Annals of Botany **122**: 649–668, 2018
doi: 10.1093/aob/mcy104, available online at www.academic.oup.com/aob

ANNALS OF BOTANY
Founded 1887

Phylogeny and staminal evolution of *Salvia* (Lamiaceae, Nepetoideae) in East Asia

Guo-Xiong Hu[1,2], Atsuko Takano[3], Bryan T. Drew[4], En-De Liu[1], Douglas E. Soltis[5,6], Pamela S. Soltis[5], Hua Peng[1,*] and Chun-Lei Xiang[1,*]

[1]Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China, [2]College of Life Sciences, Guizhou University, Guiyang, Guizhou 550025, China, [3]Museum of Nature and Human Activities, Hyogo, 6 Chome, Yayoigaoka, Sanda, Hyogo 669-1546, Japan, [4]Department of Biology, University of Nebraska-Kearney, Kearney, NE 68849, USA, [5]Department of Biology, University of Florida, Gainesville, FL 32611, USA and [6]Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA
**For correspondence. E-mail hpeng@mail.kib.ac.cn or xiangchunlei@mail.kib.ac.cn*

Received: 10 February 2018 Returned for revision: 10 April 2018 Editorial decision: 18 May 2018 Accepted: 21 May 2018
Published electronically 25 June 2018

• **Background and Aims** *Salvia* is the largest genus within Lamiaceae, with about 980 species currently recognized. East Asia, with approx. 100 species, is one of the three major biodiversity centres of *Salvia*. However, relationships within this lineage remain unclear, and the staminal lever mechanism, which may represent a key innovation within the genus, has been understudied. By using six genetic markers and nearly comprehensive taxon sampling, this study attempts to elucidate relationships and examine evolutionary trends of staminal development within the East Asia (EA) *Salvia* clade.
• **Methods** Ninety-one taxa of EA *Salvia* were sampled and 34 taxa representing all other major lineages of *Salvia* were included for analysis. Two nuclear [internal transcribed spacer (ITS) and external transcribed spacer (ETS)] and four chloroplast (*psbA–trnH*, *ycf1–rps15*, *trnL–trnF* and *rbcL*) DNA markers were used for phylogenetic analysis employing maximum parsimony (MP), maximum likelihood (ML) and BEAST, with the latter also used to estimate divergence times.
• **Key Results** All *Salvia* species native to East Asia form a clade, and eight major subclades (A–G) were recognized. Subclade A, comprising two limestone endemics (*S. sonchifolia* and *S. petrophila*), is sister to the remainder of EA *Salvia*. Six distinct stamen types were observed within the EA clade. Stamen type A, with two fully fertile posterior thecae, only occurs in *S. sonchifolia* and may represent the ancestral stamen type within EA *Salvia*. Divergence time estimates showed that the crown of EA *Salvia* began to diversify approx. 17.4 million years ago.
• **Conclusions** This study supports the adoption of a broadly defined *Salvia* and treats EA *Salvia* as a subgenus, *Glutinaria*, recognizing eight sections within this subgenus. Stamen type A is ostensibly plesiomorphic within EA *Salvia*, and the other five types may have been derived from it. Staminal morphology has evolved in parallel within the EA *Salvia*, and staminal structure alone is inadequate to delimit infrageneric categories.

Key words: *Salvia*, phylogeny, staminal evolution, stamen movement, Mentheae, *Salvia sonchifolia*, *Salvia plebeia*, subg. *Glutinaria*, sect. *Sobiso*.

INTRODUCTION

Salvia, with about 980 species and a nearly cosmopolitan distribution, is the largest genus in the angiosperm family Lamiaceae (Alziar, 1988–1993; Walker *et al.*, 2004; Wei *et al.*, 2015; Drew *et al.*, 2017; Will and Claßen-Bockhoff, 2017). The genus has undergone major species radiations in Mesoamerica/South America (at least 500 spp.), south-western Asia and the Mediterranean region (approx. 250 spp.), and Eastern Asia (approx. 100 spp.) (Alziar, 1988–1993; Walker and Sytsma, 2007). The genus is utilized throughout its range for medicinal purposes, and many species are of economic importance. For instance, *Salvia miltiorrhiza* ('Danshen' in Chinese), endemic to China, is a traditional Chinese medicine that is widely used to treat cardiovascular and cerebrovascular diseases and hyperlipidaemia (Wang, 2010). *Salvia hispanica*, an important Mesoamerican staple food and medicinal plant in pre-Columbian times, is now available commercially worldwide as a 'superfood' (Ali *et al.*, 2012). Additionally, at least 150 species are widely marketed in the horticultural trade (Clebsch, 2008).

Salvia has traditionally been distinguished from other genera of Lamiaceae by an unusual morphological character in which two fertile stamens are separated by a significantly elongated connective tissue. Based on calyx, corolla and stamen morphology, Bentham (1832–1836) first established an infrageneric classification for *Salvia.* His treatment placed the 266 species known at the time into 14 sections. In subsequent updates, he classified 406 *Salvia* species into 12 sections (Bentham, 1848), and eventually these sections were organized into four subgenera (Bentham, 1876), thus forming the first comprehensive subgeneric classification of *Salvia*. Subsequently, Briquet (1897)

provided an updated global synopsis of the genus, recognizing eight subgenera and 17 sections. These two comprehensive classifications were subsequently modified by various authors (e.g. Stibal, 1934; Epling, 1938, 1939; Pobedimova, 1954; Wu, 1977; Murata and Yamazaki, 1993).

Due to tremendous diversity in habit, floral morphology and staminal morphology across *Salvia*, infrageneric boundaries within the genus have been notoriously difficult to define (Bentham, 1876; Briquet, 1897; Stibal, 1934; Pobedimova, 1954; Wu, 1977; Murata and Yamazaki, 1993; Drew *et al.*, 2017; Will and Claßen-Bockhoff, 2017). To avoid troublesome issues resulting from subgeneric circumscriptions, some researchers adopted 'species-groups' or sections (often monotypic) rather than explicitly defining subgenera (e.g. Epling, 1939; Hedge, 1974, 1982*a*, *b*).

Recent molecular phylogenetic studies, however, have demonstrated that traditionally defined *Salvia* is non-monophyletic, as five genera (*Dorystaechas*, *Meriandra*, *Perovskia*, *Rosmarinus* and *Zhumeria*), most lacking an elongated connective as in traditionally defined *Salvia*, are embedded within it (Walker *et al.*, 2004, 2015; Walker and Sytsma, 2007; Drew and Sytsma, 2011, 2012; Takano and Okada, 2011; Jenks *et al.*, 2012; Li *et al.*, 2013; Will and Claßen-Bockhoff, 2014, 2017; Drew *et al.*, 2017; Fragoso-Martínez *et al.*, 2018). Two competing ideas regarding circumscription of the genus have recently been proposed. One option is to name the five embedded genera as *Salvia* and maintain *Salvia* in a broad (although slightly expanded) sense (González-Gallegos, 2015; Drew *et al.*, 2017). The other is to break up *Salvia* into several smaller genera (Will *et al.*, 2015; Will and Claßen-Bockhoff, 2017) and maintain the names of the five embedded genera. Will *et al.* (2015), advocating the latter option, transferred 14 *Salvia* species (distributed from south-west Asia to northern Africa) to the resurrected genus *Pleudia*. Later, based on expanded phylogenetic sampling of Mediterranean *Salvia*, Will and Claßen-Bockhoff (2017) suggested splitting *Salvia* into six genera [i.e. *Salvia sensu stricto* (*s.s.*). *Lasemia*, *Ramona*, *Glutinaria*, *Pleudia* and *Polakia*] and retaining the generic status of the five embedded genera. Will and Claßen-Bockhoff (2017) did not provide formal taxonomic treatments, but offered suggestions for future nomenclatural revisions. Conversely, based on phylogenetic, taxonomic, morphological and practical considerations, Drew *et al.* (2017) treated the five embedded genera as subgenera within *Salvia* (subg. *Dorystaechas*, subg. *Meriandra*, subg. *Perovskia*, subg. *Rosmarinus* and subg. *Zhumeria*) to maintain a broadly defined *Salvia*, and provided nomenclatural revisions for the 15 species belonging to the five embedded genera. Based upon the taxonomic treatment suggested by Will and Claßen-Bockhoff (2017), about 750 *Salvia* species would be transferred to the resurrected genera *Glutinaria*, *Lasemia*, *Ramona*, *Pleudia* and *Polakia*. Consequent nomenclatural changes would lead to confusion in other subjects such as horticulture, ecology and phytochemistry. Furthermore, the boundaries between the genera advocated by Will and Claßen-Bockhoff (2017) are not morphologically distinct, which would lead to ongoing taxonomic confusion. Inclusion of the five embedded genera in a broadly defined *Salvia* is phylogenetically legitimate and will probably be accepted by botanists as well as workers in other disciplines. Therefore, we prefer to maintain a broadly defined *Salvia* following Drew *et al.* (2017).

