertia)*	*Salvia mohavensis*	Walker et al. (2015)	KP852828	KP852921	KP852648
outgroup *(Audibertia)*	*Salvia munzii*	1) Walker and Sytsma (2007); 2), 3) *I. Fragoso-Martínez & R. Middleton 246* (cult.)	DQ667224	MF664026	MF663876

outgroup *(Audibertia)*	*Salvia spathacea*	Walker et al. (2015)	KP852839	KP852929	KP852657
Sphacelioides	*Salvia acerifolia*	Mexico. Guerrero, *L. Lozada 55345* (FCME)	MF664540	MF663939	MF663799
Holwaya	*Salvia adenophora*	1), 3) Jenks et al. (2013); 2) *I. Fragoso-Martínez & R. Middleton 216* (cult.)	MF622100	MF663940	MF623919
Scorodonia	*Salvia aequidistans*	Jenks et al. (2013)	MF622101	/	MF623920
Lavanduloideae	*Salvia agnes*	Mexico. Jalisco, *M. Harker 2986* (IBUG)	MF664541	MF663941	MF663800
Sigmoideae	*Salvia alamosana*	Mexico. Sinaloa, *J. González 525* (MEXU)	MF664542	MF663942	MF663801
Angulatae	*Salvia alba*	Bolivia. Cochabamba, *A. Cocucci 5522* (CORD)	MF664543	MF663943	MF663802
*Fernaldia**	*Salvia albocaerulea*	1), 3) Jenks et al. (2013); 2) Mexico. Guerrero, J. Calónico 14716 (FCME)	MF622104	MF663944	MF623923
*Lopeziana**	*Salvia alborosea*	Jenks et al. (2013)	MF622105	/	MF623924
Scorodonia	*Salvia amarissima*	Mexico. Distrito Federal, *M. Castañeda-Zarate 837* (MEXU)	MF664544	MF663945	MF663803
Rubescentes	*Salvia amethystina*	I. Fragoso-Martínez & R. Middleton 197 (cult.)	MF664545	MF663946	MF663804
Angulatae	*Salvia amplifrons*	Bolivia. Cochabamba, *A. Cocucci 5523* (CORD)	MF664546	MF663947	MF663805
Angulatae	*Salvia angulata*	Jenks et al. (2013)	MF622108	/	MF623927
Brandegeia	*Salvia angustiarum*	Jenks et al. (2013)	MF622109	/	MF623928
Wrightiana	*Salvia arborescens*	Dominican Republic. *I. Fragoso Martínez et al. 459* (JBSD, MEXU)			
Iodanthae	*Salvia arbuscula*	Jenks et al. (2011)	HQ418846	HQ418949	HQ418898
Ekmania	*Salvia arduinervis*	Haiti. *Ekman 3168* (TEX)			
Purpureae	*Salvia areolata*	Jenks et al. (2013)	MF622110	/	MF623929
Nobiles	*Salvia articulata*	Jenks et al. (2013)	MF622111	/	MF623930
Angulatae	*Salvia arthrocoma*	México. Puebla, *I. Fragoso-Martínez et al. 416* (MEXU)			
Conzattiana	*Salvia aspera*	Mexico. Oaxaca, *F. Sazatornil 22* (MEXU)	MF664547	MF663948	MF663806
Coeruleae	*Salvia atrocyanea*	Bolivia. Santa Cruz, *A. Cocucci 5516* (CORD)	MF664548	MF663949	MF663807
*Axillares**	*Salvia axillaris*	Mexico. Oaxaca, *C. Martorell & A. Martínez s.n.* (cult.)	MF664549	MF663950	MF663808
Farinaceae	*Salvia azurea*	Walker and Sytsma (2007)	DQ667317	DQ667505	DQ667404
Ekmania	*Salvia bahorucona*	Dominican Republic, Bahoruco. *I. Fragoso Martínez et al. 517* (JBSD, MEXU)			
Tomentellae	*Salvia ballotiflora*	Jenks et al. (2011)	HQ418849	HQ418952	HQ418901
Erythrostachys	*Salvia betulifolia*	Jenks et al. (2013)	MF622113	/	MF623932
Brandegeia	*Salvia blepharophylla*	Jenks et al. (2011)	HQ418850	HQ418953	HQ418902
Angulatae	*Salvia bogotensis*	*I. Fragoso-Martínez & R. Middleton 221* (cult.)	MF664550	MF663951	MF663809
Fulgentes	*Salvia booleana*	Mexico. Puebla, *Palzkill sn.* (HUAP)			
Gardoquiiflorae	*Salvia brachyloba*	Dominican Republic. *I. Fragoso Martínez et al. 509* (JBSD, MEXU)			

Scorodonia	*Salvia breviflora*	Mexico. Guerrero, *L. Lozada 3775* (FCME)	MF664551	MF663952	MF663810
Rudes	*Salvia brevipes*	Argentina. Corrientes, *S. Benitez 16* (CORD)	MF664552	MF663953	MF663811
Brandegeia	*Salvia buchananii*	*I. Fragoso-Martínez & R. Middleton 252* (cult.)	MF664553	MF663954	MF663812
Corrugatae	*Salvia bullulata*	*I. Fragoso-Martínez & R. Middleton 219* (cult.)	MF664554	MF663955	MF663813
Membranaceae	*Salvia bupleuroides*	Mexico. Guerrero, *J. Rojas et al. 411* (FCME)	MF664555	MF663956	MF663814
Standleyana	*Salvia cacaliifolia*	1), 3) Walker and Sytsma (2007); 2) Jenks et al. (2011)	DQ667259	HQ418954	DQ667367
Urbania	*Salvia calaminthifolia*	Haiti. *Ekman 9443* (TEX)			
Tomentellae	*Salvia calolophos*	Argentina. Jujuy, *S. Benitez-Vieyra 2* (CORD)	MF664556	MF663957	MF663815
Tubiflorae	*Salvia camarifolia*	Colombia, *Wood 4985* (TEX)			
Tomentellae	*Salvia candicans*	Mexico, Puebla, *M. Martínez s.n*. (FCME)	MF664557	MF663958	MF663816
Carneae	*Salvia carnea*	Ecuador. *I. Fragoso Martínez et al. 621* (QCNE, MEXU)			
Fulgentes	*Salvia carranzae*	Mexico. Queretaro, 1, 2) *Zamudio et al. 11189* (IEB); 3) *Zamudio et al. 10882* (IEB)			
Micranthae	*Salvia caymanensis*	*I. Fragoso-Martínez & R. Middleton 309* (cult.)			