The East Asian (EA) radiation of *Salvia* comprises 82 species native (72 endemic) to China, 12 species native (nine endemic) to Japan and three species native (one endemic) to the Korean Peninsula (Murata and Yamazaki, 1993; Li and Hedge, 1994; Lee, 2004; Hu *et al.*, 2013, 2014, 2017; Takano *et al.*, 2014; Hu and Peng, 2015; Xiang, 2016; Xiang *et al.*, 2016*a*). The EA *Salvia* are highly diverse in terms of root, leaf, calyx, corolla and staminal morphology and habitat (Fig. 1). Additionally, all EA *Salvia* are herbaceous, contrasting with the other two major centres of diversity where shrubs are common. Based on staminal morphology, EA *Salvia* have been classified into three subgenera: subg. *Salvia*, subg. *Sclarea* and subg. *Allagospadonopsis* (Wu, 1977; Murata and Yamazaki, 1993).

Early molecular phylogenetic studies, focusing on *Salvia* as a whole (Walker *et al.*, 2004; Walker and Sytsma, 2007), indicated that EA *Salvia* may represent an independent lineage, but these studies had sparse sampling within EA *Salvia*. Subsequently, two phylogenetic studies focusing on EA *Salvia* have been conducted. Using three DNA makers [*rbcL*, *trnL–trnF* and an internal transcribed spacer (ITS)], Takano and Okada (2011) first reported that the 11 species of *Salvia* from Japan are monophyletic. Later, based upon four DNA markers (*psbA–trnH*, *rbcL*, *matK* and ITS), Li *et al.* (2013) inferred phylogenetic relationships of 37 Chinese and four Japanese *Salvia* species and found that the Chinese (except for *S. deserta*, widespread in Xinjiang Province of China, Russia, Kyrgyzstan and Kazakhstan) and Japanese species of *Salvia* formed a clade. Unfortunately, due to limited sampling and misidentification of a few key species (this remark is based on results presented here), the phylogenetic backbone of EA *Salvia* was not resolved.

Additionally, Li *et al.* (2013) suggested discordance between phylogenetic results and traditional classifications, although morphological evidence was not provided. Recently, Will and Claßen-Bockhoff (2017), based on ITS sequences, recognized three clades of EA *Salvia.* Most of the the 46 EA *Salvia* ITS sequences used by Will and Claßen-Bockhoff (2017) were from GenBank. However, based on phylogenetic analyses presented here, we found that some ITS sequences (e.g. sequences of *S. plectranthoides* 247, *S. chienii* DQ123828# and *S. evansiana* FJ593405#) used in Will and Claßen-Bockhoff (2017) were questionable, which may have resulted from species misindentification and/or GenBank uploading errors.

Traditionally, *Salvia* has been defined largely by a lever-like staminal feature that is formed by elongate connectives and filaments (Himmelbaur and Stibal, 1932–1934; Claßen-Bockhoff *et al.*, 2004*a*). Staminal morphology in *Salvia* is highly diverse, and up to 11 distinct stamen types have been described within traditionally defined *Salvia* (Himmelbaur and Stibal, 1932–1934; Claßen-Bockhoff *et al.*, 2003, 2004*a*, *b*; Walker and Sytsma, 2007; Will and Claßen-Bockhoff, 2014; Walker *et al.*, 2015). Himmelbaur and Stibal (1932–1934) first hypothesized parallel evolution of the lever mechanism within *Salvia*, and this has been repeatedly corroborated by molecular phylogenetic studies (Walker *et al.*, 2004, 2015; Walker and Sytsma, 2007; Will and Claßen-Bockhoff, 2014; Drew *et al.*, 2017).

Wu (1977) recognized three distinct stamen types for EA *Salvia* species: (1) connectives ± curved, upper arms longer than or equal to lower arms, posterior thecae fertile, fused; (2) connectives ± straight, not curved, posterior thecae sterile, fused; and (3) connectives ± straight, not curved, posterior thecae

FIG. 1. Morphological diversity of EA *Salvia*. 1–4, Root morphology (1, *S. castanea*; 2, *S. miltiorrhiza*; 3, *S. plectranthoides*; 4, *S. cavaleriei* var. *erythrophylla*). 5–10, Leaf morphology (5, *S. sonchifolia*; 6, *S. luteistriata*; 7, *S. wardii*; 8, *S. prionitis*; 9, *S. bowleyana*; 10, *S. japonica*). 11–13, Bract morphology (11, *S. scapiformis*; 12, *S. trijuga*; 13, *S. atropurpurea*). 14–17, Calyx morphology (14, *S. scapiformis*; 15, *S. sonchifolia*; 16, *S. substolonifera*; 17, *S. hylocharis*). 18–24, Corolla diversity (18, *S. sonchifolia*; 19, *S. miltiorrhiza*; 20, *S. honania*; 21, *S. japonica*; 22, *S. liguliloba*; 23, *S. campanulata*; 24, *S. prattii*). 25–34, Stamen diversity (25, *S. sonchifolia*; 26, *S. luteistriata*; 27, *S. plebeia*; 28 and 29, *S. plectranthoides*; 30, *S. petrophila*; 31, *S. bowleyana*; 32, *S. honania*; 33, *S. cavaleriei*; 34, *S. scapiformis*). Photographs: 12–13 by E. D. Liu, 24 by Y. P. Chen, 29 by X. X. Zhu, others by G. X. Hu.

sterile, separated. Based on the staminal morphology alone, Wu (1977) classified EA *Salvia* into three subgenera (subg. *Salvia*, *Sclarea* and *Allagospadonopsis*). Based on floral morphology and Wu's (1977) treatment, Huang *et al.* (2014) hypothesized an evolutionary trend of stamen types for EA *Salvia*, from a 'short-lever type' (subg. *Salvia*), to a 'long-lever type' (subg. *Sclarea*), to a 'degraded-lever type' (subg. *Allagospadonopsis*). However, while preparing this paper, we observed additional stamen types within EA *Salvia* and found that the stamen types defined by Wu (1977) are too general to describe stamen morphology accurately within EA *Salvia* [see Fig. 1 (25–34)]. Additionally, the three subgenera circumscribed by Wu (1977) have been demonstrated to be non-monophyletic. The evolutionary trajectory of stamen types for EA *Salvia* therefore needs to be re-evaluated. Additionally, it remains unclear whether parallel evolution of staminal morphology has occurred within EA *Salvia*, as sampling within EA *Salvia* was limited in previous phylogenetic research regarding staminal evolution (Walker and Sytsma, 2007; Will and Claßen-Bockhoff, 2014).

In this study, based on the most comprehensive geographic, taxonomic and genetic sampling to date, we reconstruct the phylogeny of EA *Salvia* and clarify inter-relationships within the group. Furthermore, we present a detailed summary of staminal morphology within EA *Salvia* and elucidate evolutionary trends in staminal morphology in the context of a phylogenetic framework. Finally, based on phylogenetic and morphological considerations, we provide an updated taxonomic treatment for EA *Salvia*.

MATERIALS AND METHODS

Nomenclature and taxon sampling

Names of *Salvia* subgenera and major clades follow Drew *et al.* (2017). A total of 91 taxa, representing 78 species, ten varieties and three forms from China, Japan and the Korean Peninsula, were sampled for this study. Except for the monotypic sect. *Aethiopis* and ser. *Piasezkianae*, our sampling represents all subgenera, sections and series *sensu* Wu (1977) and Murata and Yamazaki (1993). In addition, 33 species of *Salvia* from other major lineages of *Salvia* (subg. *Calosphace*, *Audibertia*, *Dorystaechas*, *Meriandra*, *Perovskia*, *Rosmarinus*, *Zhumeria*, the '*S. aegyptiaca* clade' and the '*S. officinalis* clade') were sampled. *Melissa* and *Lepechinia* from subtribe Salviinae were also sampled. *Horminum* and *Hedeoma* were selected as outgroups based on Drew and Sytsma (2012). In total, our data set included 172 accessions, of which 145 were newly generated for this study (see Supplementary Data Appendix). Although sequences of many Chinese taxa are available from GenBank, we sampled and identified all Chinese taxa used in this study, and all sequences of Chinese taxa used here were independently produced to ensure independent results and accuracy.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from silica gel-dried leaf materials or herbarium specimens (*S. brachyloma*, *S. filicifolia* and *S. potaninii*) using the modified cetyltrimethylammoniun bromide (CTAB) method of Doyle and Doyle (1987). Four chloroplast DNA markers (*psbA–trnH*, *ycf1–rps15*, *trnL–trnF* and *rbcL*) and two nuclear regions [the ITS and the external transcribed sequence (ETS)] were selected for phylogenetic analyses. Primers used in this study are listed in Table 1.

The standard 25 μL PCR mixtures contained 1 μL of each primer (10 μM, Sangon Biotechnology, Shanghai, China), 2.5 μL of 10× reaction buffer (Mg^{2+} free), 2.5 μL of dNTP mixture, 1.5 μL of $MgCl_2$, 0.3 μL of *Taq* polymerase (2.5 U μL^{-1}, Tiangen Biotech, Beijing, China), 2 μL of unquantified template DNA, 1 μL of bovine serum albumin (BSA, 20 mg ml^{-1}) and deionized water added to achieve a final volume of 25 μL. Amplification for all six markers was performed as follows: an initial denaturation at 94 °C for 4 min, followed by 35 cycles of 30 s denaturation (94 °C), 90 s annealing (50 °C) and 2.5 min extension (72 °C), ending with a final extension at 72 °C for 7 min.

Amplification products were checked on 1 % TAE agarose gels and purified using the QIAquick PCR purification kit (BioTeke, Beijing, China) following the manufacturer's instructions. Sequencing reactions were performed with the dideoxy chain termination method running on an ABI-PRISM-3730 automated sequencer (Sangon Biotechnology). Sequencing primers for DNA markers were the same as for the PCR primers.

Table 1. *List of primers used in this study (F represents a forward primer and R represents a reverse primer)*

DNA markers	Sequences (5′–3′)	References
ITS	ITS5 (F): GGAAGTAAAAGTCGTAACAAGG	White *et al.* (1990)
	ITS4 (R): TCCTCCGCTTATTGATATGC	White *et al.* (1990)
	ITSA (F): GGAAGGAGAAGTCGTAACAAGG	Blattner (1999)
	ITSB (R): CTTTTCCTCCGCTTATTGATATG	Blattner (1999)
ETS	ETS-bdf1 (F): GTGAGTGGTGKTTGGCGYGT	Drew and Sytsma (2011)
	18S-IGS(R): GAGACAAGCATATGACTACTGGCAGGATCAACCAG	Baldwin and Markos (1998)
	ETS-B (F): ATAGAGCGCGTGAGTGGTG	Beardsley and Olmstead (2002)
	18S-E (R): GCAGGATCAACCAGGTAGCA	Baldwin and Markos (1998)
psbA–trnH	psbAF (F): GTTATGCATGAACGTAATGCTC	Sang *et al.* (1997)
	trnHR (R): CGCGCATGGTGGATTCACAAATC	Sang *et al.* (1997)
rbcL	Z1F (F): ATGTCACCACAAACAGAAACTAAAGCAAGT	Soltis *et al.* (1992)
	Z1351R(R): CTTCACAAGCAGCAGCTAGTTCAGGACTCC	Soltis *et al.* (1992)
trnL–trnF	trn-c (F): CGAAATCGGTAGACGCTACG	Taberlet *et al.* (1991)
	trn-f (R): ATTTGAACTGGTGACACGAG	Taberlet *et al.* (1991)
ycf1–rps15	ycf1 5711f (F): CTTGTATGRATCGTTATTGKTTTG	Drew and Sytsma (2011)
	ycf1 rps15r (R): CAATTYCAAATGTGAAGTAAGTCTCC	Drew and Sytsma (2011)

Sequence alignment and phylogenetic analyses

Sequences were checked and assembled using Sequencher v.4.1.4 (Gene Codes, Ann Arbor, MI, USA). Alignments were initially performed using MUSCLE (Edgar, 2004) as implemented in MEGA v.6.0 (Tamura *et al.*, 2013) and then manually adjusted using PhyDE v.0.9971 (Müller *et al.*, 2010). Two separate matrices [combined nuclear ribosomal DNA (nrDNA) and combined chloroplast DNA (cpDNA)] were used for phylogenetic analyses using the following approaches: maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BEAST). To assess incongruence between the combined nrDNA and combined cpDNA, we compared the resultant trees from the two genetic compartments for supported incongruence, and also performed the incongruence length difference (ILD) test (Farris *et al.*, 1994) as implemented in PAUP* version 4.0b10 (Swofford, 2003). While Yoder *et al.* (2001) suggested that the ILD test was not useful in testing data partition compatibility, Hipp *et al.* (2004) demonstrated this argument to be unconvincing and pointed out that the ILD test has value as a tool for assessing data partition congruence.

The MP analyses were performed using PAUP* v.4.0b10 (Swofford, 2003) with the following settings: heuristic search option, tree-bisection-reconnection (TBR) branch swapping with 1000 random sequence addition replicates and ten trees saved per replicate. All characters were unordered and equally weighted; gaps were treated as missing data. A strict consensus tree was summarized from all of the most parsimonious trees retained. Bootstrap support values were calculated from 1000 rapid bootstrap replicates, with each comprising ten random sequence addition replicates, with only one tree saved per replicate.

Partitioned ML analyses were performed using RAxML-HPC2 on XSEDE v.8.1.11 (Stamatakis, 2014) as implemented on the CIPRES computer cluster (http://www.phylo.org/) (Miller *et al.*, 2010). The GTRCAT model was used for analyses and bootstrapping; bootstrap iterations (–#/–N) was set to 1000, and other parameters followed default settings. All trees were visualized using TreeGraph v.2 (Stöver and Müller, 2010).