Flocculosae	*Salvia cedrosensis*	1) Walker and Sytsma (2007); 2) Walker et al. (2004); 3) Jenks et al. (2013)	DQ667228	AY570470	MF623934
Rudes	*Salvia cerradicola*	Jenks et al. (2013)	MF622116	/	MF623935
Flocculosae	*Salvia chamaedryoides*	Jenks et al. (2011)	HQ418836	HQ418958	HQ418907
Sigmoideae	*Salvia chapalensis*	Jenks et al. (2013)	MF622117	/	MF623936
Maxonia	*Salvia chiapensis*	Mexico. Chiapas, *H. Mejía & A. Luna 629* (MEXU)	MF664558	MF663959	MF663817
Flocculosae	*Salvia chionophylla*	Jenks et al. (2013)	MF622119	/	MF623938
Incarnatae	*Salvia cinnabarina*	Mexico. Oaxaca, *G. Salazar-Chávez 9455* (MEXU)	MF664559	MF663960	MF663818
Purpureae	*Salvia clarkcowanii*	Mexico. Guerrero, *de Santiago s.n.* (FCME)			
*Cucullatae**	*Salvia clinopodioides*	Mexico. Estado de Mexico, *F. Sazatornil 10* (MEXU)	MF664561	MF663962	MF663820
Flocculosae	*Salvia coahuilensis*	Jenks et al. (2013)	MF622120	/	MF623939
Subrotundae	*Salvia coccinea*	1), 3) Jenks et al. (2013); 2) Walker et al. (2004)	EU169484	AY570474	MF623940
Membranaceae	*Salvia compsostachys*	Mexico. Tamaulipas, *Hinton et al. 24799* (MEXU)	MF664562	MF663963	MF663821
Dusenostachys	*Salvia concolor*	Jenks et al. (2011)	HQ418858	HQ418961	HQ418910
Secundae	*Salvia confertiflora*	Jenks et al. (2011)	HQ418859	HQ418962	HQ418911
Membranaceae	*Salvia confertispicata*	Mexico. Guerrero, *E. López 5744* (FCME)	MF664563	MF663964	MF663822
Uliginosae	*Salvia congestiflora*	Brazil. Torres, *M. Sobral et al. 8343* (MEXU)	MF664564	MF663965	MF663823
Polystachyae	*Salvia connivens*	Mexico. Hidalgo, *F. Sazatornil 18* (MEXU)	MF664565	MF663966	MF663824
Corrugatae	*Salvia corrugata*	1), 3) Jenks et al. (2013); 2) Walker et al. (2004)	MF622122	AY570476	MF623941

Lavanduloideae	*Salvia cryptodonta*	Mexico. Jalisco, *F. Santana Michel et al. 1744* (MEXU)	MF664566	MF663967	MF663825
Maxonia	*Salvia cuatrecasana*	1), 3) Jenks et al. (2013); 2) *I. Fragoso-Martínez & R. Middleton 198* (cult.)	MF622123	MF663968	MF623942
Flocculosae	*Salvia curticalyx*	Ecuador. Loja, *I. Fragoso-Martínez et al. 764* (QCNE, MEXU)			
Purpureae	*Salvia curviflora*	*I. Fragoso-Martínez & R. Middleton 230* (cult.)	MF664567	MF663969	MF663826
Tomentellae	*Salvia cuspidata* var. *gilliesii*	Argentina. Tucumán, *A. Cocucci 5253* (CORD)	MF664568	MF663970	MF663827
Tomentellae	*Salvia cuspidata* var. *rosea*	Bolivia. Chuquisaca, *A. Cocucci 5547* (CORD)	MF664569	MF663971	MF663828
Angulatae	*Salvia cyanicalyx*	Jenks et al. (2013)	MF622125	/	MF623944
Holwaya	*Salvia darcyi*	Cambridge University Botanic Garden 20120498			
Flocculosae	*Salvia decumbens*	Dominican Republic, Pelempito. *I. Fragoso Martínez et al. 519* (JBSD, MEXU)	KU358695	/	KU358701
Wrightiana	*Salvia densiflora*	Dominican Republic. Constanza, *Liogier 13064* (MEXU)	MF664570	MF663972	MF663829
Fulgentes	*Salvia dichlamys*	Mexico. Mexico, *J. Rojas et al. 5411* (FCME)			
Nivalis	*Salvia diegoae*	Mexico. Guerrero, *J. Soto 20396* (FCME)	MF664571	MF663973	MF663830
*Discolores**	*Salvia discolor*	*I. Fragoso-Martínez & R. Middleton 224* (cult.)	MF664572	MF663974	MF663831
Holwaya	*Salvia disjuncta*	1), 3) Jenks et al. (2013); 2) *I. Fragoso-Martínez & R. Middleton 257* (cult.)	MF622126	MF663975	MF623945
Dusenostachys	*Salvia divinorum*	Mexico. Oaxaca, *I. Fragoso-Martínez & M. Martínez 176* (FCME)	MF664573	MF663976	MF663832
Longiflorae	*Salvia dombeyi*	Jenks et al. (2011)	HQ418862	HQ418965	HQ418914
Holwaya	*Salvia dorisiana*	Jenks et al. (2011)	HQ418863	HQ418966	HQ418915
Rudes	*Salvia durifolia*	*I. Fragoso-Martínez & R. Middleton 265* (cult.)	MF664574	MF663977	MF663833
Incarnatae	*Salvia elegans*	1) Jenks et al. (2013); 2), 3) Mexico. Hidalgo, *F. Sazatornil 14* (MEXU)	MF622127	MF663978	MF663834
Longipes	*Salvia erythrostoma*	Jenks et al. (2011)	HQ418864	HQ418967	HQ418967
*Mineatae**	*Salvia exserta*	Bolivia. Cochabamba, *A. Cocucci 5537* (CORD)	MF664575	MF663979	MF663835
Farinaceae	*Salvia farinacea*	1), 3) Jenks et al. (2013); 2) Walker et al. (2004)	EU169483	AY570479	MF623948
Uliginosae	*Salvia filifolia*	Mexico. Tlaxcala, *M. Corona 43* (MEXU)	MF664576	MF663980	MF663836
Polystachyae	*Salvia filipes*	Mexico. Guerrero, *R. Trujillo-Vázquez 1071* (FCME)	MF664577	MF663981	MF663837
Dusenostachys	*Salvia flaccidifolia*	Jenks et al. (2011)	HQ418865	HQ418968	HQ418917
Flocculosae	*Salvia flocculosa*	Ecuador, *I. Fragoso-Martínez et al. 746* (QCNE, MEXU)			
Floridae	*Salvia florida*	Jenks et al. (2013)	MF622130	/	MF623949
Leonuroideae	*Salvia formosa*	Jenks et al. (2013)	MF622131	/	MF623950
Uliginosae	*Salvia forreri*	Jenks et al. (2013)	MF622132	/	MF623951
Ekmania	*Salvia foveolata*	Dominican Republic. *I. Fragoso Martínez et al. 508* (JBSD, MEXU)			

Tomentellae	*Salvia fruticulosa*	Jenks et al. (2011)	HQ418866	HQ418969	HQ418918
Fulgentes	*Salvia fulgens*	Mexico. Distrito Federal, *F. Sazatornil 5* (MEXU)	MF664579	MF663983	MF663839
Rubescentes	*Salvia gachantivana*	*I. Fragoso-Martínez & R. Middleton 205* (cult.)	MF664580	MF663984	MF663840