Divergence time estimation

Divergence time analyses were performed using BEAST v.1.8.3 (Drummond *et al.*, 2012) as implemented on the CIPRES computer cluster (http://www.phylo.org/) (Miller *et al.*, 2010). Dates were estimated using a lognormal relaxed molecular clock and the Yule model of speciation. Models of nucleotide evolution were evaluated with jModelTest2 (Darriba *et al.*, 2012) using the Akaike information criterion (AIC).

Although divergence times of the *Salvia* crown have been investigated previously (e.g. Drew and Sytsma, 2012; Drew *et al.*, 2017), sampling within EA *Salvia* was limited, and consequently the divergence times of the main clades of EA *Salvia* are not clear. Here, based on broad sampling of EA *Salvia*, we employed a two-step scheme to estimate divergence times within *Salvia* independently. For the initial step, we used the Lamiaceae-wide data set from Drew and Sytsma (2012) and used two constraint strategies. (1) We used the minimum (48.3) and maximum (71.9) Lamiaceae crown dates from Yao *et al.* (2016) as an age constraint for the Lamiaceae crown. This constraint was applied using a truncated normal prior (with the aforementioned minimum and maximum dates), a mean of 60.1 and an s.d. of 15. This approach approximates a uniform distribution, but allows for a slightly higher prior probability in the centre of the curve relative to the edges. (2) We combined the above-described and the constraint strategy used by Drew and Sytsma (2012).

In the second step, we used the 95 % height probability distribution (HPD) from the Lamiaceae-wide analyses as a constraint for the root of the tree and for the crown of *Salvia*. We constrained the root of the tree using a truncated normal distribution, with a lower limit of 26.6 and an upper limit of 46.3, a mean of 36.45 and an s.d. of 13. We constrained the crown of *Salvia* with a uniform distribution that had a lower age of 18.5 and an upper age of 34.1.

For both Lamiaceae-wide analyses we conducted two separate runs of 60 million generations and saved samples every 5000 generations. After assessing results in Tracer v1.6 (Rambaut *et al.*, 2014), we discarded the first 6 million generations as burn-in. For the *Salvia* data set analyses, we conducted two separate runs of 100 million generations, with samples saved every 5000 generations. After assessing the results in Tracer, we discarded the first 10 % of the trees as burn-in. The log files were checked for convergence using Tracer. In both steps of our analyses, all ESS (explained sum of squares) values were well over 200; trees from separate runs were combined with LogCombiner v 1.8.4 and summarized with Tree Annotator v. 1.8.4 (both included in the BEAST package), and the chronogram was visualized using FigTree v. 1.4.2 (Rambaut, 2014).

Stamen morphology

Staminal morphology of most EA *Salvia* was observed based on fresh specimens collected in the field, with a few observations based on herbarium specimens (*Salvia brachyloma*, *S. filicifolia* and *S. potaninii*). Stamen type was summarized based on observation results. To better understand staminal morphology of EA *Salvia*, we provide stamen type schematics. In terms of morphology and size, there is no major differences among species with the same stamen type, and therefore each type is illustrated referring to a single species.

Walker and Sytsma (2007) listed 14 stamen types within *Salvia* and named them using Latin upper case letters ranging from A to N, in which only one stamen type (N) was described from EA *Salvia*. When Will and Claßen-Bockhoff (2014) showed stamen diversity of African *Salvia*, the letters A, B and C were used, but they represented different stamen types as compared with Walker and Sytsma (2007). Here, we used six upper case letters (A–F) to distinguish six distinct stamen types of EA *Salvia*. The naming system is independent of the already mentioned systems.

RESULTS

Alignment and phylogenetic reconstruction

After inspecting the cpDNA and nrDNA trees from our various analyses, it was apparent that these genomes do not exhibit the

same gene tree histories within EA *Salvia*. There were myriad well-supported differences between the two data sets, with the nrDNA phylogeny more in accordance with morphology (see the Discussion). Additionally, ILD *P*-values were <0.01, a threshold that can indicate significant incongruence between data sets (Cunningham, 1997). Thus, we did not perform analyses on a combined cpDNA and nrDNA data set.

Nuclear DNA analysis. After removing ambiguously aligned sites, the aligned length of the combined nuclear data set included 1109 bp (ITS, 672 bp; ETS, 437 bp), of which 494 bp (44.5 %) were parsimony informative. Apart from collapsed or weakly supported nodes, MP, ML and BEAST trees generated similar topologies. Therefore, only the BEAST tree is shown, with posterior probabilities (PP) and ML bootstrap (MLBS) values given above branches, and MP bootstrap (MPBS) values below branches (Fig. 2).

The monophyly of subtribe Salviinae was well supported (Fig. 2; PP = 1.00, MLBS = 85 %, MPBS = 99 %; all values are reported in this order below), in which *Melissa* was sister to *Lepechinia*, and these together were sister to *Salvia*. Within *Salvia*, subg. *Perovskia* was sister to subg. *Rosmarinus*, and this clade (subg. *Perovskia* + subg. *Rosmarinus*) was then sister to the '*Salvia officinalis* clade' (0.85, –, –); subg. *Zhumeria* and the *S. aegyptiaca* clade formed a clade (1.00, 100 %, 91 %); subg. *Dorystaechas* was sister to subg. *Meriandra*, and this clade was sister to subg. *Audibertia* + subg. *Calosphace* (1.00, 99 %, 83 %).

All EA *Salvia* formed a well-supported clade (EA clade: 1.00, 100 %, 100 %). *Salvia glutinosa*, a widely distributed species ranging from western Asia to Europe, was embedded in the EA clade. Within the EA clade, eight subclades (G1–G8) were recognized: (G1) subclade Sonchifoliae (1.00, 100 %, 100 %), including *S. sonchifolia* and *S. petrophila*, which are sister to the rest of the EA clade; (G2) subclade Notiosphace (1.00, 100 %, 100 %) containing only the widespread and enigmatic *S. plebeia*; (G3) subclade Substoloniferae (1.00, 100 %, 99 %) including *S. trijuga* and *S. substolonifera*; (G4) subclade Glutinaria (1.00, 97 %, 93 %) consisting of two species distributed from Europe to western China (*S. glutinosa* and *S. nubicola*) and another four species endemic to Japan and Taiwan Island (*S. koyamae*, *S. glabrescens*, *S. sakuensis* and *S. nipponica*); (G5) subclade Annuae (1.00, 95 %, 90 %) comprising three morphologically similar species (*S. roborowskii*, *S. umbratica* and *S. tricuspis*); (G6) subclade Eurysphace (1.00, 82 %, 78 %) including 33 species of subg. *Salvia sensu* Wu (1977); (G7) subclade Drymosphace (0.99, –, 62 %), consisting of ten species of subg. *Sclarea sensu* Wu (1977). Subclade G7 can be further divided into two groups: (1) the *Salvia miltiorrhiza* group (0.94, 72 %, 88 %), comprising *S. miltiorrhiza* and its morphologically similar species (*S. sinica*, *S, bowleyana*, *S. paramiltiorrhiza* and *S. dabieshanensis*) as well as two other morphologically unique species (*S. honania* and *S. meiliensis*); and (2) *Salvia yunnanensis*, *S. plectranthoides* and *S. nanchuanensis*, three morphologically similar species which might represent another group, the '*S. plectranthoides* group' (0.51, –, –). The final subclade (G8), subclade Sobiso (1.00, 92 %, 78 %), consists of 21 species of subg. *Allagospadonopsis* and subg. *Sclarea sensu* Wu (1977) and includes two major lineages: (1) the *S. lutescens* group (1.00, 87 %, 66 %), comprising six species (*S. hayatana* endemic to Taiwan Island and the other five endemic to Japan); and (2) the *S. chinensis* group (1.00, 94 %, 88 %), including 15 species (with the exception of *S. japonica*, the other 14 species are endemic to Japan or China).