Uliginosae	*Salvia galloana*	Mexico. Guerrero, *L. Lozada 2929* (FCME)	MF664581	MF663985	MF663841
Holwaya	*Salvia gesneriiflora*	1) Jenks et al. (2013); 2), 3)Mexico. Michoacán, *G. Salazar-Chávez 9217* (MEXU)	MF622133	MF663986	MF663842
Uliginosae	*Salvia glechomifolia*	Jenks et al. (2013)	MF622134	/	MF623953
Longipes	*Salvia gracilipes*	Jenks et al. (2013)	MF622135	/	MF623954
Carneae	*Salvia gracilis*	Mexico, Oaxaca. *I. Fragoso Martínez et al. 648* (QCNE, MEXU)			
*Exiles**	*Salvia graciliramulosa*	Bolivia. Chuquisaca, *A. Cocucci 5541* (CORD)	MF664582	MF663987	MF663843
Skeptostachys	*Salvia gravida*	Mexico. Michoacán, *Tenorio & Flores 19623* (MEXU)	MF664583	MF663988	MF663844
Flocculosae	*Salvia greggii*	Jenks et al. (2011)	HQ418870	HQ418972	HQ418922
Hoehneana	*Salvia grewiifolia*	Jenks et al. (2011)	HQ418871	HQ4118973	HQ418923
Lavanduloideae	*Salvia guadalajarensis*	Mexico. Jalisco, *J. González 1165* (MEXU)	MF664584	MF663989	MF663845
Coeruleae	*Salvia guaranitica*	Argentina. Córdoba, *S. Benitez-Vieyra 15* (CORD)	MF664585	MF663990	MF663846
Fulgentes	*Salvia guevarae*	Mexico. Queretaro, *Zamudio & Servin 14465* (IEB)			
Cylindriflorae	*Salvia haenkei*	Bolivia. Cochabamba, *A. Cocucci 5540* (CORD)	MF664586	MF663991	MF663847
Cylindriflorae	*Salvia heerii*	*I. Fragoso-Martínez & R. Middleton 196* (cult.)	MF664587	MF663992	MF663848
Lavanduloideae	*Salvia helianthemifolia*	Mexico. Estado de Mexico, *I. Fragoso-Martínez 100* (FCME)	MF664588	MF663993	MF663849
*Bracteata**	*Salvia herbacea*	Mexico. Oaxaca, *E. Martínez et al. 32508* (MEXU)	MF664589	MF663994	MF663850
Angulatae	*Salvia heterofolia*	Mexico. Estado de Mexico, *I. Fragoso-Martínez 79* (FCME)	MF664590	MF663995	MF663851
Tomentellae	*Salvia hidalgensis*	Mexico. Hidalgo, *V. Juárez & M. Ortíz 879* (MEXU)	MF664591	MF663996	MF663852
Nobiles	*Salvia hilarii*	Jenks et al. (2013)	MF622137	/	MF623956
Uliginosae	*Salvia hintonii*	Mexico. Guerrero, *J. Calónico 20748* (FCME)	MF664592	MF663997	MF663853
Cylindriflorae	*Salvia hirta*	Ecuador, *I. Fragoso-Martínez & K. Montesinos* (QCNE, MEXU)			
*Phoeniceae**	*Salvia hirtella*	Walker and Sytsma (2007)	DQ667326	DQ667515	DQ667411
*Potiles**	*Salvia hispanica*	Mexico. Distrito Federal, *I.Fragoso-Martínez 4* (FCME)	MF664593	MF663998	MF663854
Holwaya	*Salvia holwayi*	1), 3) Jenks et al. (2013); 2) G. Salazar-Chávez 9561 (MEXU)	MF622138		MF623957
Flocculosae	*Salvia incumbens*	Dominican Republic, *T. Clase 7990* (JBSD)			
Donnellsmithia	*Salvia infuscata*	Jenks et al. (2013)	MF622139	/	MF623958
Cylindriflorae	*Salvia integrifolia*	*I. Fragoso-Martínez & R. Middleton 651* (cult.)			
Holwaya	*Salvia involucrata*	1) Jenks et al. (2013); 2), 3) Mexico. Hidalgo, *I. Fragoso-Martínez 291* (MEXU)	MF622140	MF663999	MF663855

Iodanthae	*Salvia iodantha*	Mexico. Michoacán, *G. Salazar-Chávez 9215* (MEXU)	MF664594	MF664000	MF663856
Carneae	*Salvia ionocalyx*	1), 2) *I. Fragoso-Martínez & R. Middleton 206* (cult.); 3) Jenks et al. (2013)	MF664595	MF664001	MF623961
Farinaceae	*Salvia jaimehintoniana*	Mexico. Nuevo Leon, *G. Cornejo 4352* (IEB)			
Curtiflorae	*Salvia jorgehintoniana*	*I. Fragoso-Martínez & R. Middleton 245* (cult.)	MF664596	MF664002	MF663857
Holwaya	*Salvia karwinskii*	1), 3) Jenks et al. (2013); 2) Mexico. Oaxaca, *I. Fragoso-Martínez 185* (FCME)	MF622144	MF664003	MF623963
Scorodonia	*Salvia keerlii*	*I. Fragoso-Martínez & R. Middleton 240* (cult.)	MF664597	MF664004	MF663858
Carneae	*Salvia killipiana*	Ecuador, *I. Fragoso-Martínez et al. 718* (QCNE, MEXU)			
Uliginosae	*Salvia lachnostachys*	Brazil. Paraná, *A. Krapovickas & C. Cristobal 39715* (MEXU)	MF664598	MF664005	MF663859
Uliginosae	*Salvia laevis*	Mexico. Hidalgo, *I. Fragoso-Martínez 284* (MEXU)	MF664599	MF664006	MF663860
Ekmania	*Salvia laichnaiaclada*	Dominican Republic. *I. Fragoso Martínez et al. 608* (JBSD, MEXU)			
Membranaceae	*Salvia langlassei*	Mexico. Guerrero, *M. 41561* (MEXU)	MF664600	MF664007	MF663861
Angulatae	*Salvia languidula*	Jenks et al. (2013)	MF622146	/	MF623965
*Mitratae**	*Salvia lasiantha*	1) Walker and Sytsma (2007); 2), 3) Jenks et al. (2011)	DQ667300	HQ418976	HQ418926
Membranaceae	*Salvia lasiocephala*	Mexico. Puebla, *J. Contreras 9202* (FCME)	MF664601	MF664008	MF663862
Lavanduloideae	*Salvia lavanduloides*	Mexico. Guerrero, *M. Martínez 5649* (FCME)	MF664602	MF664009	MF663863
Wrightiana	*Salvia lavendula*	Dominican Republic. *T. Clase 1059* (JBSD)			
Angulatae	*Salvia leptostachys*	Mexico. Guerrero, *E. Mendoza 8006* (FCME)	MF664603	MF664010	MF663864
Albolanatae	*Salvia leucantha*	Mexico. Distrito Federal, *I. Fragoso-Martínez & M. Martínez 268* (FCME)	MF664604	MF664011	MF663865
Leucocephalae	*Salvia leucocephala*	1), 3) Jenks et al. (2013); 2) *I. Fragoso-Martínez & R. Middleton 215* (cult.)	MF622147	MF664012	MF623966
*Lanatae**	*Salvia leucochlamys*	Jenks et al. (2013)	MF622148	/	MF623967
Erythrostachys	*Salvia libanensis*	*I. Fragoso Martínez & R. Middleton 306* (cult.)			
Fulgentes	*Salvia lineata*	1), 3) Jenks et al. (2013) ;2) Mexico. Distrito Federal, *I. Fragoso-Martínez 270* (FCME)	MF622149	MF664013	MF623968
Purpureae	*Salvia littae*	Mexico, *I. Fragoso-Martínez 357* (MEXU)			
Siphonantha	*Salvia lobbii*	Jenks et al. (2013)	MF622151	/	MF623970
Angulatae	*Salvia longispicata*	Mexico. Puebla, *I. Fragoso-Martínez 273* (FCME)	MF664605	MF664015	MF663866
Curtiflorae	*Salvia longistyla*	Mexico. Guerrero, *J. Rojas et al. 3931*(FCME)	MF664606	MF664016	MF663867
Membranaceae	*Salvia lophanthoides*	Mexico. Oaxaca, *A. Nava 2240* (MEXU)	MF664607	MF664017	MF663868
Malacophyllae	*Salvia loxensis*	Ecuador, *I. Fragoso-Martínez & D. Veintimilla 685* (QCNE, MEXU)			
Flocculosae	*Salvia lycioides*	1),3) Jenks et al. (2013); 2) Walker et al. (2004)	MF622153	AY570484	MF623972
Flocculosae	*Salvia macellaria*	Mexico. San Luis Potosí, *E. Martínez-Ambríz et al. 543* (FCME)			
Hastatae	*Salvia macrophylla*	Jenks et al. (2011)	HQ418877	HQ418979	HQ418929

Macrostachyae	*Salvia macrostachya*	Ecuador, *I. Fragoso-Martínez et al. 614* (QCNE, MEXU)			
Longipes	*Salvia madrensis*	Jenks et al. (2011)	HQ418878	HQ418980	HQ418930
Rubescentes	*Salvia melaleuca*	Colombia. Cundinamarca, *J. Fernández-Alonso &* A. *Castillo 14828* (MEXU)	MF664608	MF664018	MF663869
Secundae	*Salvia melissiflora*	Brazil. Paraná, *J.Silva & J. Saldanha 1788* (MEXU)	MF664609	MF664019	MF663870
Scorodonia	*Salvia melissodora*	Mexico. Guerrero, *M. Martínez 4926* (FCME)	MF664610	MF664020	MF663871
Briquetia	*Salvia mexicana*	Mexico. Guerrero, *M. Martínez 5599* (FCME)	MF664611	MF664021	MF663872
Fulgentes	*Salvia microphylla*	Mexico. Puebla, *F. Sazatornil 2* (MEXU)	MF664612	MF664022	MF663873
Rudes	*Salvia minarum*	Paraguay. Amambay, *M. Ferrucci et al. 1610* (MEXU)	MF664613	MF664023	MF663874
Microsphace	*Salvia misella*	1), 2) Mexico. Guerrero, *I. Briseño 4550* (FCME); 3) Benitez-Vieyra et al. (2014)	MF664614	MF664024	KJ473958
Membranaceae	*Salvia mocinoi*	Mexico. Estado de Mexico, *I. Fragoso-Martínez 98* (FCME)	MF664615	MF664025	MF663875
Fulgentes	*Salvia modica*	1), 3) *Acevedo* 45; 2) *Davender2000817*			
Urbania	*Salvia montecristina*	Botanical Garden of Santo Domingo, Cult., *I. Fragoso Martínez et al. 527* (JBSD, MEXU)			
Flocculosae	*Salvia muelleri*	Jenks et al. (2013)	MF622161	/	MF623980
Lavanduloideae	*Salvia muscarioides*	Mexico. Sonora, *Fishbein et al. 2594* (MEXU)	MF664616	MF664027	MF663877
Angulatae	*Salvia myriantha*	*I. Fragoso-Martínez & R. Middleton 199* (cult.)	MF664617	MF664028	MF663878
Uliginosae	*Salvia nana*	Mexico. Oaxaca. *J. Reyes 8521* (MEXU)	MF664618	MF664029	MF663879
Curtiflorae	*Salvia nervata*	Mexico. Oaxaca, *G. Salazar-Chávez 9450* (MEXU)		MF664030	
Rudes	*Salvia nervosa*	Argentina. Corrientes, *M. Ferrucci et al. 6424* (MEXU)	MF664619	MF664031	MF663880
Membranaceae	*Salvia nitida*	1) Mexico. Oaxaca, *D. Saavedra 302* (FCME); 2), 3) Mexico. *Guerrero, E. Velázquez* 2490 (FCME)	MF664620	MF664032	MF663881
Rubescentes	*Salvia nubigena*	Colombia, *Wood 5269* (TEX)			
Conzattiana	*Salvia oaxacana*	Jenks et al. (2011)	HQ418881	HQ418983	HQ418933
Microsphace	*Salvia occidentalis*	Jenks et al. (2011)	HQ418882	HQ418984	HQ418934
Biflorae	*Salvia oppositiflora*	Jenks et al. (2011)	HQ418883	HQ418985	HQ418935
*Pavonia**	*Salvia orbignaei*	Bolivia. Cochabamba, *A. Cocucci 5531* (CORD)	MF664621	MF664033	MF663882
Rudes	*Salvia ovalifolia*	1), 2) Walker and Sytsma (2007); 3) Jenks et al. (2013)	DQ667315	DQ667502	MF624020
*Tuberosae**	*Salvia oxyphora*	1), 2) Walker and Sytsma (2007); 3) Jenks et al. (2011)	DQ667262	DQ667448	HQ418936
*Pedicellata**	*Salvia palealis*	Mexico. Guerrero, *J. Soto-Núñez 11476* (MEXU)	MF664622	MF664034	MF663883
Farinaceae	*Salvia pallida*	Argentina. Entre Ríos, *S. Benitez-Vieyra 10* (CORD)	MF664623	MF664035	MF663884
*Palmerostachys**	*Salvia palmeri*	Mexico. Sonora, *A. Reina et al. 2004-1271* (MEXU)	MF664624	MF664036	MF663885
*Insigniflorae**	*Salvia pansamalensis*	Jenks et al. (2013)	MF622163	/	MF623982

Tomentellae	*Salvia parryi*	Jenks et al. (2013)	MF622164	/	MF623983
Ekmania	*Salvia parryskii*	1) Zona et al. (2016); 2), 3) Haiti. *W. Judd 5773* (MO)	KU358698		