Chloroplast DNA analysis. After removing ambiguous sites, the aligned length of the combined cpDNA data set was 3204 bp (*psbA–trnH*, 461 bp; *rbcL*, 1239 bp; *ycf1–rps15*, 657 bp; and *trnL–trnF*, 847 bp), of which 384 bp (12 %) were parsimony informative. Apart from collapsed or weakly supported nodes, MP, ML and BEAST trees generated similar topologies. Therefore, only the BEAST tree is shown, with PP and MLBS values given above branches and MPBS values below branches (Supplementary Data Fig. S1).

The monophyly of subtribe Salviinae was supported in BEAST and ML analyses (Supplementary Data Fig. S1; 1.00, 71 %, –); *Melissa* was sister to *Lepechinia*, and these together were sister to *Salvia*. Within *Salvia*, the *Salvia officinalis* clade was sister to subg. *Rosmarinus* (0.56, 84 %, 51 %), and this clade was then sister to subg. *Perovskia* (1.00, 85 %, 89 %); subg. *Dorystaechas*, subg. *Audibertia* and subg. *Calosphace* formed a clade, which was then sister to subg. *Meriandra* (1.00, 93 %, 98 %); subg. *Zhumeria* and the *S. aegyptiaca* clade formed a clade (1.00, 100 %, 100 %). Monophyly of the EA clade was again supported in cpDNA analyses (1.00, 100 %, 99 %). The cpDNA tree recovered the subclades G1, G2 and G4 of the nrDNA tree, but failed to recover the other five subclades (G3, G5, G6, G7 and G8). In the cpDNA tree, subclade G1 was sister to the rest of the EA clade, as in the nrDNA tree. Within this subclade, however, the monophyly of *S. petrophila* was not supported, as the accession from Guangxi (*S. petrophila* 1) was sister to *S. sonchifolia* instead of grouping with another accession from Guizhou (*S. petrophila* 2). Subclade G2 was recovered in the cpDNA tree (1.00, 100 %, 100 %). The sister relationship between *S. trijuga* and *S. substolonifera* (i.e. subclade G3) was not recovered in the cpDNA tree, but the monophyly of each species was supported (1.00, 100 %, 100 %). Subclade G4 was supported (1.00, 99 %, 98 %), and this clade had *S. glutinosa* as sister to the remaining five species. Subclade G5 (*S. roborowskii*, *S. tricuspis* and *S. umbratica*) was not recovered as *S. umbratica* did not form a clade with the two former species. Subclade G6 was split into two lineages, and taxa of subclade G5 were embedded in one of the lineages. Taxa of subclades G7 and G8 nested together and formed a clade (1.00, 92 %, 62 %).

Staminal morphology

To better elucidate staminal morphology within EA *Salvia*, we provided schematics illustrating EA *Salvia* staminal diversity rather than photographs. Six distinct stamen types (A–F) were observed from EA *Salvia* (Fig. 3). Stamen type A was only found in *Salvia sonchifolia*. In stamen type A [Figs 1 (25) and 3], the filaments are clearly longer than the connectives, with arms sub-equal, the anterior thecae fused, the posterior thecae similar to the anterior thecae in size and fertility, both thecae fertile, fused and in line with the lower arms distinguished from those with posterior thecae that are vertical to the lower arms [see Fig. 1 (25–34)]. Stamen type B is common in subclades G2, G3, G5 and G6. In stamen type B [Figs 1 (26,

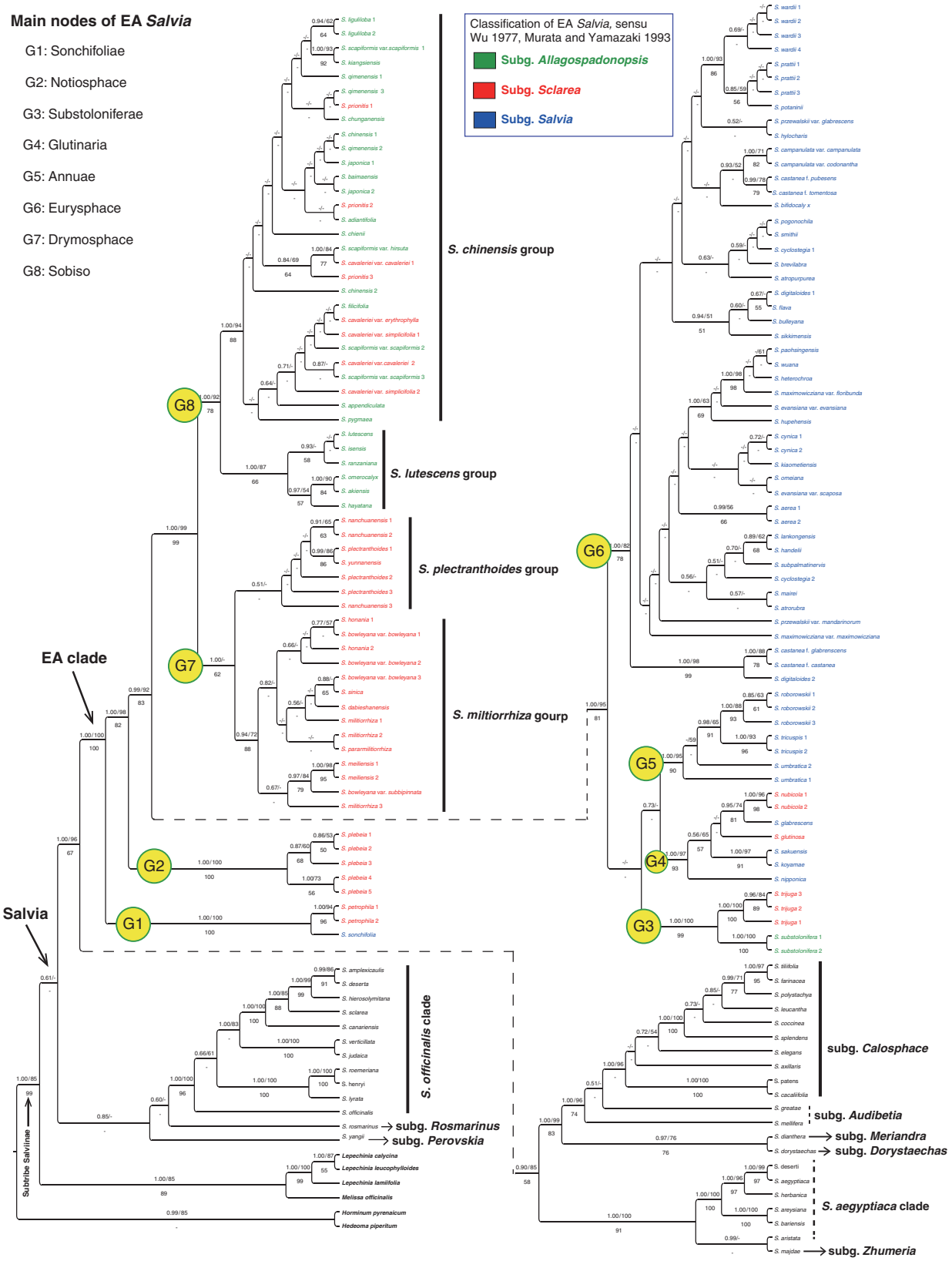

FIG. 2. Cladogram based on BEAST analysis of the combined nrDNA (ITS and ETS) matrix. Non-*Salvia* taxa are in bold. Posterior probabilities (PP) values followed by ML bootstrap values (MLBS) are given above branches, and MPBS values are indicated below. MLBS and MPBS values <50 % and PP <0.5 are indicated by '–'. Species of EA *Salvia* belonging to subgenera *sensu* Wu (1997) and Murata and Yamazaki (1993) are marked with different colours. Clades marked with dotted lines indicate the unsupported clades defined by Drew *et al.* (2017).