Blakea	*Salvia patens*	1) Jenks et al. (2011); 2, 3) Mexico. Hidalgo, *F. Sazatornil 13* (MEXU)	DQ667253	MF664037	MF663886
*Flexuosae**	*Salvia pauciserrata*	Colombia. Cundinamarca, *J. Fernández et al. 12573* (MEXU)	MF664625	MF664038	MF663887
*Peninsulares**	*Salvia peninsularis*	Jenks et al. (2013)	MF622165	/	MF623984
*Pennellia**	*Salvia pennellii*	Mexico. Tamaulipas, *L. Hernández et al. 1606* (MEXU)	MF664626	MF664039	MF663888
*Nelsonia**	*Salvia perlonga*	Mexico. Guerrero, *J. Calónico 11462* (MEXU)	MF664627	MF664040	MF663889
Angulatae	*Salvia personata*	Argentina. Jujuy, *S. Benitez-Vieyra 5* (CORD)	MF664628	MF664041	MF663890
Conzattiana	*Salvia pexa*	Mexico, *E. Martínez-Ambríz et al. 540* (FCME)			
Siphonantha	*Salvia pichinchensis*	Ecuador, Loja. *I. Fragoso-Martínez et al. 634* (QCNE, MEXU)			
Tomentellae	*Salvia pinguifolia*	Jenks et al. (2013)	MF622167	/	MF623986
Polystachyae	*Salvia plurispicata*	Mexico. Michoacán. *G. Cornejo 4298* (MEXU)	MF664629	MF664042	MF663891
Micranthae	*Salvia podadena*	Mexico. San Luis Potosí, *R. Torres 15270* (MEXU)	MF664630	MF664043	MF663892
Polystachyae	*Salvia polystachya*	Mexico. Morelos, *F. Sazatornil 4* (MEXU)	MF664631	MF664044	MF663893
*Hintoniana**	*Salvia praestans*	Mexico. Guerrero, *Ramamoorthy s.n.* (MEXU)	MF664632	MF664045	MF663894
Urbania	*Salvia praeterita*	Dominican Republic. *I. Fragoso Martínez et al.* 607 (JBSD, MEXU)			
Uliginosae	*Salvia procurrens*	*I. Fragoso-Martínez & R. Middleton 247* (cult.)	MF664633	MF664046	MF663895
Sigmoideae	*Salvia protracta*	Mexico. Oaxaca, *G. Salazar-Chávez 9463* (MEXU)	MF664634	MF664047	MF663896
Uliginosae	*Salvia prunelloides*	Mexico. Morelos, *F. Sazatornil 3* (MEXU)	MF664635	MF664048	MF663897
Holwaya	*Salvia puberula*	Mexico. San Luis Potosí, *R. Hernández 175* (MEXU)			
Erythrostachys	*Salvia pubescens*	Jenks et al. (2011)	HQ418887	HQ418990	HQ418939
Sigmoideae	*Salvia pugana*	Mexico. Jalisco, *P. Tenorio-L. & P.T. Ramamoorthy 9214* (IEB)			
Fulgentes	*Salvia pulchella*	Jenks et al. (2013)	MF622169	/	MF623988
Polystachyae	*Salvia purepecha*	Mexico. Michoacan, *B. Bedolla-García et al.* 123 (IEB)			
Purpureae	*Salvia purpurea*	Mexico. Guerrero, *M. Martínez 5109* (FCME)	MF664636	MF664049	MF663898
*Purpusiana**	*Salvia purpusii*	Mexico. Coahuila, *J. Henrickson 13702* (MEXU)	MF664637	MF664050	MF663899
Uliginosae	*Salvia pusilla*	Jenks et al. (2013)	MF622171	/	MF623990
Cylindriflorae	*Salvia quitensis*	Ecuador. Azuay, *I. Fragoso-Martínez& K. Montesinos 678* (QCNE, MEXU)			
Sigmoideae	*Salvia ramamoorthyana*	Mexico. Jalisco, *A. Castro-Castro & J. Villa-Vázquez 2274* (MEXU)	MF664638	MF664051	MF663900
Scorodonia	*Salvia ramosa*	1), 3) Jenks et al. (2013); 2) *I. Fragoso-Martínez & R. Middleton 210* (cult.)	MF622172	MF664052	MF623991
Angulatae	*Salvia raymondii*	Bolivia. Cochabamba, *A. Cocucci 5521* (CORD)	MF664639	MF664053	MF663901

Dusenostachys	*Salvia recurva*	1) 3) Jenks et al. (2013); 2) Mexico. Hidalgo, *I. Fragoso-Martínez 285* (MEXU)	MF622173	MF664054	MF623992
Glareosae	*Salvia reflexa*	Mexico. Querétaro, *H. Vibrans 6976* (MEXU)	MF664640	MF664055	MF663902
Erythrostachys	*Salvia regla*	Mexico. Hidalgo, *F. Sazatornil 15* (MEXU)	MF664641	MF664056	MF663903
Farinaceae	*Salvia reptans*	Mexico. Querétaro, *J. Rojas 6282* (FCME)	MF664642	MF664057	MF663904
Tomentellae	*Salvia retinervia*	1) Jenks et al. (2011); 2), 3) Bolivia. Santa Cruz, *A. Cocucci 5506* (CORD)	HQ418888	MF664058	MF663905
Rhombifoliae	*Salvia rhombifolia*	Jenks et al. (2013)	MF622177	/	MF623996
Angulatae	*Salvia rhyacophila*	Mexico. Michoacán, *G. Salazar-Chávez 9208* (MEXU)	MF664643	MF664059	MF663906
Farinaceae	*Salvia richardsonii*	Mexico. Tamaulipas, *P. Carrillo & E. Rojas 5644* (IEB)	MF664644	MF664060	MF663907
Angulatae	*Salvia roscida*	Mexico. Puebla. *Amith 20364* (MEXU)			
*Pruinosae**	*Salvia rosei*	Mexico. Zacatecas, *J. Balleza & M. Adame 11532* (MEXU)	MF664645	MF664061	MF663908
Rubescentes	*Salvia rubescens*	Jenks et al. (2013)	MF622178	/	MF623997
Membranaceae	*Salvia rubiginosa*	Mexico. Veracruz, *M. Tlapa & H. Morales 337* (FCME)	MF664646	MF664062	MF663909
Hastatae	*Salvia rubriflora*	*I. Fragoso-Martínez & R. Middleton 649* (cult.)			
Killipiana	*Salvia rufula*	1), 3) Jenks et al. (2013); 2) *I. Fragoso-Martínez & R. Middleton 220* (cult.)	MF622179	MF664063	MF623998
Scorodonia	*Salvia rupicola*	Mexico. Hidalgo, *F. Sazatornil 17* (MEXU)	MF664647	MF664064	MF663910
Cylindriflorae	*Salvia rusbyi*	1), 2) Walker and Sytsma (2007); 3) Jenks et al. (2013)	DQ667278	DQ667463	MF624023
Malacophyllae	*Salvia rypara* var. *platystoma*	Bolivia. Chuquisaca, *A. Cocucci 5545* (CORD)	MF664648	MF664065	MF663911
Malacophyllae	*Salvia rypara* var. *rypara*	Argentina. Jujuy, *A. Cocucci 5467* (CORD)	MF664649	MF664066	MF663912
Unassigned	*Salvia rzedowskii*	Mexico. Estado de Mexico, *J. Rojas 2038* (FCME)	MF664650	MF664067	MF663913
Hastatae	*Salvia sagittata*	1), 2) Walker and Sytsma (2007); 3) Jenks et al. (2013)	DQ667260	DQ667446	MF623999
Membranaceae	*Salvia sanctae-luciae*	Mexico. Nayarit, *J. Calzada et al. 19240* (FCME)	MF664651	MF664068	MF663914
Scorodonia	*Salvia sapinea*	Mexico. Oaxaca, *G. Salazar-Chávez 9441* (MEXU)	MF664652	MF664069	MF663915
Flocculosae	*Salvia sarmentosa*	Peru. Cuzco, *A. Tupayachi & W. Galiano 796* (MEXU)	MF664653	MF664070	MF663916
Nobiles	*Salvia scabrida*	Jenks et al. (2013)	MF622181	/	MF624000
Hastatae	*Salvia scutellarioides*	Walker and Sytsma (2007)	DQ667327	DQ667516	DQ667412
Gardoquiiflorae	*Salvia selleana*	Dominican Republic. *I. Fragoso Martínez et al. 503* (JBSD, MEXU)			
*Atratae**	*Salvia semiatrata*	1), 2)Walker and Sytsma (2007); 3) Jenks et al. (2013)	DQ667295	DQ667481	MF664098
Micranthae	*Salvia serotina*	Dominican Republic, *I. Fragoso-Martínez et al. 506* (JBSD, MEXU)			
Uliginosae	*Salvia setulosa*	1), 3) Jenks et al. (2013); 2) Mexico. Estado de Mexico, *J. Rojas et al. 3944* (FCME)	MF622182	MF664071	MF624001
Fulgentes	*Salvia sharpii*	Jenks et al. (2013)	MF622183	/	MF624002

Siphonantha	*Salvia sigchosica*	Ecuador. Chimborazo, *I. Fragoso-Martínez et al. 732* (QCNE, MEXU)			
Carneae	*Salvia simulans*	Mexico. Hidalgo, *I. Fragoso-Martínez 288* (MEXU)	MF664654	MF664072	MF663917
Uliginosae	*Salvia sinaloensis*	Jenks et al. (2013)	MF622184	/	MF624003
Malacophyllae	*Salvia sophrona*	Bolivia. Cochabamba, *A. Cocucci 5533* (CORD)	MF664655	MF664073	MF663918
Purpureae	*Salvia sordida*	*I. Fragoso-Martínez & R. Middleton 218* (cult.)	MF664656	MF664074	MF663919
Macrostachyae	*Salvia speciosa*	Jenks et al. (2013)	MF622185	/	MF624004
Angulatae	*Salvia sphacelioides*	*I. Fragoso-Martínez & R. Middleton 203* (cult.)	MF664657	MF664075	MF663920
Secundae	*Salvia splendens*	1), 3) Jenks et al. (2013); 2) Mexico. Puebla, *I. Fragoso-Martínez 274* (FCME)	MF622186	MF664076	MF624005
Cylindriflorae	*Salvia sprucei*	I. Fragoso-Martínez & R. Middleton 200 (cult.)	MF664658	MF664077	MF663921
Biflorae	*Salvia squalens*	I. Fragoso-Martínez & R. Middleton 213 (cult.)	MF664659	MF664078	MF663922
Malacophyllae	*Salvia stachydifolia*	Argentina. Jujuy, *A. Cocucci 5552* (CORD)	MF664660	MF664079	MF663923
Lavanduloideae	*Salvia stachyoides*	Mexico. Tlaxcala, *G. Flores 5372 et al.* (FCME)	MF664661	MF664080	MF663924
Holwaya	*Salvia stolonifera*	1), 3) Jenks et al. (2013); 2) *I. Fragoso-Martínez & R. Middleton 227* (cult.)	MF622187	MF664081	MF624006
Biflorae	*Salvia striata*	*I. Fragoso-Martínez & R. Middleton 258* (cult.)	MF664662	MF664082	MF663925
Corrugatae	*Salvia styphelus*	Jenks et al. (2011)	HQ418891	HQ418993	HQ418943
Subrotundae	*Salvia subrotunda*	*I. Fragoso-Martínez & R. Middleton 238* (cult.)	MF664663	MF664083	MF663926
Malacophyllae	*Salvia sucrensis*	Bolivia. Chuquisaca, *A. Cocucci 5543* (CORD)	MF664664	MF664084	MF663927
Caducae	*Salvia tehuacana*	Mexico. Puebla, *M. Castañeda-Zarate 801* (FCME)	MF664665	MF664085	MF663928
Scorodonia	*Salvia tepicensis*	Jenks et al. (2013)	MF622188	/	MF624007
Scorodonia	*Salvia tenorioi*	Mexico. Puebla, *M. Castañeda-Zarate 1179* (MEXU)			
Uliginosae	*Salvia textitlana*	Mexico. Oaxaca, *I. Trujillo-Olazo 1510* (FCME)	MF664666	MF664086	MF663929