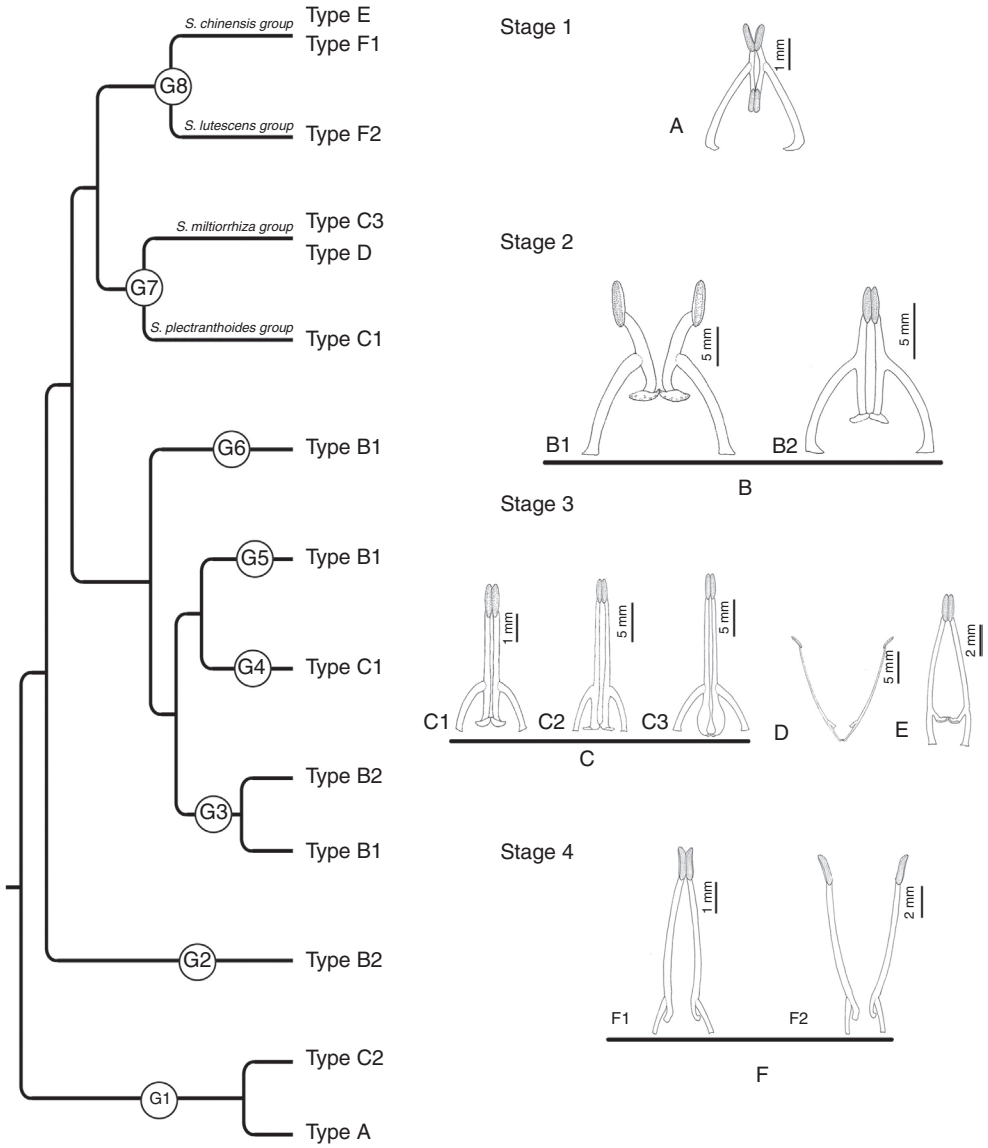

FIG. 3. Morphological diversity and hypothetical evolutionary trends of stamen types of EA *Salvia*. Parallel evolution occurs in types B, C and F. Type A is illustrated referring to *S. sonchifolia*, B1 to *S. campanulata*, B2 to *S. trijuga*, C1 to *S. plectranthoides*, C2 to *S. petrophila*, C3 to *S. bowleyana*, D to *S. honania*, E to *S. cavaleriei* var. *simplicifolia*, F1 to *S. scapiformis*, and F2 to *S. omerocalyx*.

27) and 3), the filaments are slightly shorter than or sub-equal to the connectives, the arms sub-equal, the anterior thecae connivent or slightly separated, the posterior thecae developed but clearly smaller than the upper thecae, and the posterior thecae fused with sparse (type B1) to rarely no pollen (type B2) present. *Salvia plebeia* (G2) and *S. trijuga* (G3) lack pollen (type B2), but the other taxa within the four subclades possess some pollen on the posterior thecae (type B1). Stamen type C was common in subclades G1, G4 and G7, and was variable. In stamen type C [Figs 1 (28–31) and 3], the filaments are clearly shorter than the connectives, the upper arms clearly longer than the lower arms, the anterior usually connivent but easily separated, and the posterior thecae poorly developed (type C1) or obviously reduced (types C2 and C3), and fused with no pollen present. Stamen type C1 [Figs 1 (28, 29) and 3], with obvious posterior thecae, was found in all taxa of subclade G4 and the *S. plectranthoides* group of subclade G7. Stamen type C2 [Figs 1 (30) and 3], with two obsolete and outwardly reflexed posterior thecae, was only found in *S. petrophila* of subclade G1. Stamen type C3 [Figs 1 (31) and 3], with inflated lower arms and extremely reduced posterior thecae, was found in species of the *S. miltiorrhiza* group of subclade G7, with the exception of *S. honania* and *S. meiliensis*. Stamen type D was only present in two morphologically similar species (*S. honania* and *S. meiliensis*). The structure of the type D stamen [Figs 1 (32) and 3] is similar to that of type C, but the type D has the smallest posterior thecae of all EA *Salvia*, extremely divaricate and opposite anterior thecae, and the lower arms are not inflated. Stamen type E was only observed in two species (*S. cavaleriei* and *S. prionitis*) of the *S. chinensis* group of subclade G7. In stamen type E [Figs 1 (33) and 3], the filaments are clearly shorter than the connectives, the upper arms clearly longer than

the lower arms, the anterior thecae connivent or separated, the posterior thecae slightly developed and fused, with no pollen present. Stamen type F [Figs 1 (34) and 3], restricted to subclade Sobiso, is similar to type E, but its posterior thecae are completely lost and separated, and the anterior thecae are connivent (F1) or extremely divaricate (F2). Stamen type F1 was observed for most species of the *S. chinensis* group, and type F2 was found in the *S. lutescens* group.

Divergence time estimation

The chronogram inferred from the nrDNA (ITS and ETS) data set is shown in Fig. 4 and Supplementary Data Fig. S2. Divergence times presented here are consistent with previous studies (Drew and Sytsma, 2012; Drew *et al.*, 2017). The crown age of subtribe Salviinae is estimated to be 30.15 Ma, with the 95 % HPD 23.44–37.22 Ma. The *Salvia* crown arose approx. 27.79 Ma (95 % HPD: 22.25–34.10 Ma). The crown of the *Salvia officinalis* clade + subg. *Rosmarinus* and subg. *Perovskia* clade arose approx. 23.54 Ma (95 % HPD: 16.62–30.60 Ma). The crown of the *S. aegyptiaca* + subg. *Zhumeria* clade began to diversify approx. 17.07 Ma (95 % HPD: 11.45–23.05 Ma). The crown of the subg. *Calosphace* + subg. *Audibertia* clade and subg. *Meriandra* + subg. *Dorystaechas* clade arose approx. 20.27 Ma (95 % HPD: 14.91–25.73 Ma), and the crown of subg. *Glutinaria* (EA *Salvia* clade) began to diversify approx. 17.40 Ma (95 % HPD: 12.37–23.11 Ma). The results of the divergence times for major lineages of EA *Salvia* are summarized in Table 2.