Ekmania	*Salvia thormanii*	Dominican Repuplic, Constanza. *T. Clase 8059* (JBSD)			
Flocculosae	*Salvia thymoides*	Mexico. Oaxaca, *M. Martínez s.n.* (FCME); 3) Jenks et al. (2013)	MF664667	MF664087	MF624008
Sigmoideae	*Salvia thyrsiflora*	Mexico. Guerrero, *R. Clemente 106* (FCME)	MF664668	MF664088	MF663930
Nobiles	*Salvia tilantongensis*	Mexico. Oaxaca, *E. Martínez-Ambríz et al. 521* (FCME)			
Angulatae	*Salvia tiliifolia*	Mexico, Distrito Federal, *M. Castañeda-Zarate 868* (MEXU)	MF664669	MF664089	MF663931
Purpureae	*Salvia tolimensis*	Jenks et al. (2013)	MF622190	/	MF624009
Albolanatae	*Salvia tomentella*	Jenks et al. (2013)	MF622191	/	MF624010
Polystachyae	*Salvia tonaticensis*	Mexico. Guerrero, *J. Rojas et al. 3122* (FCME)	MF664670	MF664090	MF663932
Tubiflorae	*Salvia tortuosa*	1), 3) *I. Fragoso-Martínez et al. 620* (QCCNE, MEXU); 2) Jenks et al. (2011)		HQ418995	
Cylindriflorae	*Salvia trachyphylla*	Ecuador, *G. Salazar-Chávez 9679* (QCCNE, MEXU)			

*Gentryana**	*Salvia trichostephana*	Mexico. Sinaloa, *M. Kimnach & H. Sánchez-Mejorada 2220* (MEXU)	MF664671	MF664091	MF663933
Uliginosae	*Salvia tricuspidata*	1), 2) Walker and Sytsma (2007); 3) Jenks et al. (2013)	DQ667293	DQ667479	MF624026
Curtiflorae	*Salvia tubifera*	Jenks et al. (2011)	HQ418894	HQ418996	HQ418946
Biflorae	*Salvia tubiflora*	1), 2) *I. Fragoso-Martínez & R. Middleton 263* (cult.); 3) Jenks et al. (2013)	MF664672	MF664092	MF624011
Ekmania	*Salvia tuerckheimii*	1), 2) *I. Fragoso Martínez et al. 603* (JBSD, MEXU); 3) Zona et al. (2016)			KU358704
Uliginosae	*Salvia uliginosa*	Jenks et al. (2011)	HQ418895	HQ418997	HQ418947
Ekmania	*Salvia uncinata*	Dominican Republic, *I. Fragoso Martínez et al. 575* (JBSD, MEXU)			
Holwaya	*Salvia univerticillata*	*I. Fragoso-Martínez & R. Middleton 228* (cult.)	MF664673	MF664093	MF663934
Urbania	*Salvia urbania1*	Dominican Republic, *I. Fragoso Martínez et al. 497* (JBSD, MEXU)			
Urbania	*Salvia urbania2*	Dominican Republic, *I. Fragoso Martínez et al. 529* (JBSD, MEXU)			
Angulatae	*Salvia urolepis*	México. Nuevo León, *G. Cornejo Tenorio* 4353 (IEB)		/	
Scorodonia	*Salvia urica*	1), 3) Jenks et al. (2013); 2), 3) *I. Fragoso-Martínez & R. Middleton 232* (cult.)	MF622193	MF664094	MF663935
Angulatae	*Salvia uruapana*	Mexico. Michoacán, *M. Rojas 180* (IEB)			
Cylindriflorae	*Salvia vargas-llosae*	*I. Fragoso-Martínez & R. Middleton 653* (cult.)			
Skeptostachys	*Salvia vazquezii*	1, 3) Jenks et al. (2013); 2) *I. Fragoso-Martínez & R. Middleton 305* (cult.)	MF622194		MF624013
Flocculosae	*Salvia venturana*	Mexico. Puebla, *A. Combes 1181* (BUAP)			
Tubiflorae	*Salvia venulosa*	Jenks et al. (2011)	HQ418896	HQ418998	HQ418948
Membranaceae	*Salvia verecunda*	Mexico. Chihuahua, *J. Laferriere 1035* (MEXU)	MF664674	MF664095	MF663936
Blakea	*Salvia vitifolia*	Mexico. Oaxaca, *C. Cervantes 44* (FCME)	MF664675	MF664096	MF663937
Holwaya	*Salvia wagneriana*	Jenks et al. (2013)	MF622196	/	MF624015
Angulatae	*Salvia xalapensis*	1), 3) Jenks et al. (2013); 2) Mexico, *J. Maldonado 3123* (FCME)	MF622197	MF664097	MF624016
Unassigned	*Salvia sp.*	Ecuador. Azuay, *I. Fragoso-Martínez et al. 758* (QCNE, MEXU)			

Black shaded cells indicate newly sequenced sections; gray shaded cells indicate newly sequenced accessions; an asterisk (*) in the first column indicates monotypic sections; a slash (/) in the fifth column marks missing *trn*L-*trn*F sequences

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Abiotic processes as primary drivers of the diversification of the Neotropical sages (*Salvia* subgenus *Calosphace*)

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60
50
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Dominican subclade
Angulatae clade
Lavanduloideae clade
South American clade
S. chamaedryoides + S. thymoides clade
Stem Calosphace
Calosphace+ Audibertia + Dorystaechas + Meriandra
Zhumeria + Salvia aegyptiaca clade
Salvia s.l.
Nepetoideae
Salvia officinalis clade+ Perovskia + Rosmarinus
Melissa + Lepechinia
Salvia brachyloba
Salvia selleana
Salvia paryskii
Salvia arborescens
Salvia bahorucona
Salvia foveolata
Salvia laichnaiaclada
Salvia lavendula
Salvia thormanii
Salvia uncinata
Salvia tuerckheimii
Salvia densiflora
Salvia arduinervis
Salvia praeterita
Salvia urbania2
Salvia urbania1
Salvia calaminthifolia
Salvia montecristina
Salvia amplifrons
Salvia raymondii
Salvia cyanicalyx
Salvia sordida
Salvia camarifolia
Salvia libanensis
Salvia florida
Salvia tortuosa
Salvia bogotensis
Salvia sphacelioides
Salvia cuatrecasana
Salvia rufula
Salvia tolimensis
Salvia gracilis
Salvia palealis
Salvia ionocalyx
Salvia simulans
Salvia carnea
Salvia killipiana
Salvia leptostachys
Salvia rhyacophila
Salvia hispanica
Salvia uruapana
Salvia pennellii
Salvia richardsonii
Salvia urolepis
Salvia farinacea
Salvia nervata
Salvia tubifera
Salvia jorgehintoniana
Salvia longistyla
Salvia personata
Salvia tiliifolia
Salvia connivens
Salvia iodantha
Salvia purepecha
Salvia arbuscula
Salvia trichostephana
Salvia purpurea
Salvia tonaticensis
Salvia filipes
Salvia areolata
Salvia chiapensis
Salvia plurispicata
Salvia polystachya
Salvia littae
Salvia curviflora
Salvia longispicata
Salvia xalapensis
Salvia roscida
Salvia arthrocoma
Salvia languidula
Salvia mexicana
Salvia myriantha
Salvia sapinea
Salvia calderoniae
Salvia carranzae
Salvia angulata
Salvia leucantha
Salvia peninsularis
Salvia clarkcowanii
Salvia helianthemifolia
Salvia lavanduloides
Salvia rzedowskii
Salvia semiscaposa
Salvia stachyoides
Salvia heterofolia
Salvia agnes
Salvia guadalajarensis
Salvia muscarioides
Salvia cryptodonta
Salvia palmeri
Salvia rosei
Salvia pichinchensis
Salvia sigchosica
Salvia alborosea
Salvia pauciserrata
Salvia pansamalensis
Salvia diegoae
Salvia madrigalii
Salvia gravida
Salvia vazquezii
Salvia hirtella
Salvia trachyphylla
Salvia speciosa
Salvia quitensis
Salvia hirta
Salvia sprucei
Salvia reflexa
Salvia rhombifolia
Salvia integrifolia
Salvia striata
Salvia squalens
Salvia oppositiflora
Salvia tubiflora
Salvia macrostachya
Salvia lobbii
Salvia angustiarum
Salvia cedrosensis
Salvia jaimehintoniana
Salvia reptans
Salvia buchananii
Salvia azurea
Salvia blepharophylla
Salvia sharpii
Salvia vargas llosae
Salvia chionophylla
Salvia xolocotzii
Salvia zamoranensis
Salvia ozolotepecensis
Salvia praestans
Salvia perlonga
Salvia amarissima
Salvia leucochlamys
Salvia urica
Salvia serotina
Salvia caymanensis
Salvia coccinea
Salvia herbacea
Salvia infuscata
Salvia gachantivana
Salvia melaleuca
Salvia gracilipes
Salvia nubigena
Salvia erythrostoma
Salvia rubescens
Salvia amethystina
Salvia protracta
Salvia thyrsiflora
Salvia ramamoorthyana
Salvia chapalensis
Salvia alamosana
Salvia pugana
Salvia aequidistans
Salvia dichlamys
Salvia brevipes
Salvia minarum
Salvia rypara rypara
Salvia sucrensis
Salvia exserta
Salvia subrotunda
Salvia nervosa
Salvia grewiifolia
Salvia oxyphora
Salvia tomentella
Salvia rusbyi
Salvia pallida
Salvia graciliramulosa
Salvia orbignaei
Salvia alba
Salvia splendens
Salvia haenkei
Salvia rypara platystoma
Salvia sophrona
Salvia durifolia
Salvia confertiflora
Salvia articulata
Salvia hilarii
Salvia cerradicola
Salvia melissiflora
Salvia guaranitica
Salvia scabrida
Salvia stachydifolia
Salvia atrocyanea
Salvia dombeyi
Salvia formosa
Salvia sarmentosa
Salvia aspera
Salvia pexa
Salvia keerlii
Salvia semiatrata
Salvia chamaedryoides
Salvia thymoides
Salvia lasiantha
Salvia tenorioi
Salvia ramosa
Salvia melissodora
Salvia breviflora
Salvia rupicola
Salvia discolor
Salvia leucocephala
Salvia sp
Salvia loxensis
Salvia curticalyx
Salvia flocculosa
Salvia decumbens
Salvia incumbens
Salvia guevarae
Salvia galloana
Salvia bupleuroides
Salvia compsostachys
Salvia lasiocephala
Salvia verecunda
Salvia mocinoi
Salvia rubiginosa
Salvia confertispicata
Salvia lophanthoides
Salvia langlassei
Salvia sanctaeluciae
Salvia nitida
Salvia adenophora
Salvia disjuncta
Salvia madrensis
Salvia greggii
Salvia muelleri
Salvia coahuilensis
Salvia lycioides
Salvia microphylla
Salvia macellaria
Salvia venturana
Salvia fulgens
Salvia gesneriiflora
Salvia lineata
Salvia stolonifera
Salvia booleana
Salvia darcyi
Salvia patriciae
Salvia pulchella
Salvia univerticillata
Salvia holwayi
Salvia wagneriana
Salvia involucrata
Salvia puberula
Salvia dorisiana
Salvia karwinskii
Salvia divinorum
Salvia venulosa
Salvia acerifolia
Salvia modica
Salvia podadena
Salvia tehuacana
Salvia purpusii
Salvia ballotiflora
Salvia pinguifolia
Salvia candicans
Salvia oaxacana
Salvia betulifolia
Salvia regla
Salvia pubescens
Salvia hidalgensis
Salvia tilantongensis
Salvia filifolia
Salvia nana
Salvia prunelloides
Salvia forreri
Salvia laevis
Salvia glechomifolia
Salvia setulosa
Salvia tetramerioides
Salvia pusilla
Salvia albocaerulea
Salvia sinaloensis
Salvia misella
Salvia occidentalis
Salvia bullulata
Salvia styphelus
Salvia corrugata
Salvia clinopodioides
Salvia congestiflora
Salvia uliginosa
Salvia lachnostachys
Salvia ovalifolia
Salvia procurrens
Salvia cinnabarina
Salvia elegans
Salvia textitlana
Salvia tricuspidata
Salvia hintonii
Salvia fruticulosa
Salvia parryi
Salvia calolophos
Salvia cuspidata gilliesii
Salvia heerii
Salvia retinervia
Salvia cuspidata rosea
Salvia concolor
Salvia flaccidifolia
Salvia recurva
Salvia macrophylla
Salvia sagittata
Salvia rubriflora
Salvia scutellarioides
Salvia cacaliifolia
Salvia patens
Salvia vitifolia
Salvia axillaris
Salvia mellifera
Salvia munzii
Salvia apiana
Salvia clevelandii
Salvia californica
Salvia greatae
Dorystaechas hastata
Salvia dianthera
Salvia aegyptiaca
Salvia trichocalycina
Salvia aristata
Salvia majdae
Salvia chienii
Salvia sinica
Salvia koyamae
Salvia roborowskii
Salvia sonchifolia
Salvia nemorosa
Salvia transsylvanica
Salvia austriaca
Salvia garipensis
Salvia aucheri
Salvia officinalis
Salvia cabulica
Salvia lyrata
Salvia roemeriana
Perovskia atriplicifolia
Perovskia scrophulariifolia
Salvia rosmarinus
Lepechinia caulescens
Melissa officinalis
Audibertia
Dorystaechas + Meriandra
Salvia aegyptiaca clade
Salvia glutinosa clade
Salvia officinalis clade
Perovskia + Rosmarinus
60
50
40
30
20
10
0
Paleocene
Eocene
Oligocene
Miocene
Pliocene
Pleistocene
Paleogene
Neogene
Quat.