DISCUSSION

Based on nearly comprehensive taxon sampling, we corroborated that EA *Salvia* are monophyletic (both nrDNA and cpDNA analyses) and recognized eight major lineages based on the nrDNA data set. Additionally, we provide a detailed description of staminal diversity within EA *Salvia*. Based upon phylogenetic results and morphological evidence, we maintain a broadly defined *Salvia* and treat the EA *Salvia* as a new subgenus, *Glutinaria*, and recognize eight sections within this subgenus.

Possible causes of incongruence between nuclear and plastid phylogenies

Although the cpDNA phylogeny strongly supported EA *Salvia* as monophyletic, resolution within the cpDNA tree was quite low as compared with the nrDNA tree, and topologies inferred from these two data sets displayed obvious and widespread discordance (Fig. 2; Supplementary Data Fig. S1). Similar discordances between genomes have been noted in clades elsewhere in Lamiaceae, and ancient hybridization with chloroplast capture has been hypothesized to have contributed to the discordance (Albaladejo *et al.*, 2005; Drew and Sytsma, 2013; Xiang *et al.*, 2013; Drew *et al.*, 2014; Deng *et al.*, 2015; Walker *et al.*, 2015). In this study, subclades G7 and G8 formed two distinct lineages in the nrDNA tree (Fig. 2), but taxa from these two lineages were mixed together in the cpDNA phylogeny, forming a well-supported clade (Supplementary Data Fig. S1). Additionally, previous chromosome research indicated that all taxa of G7 and G8 are diploid and have the same number of chromosomes (Funamoto *et al.*, 2000; Zhao *et al.*, 2006; Wang *et al.*, 2009; Hu *et al.*, 2016). Therefore, ancient hybridization with chloroplast capture is likely to be responsible for the discordance in the placement of these two lineages. Another case probably involving chloroplast capture is that of *S. petrophila* and S. *sonchifolia*. In the nrDNA tree, two accessions of *S. petrophila* formed a clade. In the cpDNA tree, however, *S. petrophila* was non-monophyletic, with one accession sister to *S. sonchifolia* (Supplementary Data Fig. S1). The discordance may have been caused by the accession of *S. petrophila* from Guangxi 'capturing' the chloroplast genome of *S. sonchifolia*.

Rapid speciation events have occurred throughout the tree of life (Enard and Paabo, 2004). Incomplete lineage sorting, often occurring in taxa associated with rapid radiations, may significantly influence phylogenetic relationships (Enard and Paabo, 2004; Pollard *et al.*, 2006). In EA *Salvia*, the core subclades (G6, G7 and G8) diversified recently (Fig. 4), and interspecific relationships within the three subclades remain unresolved. Recent rapid radiation may have occurred in these groups, and incomplete lineage sorting may be a possible cause resulting from incongruence between the nuclear and plastid data sets. Additionally, the relative lack of informative sites within the cpDNA matrix (12 % vs. 45 % of nrDNA) may also contribute to the conflict. Phylogenomic approaches based on next-generation sequencing (NGS) data could address this issue.

Phylogeny of East Asian Salvia

Primarily on the basis of staminal morphology, EA *Salvia* were previously classified into three subgenera: subg. *Salvia*, *Sclarea* and *Allagospadonopsis* (Wu, 1977; Murata and Yamazaki, 1993). However, our phylogenetic analyses do not support these traditionally defined subgenera. In the nrDNA tree, taxa of subg. *Salvia* are spread across four lineages (G1, G4, G5 and G6), taxa of subg. *Sclarea* across six lineages (G1, G2, G3, G4, G7 and G8) and taxa of subg. *Allagospadonopsis* across two lineages (G3 and G8) (see Fig. 2).

Compared with the nrDNA tree, the cpDNA tree with limited resolution does not accurately reflect morphological relationships (Fig. 2; Supplementary Data Fig. S1). As nrDNA trees have been shown to reflect relationships based on morphological characters more accurately, the following discussion of phylogeny within EA *Salvia* mainly refers to the nuclear topology, and naming of lineages follows the nrDNA tree.

Subclade Sonchifoliae (G1)

Both nrDNA and cpDNA trees indicate that the recently described *Salvia petrophila* (Hu *et al.*, 2014) and *S. sonchifolia* form a clade that is sister to the rest of EA *Salvia*. It was unexpected that these taxa would be phylogenetically closely related, because the species are quite different in terms of flower morphology, but they are very similar in vegetative features (see below).

Salvia sonchifolia was described by Wu (1976) based on specimens collected from limestone mountain areas of

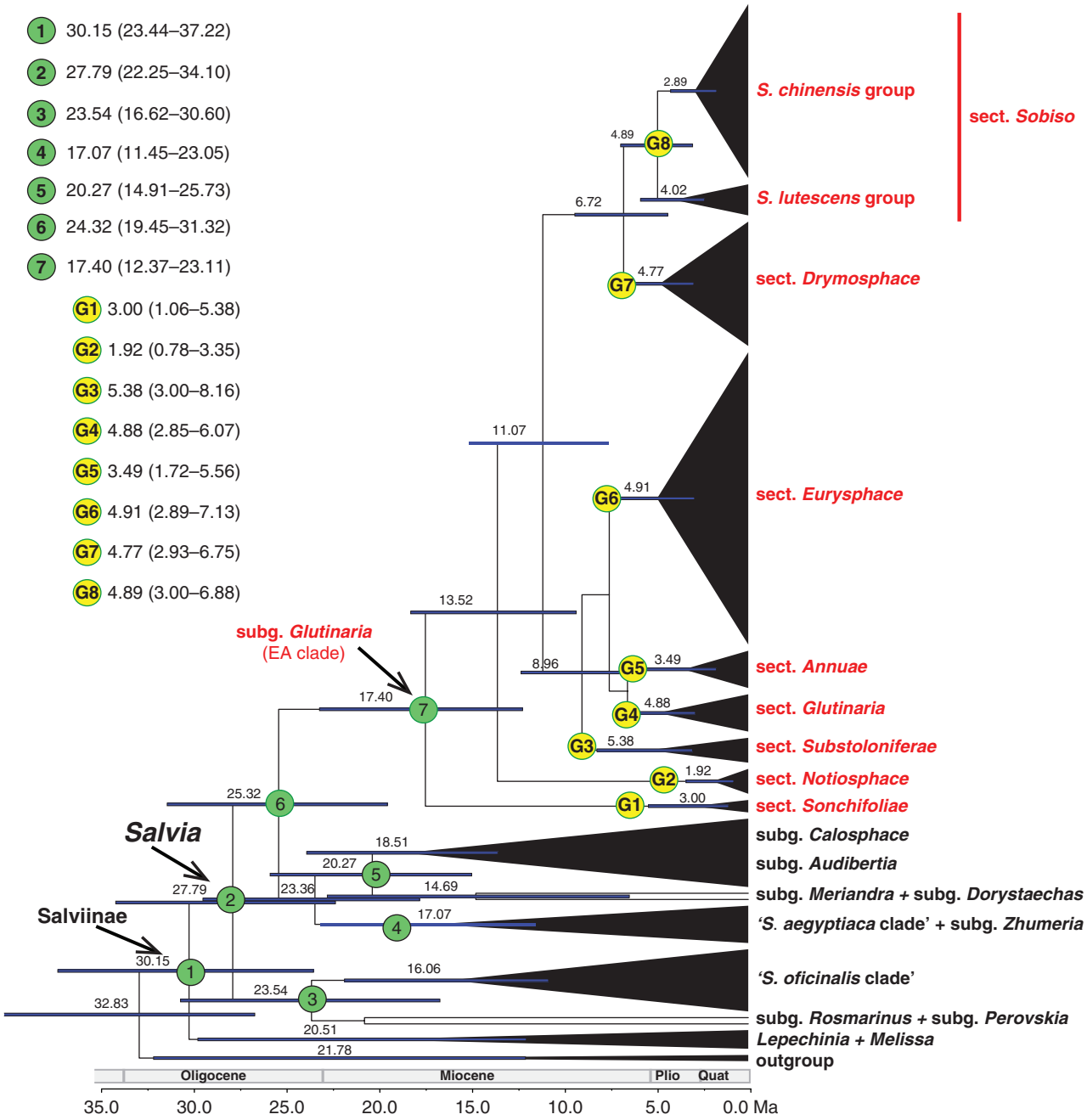

FIG. 4. Overview of the Salviinae chronogram based on the combined nrDNA (ITS and ETS) matrix. Detailed divergence times are given in Supplementary Data Fig. S2. 1, Crown of Salviinae; 2, crown of *Salvia*; 3, crown of *S. officinalis* clade and subg. *Rosmarinus* + subg. *Perovskia*. 4, crown of *S. aegyptiaca* clade and subg. *Zhumeria*; 5, crown of subg. *Calosphace* + subg. *Audibertia* and subg. *Meriandra* + subg. *Dorystaechas*; 6, stem of subg. *Glutinaria* (EA *Salvia* clade); 7, crown of subg. *Glutinaria* (EA *Salvia* clade). G1–G8, crown of sect. *Sonchifoliae*, sect. *Notiosphace*, sect. *Substoloniferae*, sect. *Glutinaria*, sect. *Annuae*, sect. *Eurysphace*, sect. *Drymosphace* and sect. *Sobiso* respectively.

south-eastern Yunnan. We recently found three new populations in western Guangxi, adjacent to south-eastern Yunnan (Hu, 2015). Wu (1977) placed this species into subg. *Salvia* based upon its curved connectives, sub-equal arms and fused posterior thecae. However, it can be easily distinguished from the rest of subg. *Salvia* due to its oblong rosettes of basal leaves, fully fertile posterior thecae and long corolla tubes, and was therefore placed into a monotypic series (ser. *Sonchifoliae*) by Wu (1977). *Salvia petrophila*, a newly described species (Hu *et al.*, 2014), occurs only on moist limestone cliffs in two adjacent national nature reserves (Maolan National Nature Reserve in southern Guizhou and Mulun National Nature Reserve in northern Guangxi) in south-west China.

TABLE 2. *Divergence time estimates, based on BEAST analyses of nrDNA, for major clades within EA* Salvia

Node	Age estimated in this study (Ma)	
	Mean	95 % HPD
Subg. *Glutinaria*	17.40	12.37–23.11
Sect. *Sonchifoliae*	3.00	1.06–5.38
Sect. *Notiosphace*	1.92	0.78–3.35
Sect. *Substoloniferae*	5.38	3.00–8.16
Sect. *Glutinaria*	4..88	2.85–6.07
Sect. *Annuae*	3.49	1.72–5.56
Sect. *Eurysphace*	4.91	2.89–7.13
Sect. *Drymosphace*	4.77	2.93–6.75
Sect. *Sobiso*	4.89	3.00–6.88
S. lutescens group	4.02	2.35–5.81
S. chinensis group	2.89	1.70–4.91

However, prior to flowering, it is difficult to distinguish *S. sonchifolia* from *S. petrophila*, as they have similar leaves and habitats. In fact, specimens of *S. petrophila* were first collected in 1984 from the Maolan National Nature Reserve (Chen 2635, HGAS!) and were misidentified as *S. sonchifolia* because the collections were in bud. In flower, however, *S. petrophila* can be easily distinguished from *S. sonchifolia* by its falcate (vs. sub-circular) and relatively long upper corolla lips (1.2–1.5 cm vs. 0.6–0.7 cm), longer connectives (15–18 mm vs. 1.4–1.7 mm), unequal arms (upper arms twice as long as the lower vs. sub-equal arms), aborted posterior thecae lacking pollen (vs. fully fertile posterior thecae) and exserted styles (vs. included styles). Based on clear differences in flower morphology, Hu *et al.* (2014) did not regard them as closely related species. Instead, Hu *et al.* (2014) placed *S. petrophila* in sect. *Drymosphace* of subg. *Sclarea sensu* Wu (1977), because its flower morphology is similar to that of *S. miltiorrhiza*. In the nrDNA analyses, *S. petrophila* is sister to *S. sonchifolia*. In the cpDNA analyses, however, *S. sonchifolia* is sister to one accession of *S. petrophila*, then these together are sister to another accession of *S. petrophila* (Supplementary Data Fig. S1). The discordance between nrDNA and cpDNA may be partially caused by the accession of *S. petrophila* from Guangxi 'capturing' the chloroplast genome of *S. sonchifolia*. Possible synapomorphies for subclade G1 include thickened fleshy roots, sub-succulent, oblong and basal leaves, and limestone habitats.

Subclade Notiosphace (G2)

This monotypic subclade was represented by five individuals of *Salvia plebeia* in our study. Thunberg (1784) first described *S. plebeia* (from Japan) as *Ocimum virgatum*. Subsequently, Brown (1810), presumably unaware of Thunberg's species delimitation, established *S. plebeia* based on collections from Australia but without type designation. Recently, Sales *et al.* (2010) made a typification of the name. The phylogenetic position of *S. plebeia* has long puzzled taxonomists. Bentham (1832–1836) was the first to place *S. plebeia*, along with *S. aegyptiaca*, in sect. *Notiosphace*, but he was unsure about the placement of the latter. In his subsequent study (Bentham, 1848), a number of species considered to be allied to *S. aegyptiaca* (including *S. japonica* and *S. chinensis* from East Asia) were added into sect. *Notiosphace*. However, Briquet (1897) excluded *S. japonica* and *S. chinensis* from sect. *Notiosphace* in his classification of *Salvia*. Based on morphological differences in habit, calyces, corollas and stamens, Stibal (1935) considered *S. plebeia* to be unrelated to *S. japonica* and its allies. Instead, he argued that his newly described species, *S. substolonifera*, was related to *S. plebeia* by virtue of sharing an annual habit, campanulate calyces and small corollas (0.4–0.6 cm long) (Stibal, 1934). Based on morphology, Sales *et al.* (2010) concluded that *S. plebeia* is a phenetically isolated species, with affinities for species from Asia. In *Flora Reipublicae Popularis Sinicae* (Wu, 1977), although *S. plebeia* is considered to be a member of subg. *Sclarea*, the species is placed in the monotypic sect. *Notiosphace* based on an annual or biennial habit, simple leaves and small corollas, and subequal connective arms (Fig. 5). Although previous molecular phylogenetic studies showed that *S. plebeia* was in the *S. glutinosa* clade (EA clade), consensus on its phylogenetic position has not been reached (Takano and Okada, 2011; Li *et al.*, 2013; Will and Claßen-Bockhoff 2017), leaving its phylogenetic position unclear. Here, our molecular phylogenetic analyses suggest that *S. plebeia* is an independent lineage in EA *Salvia*, worthy of a monotypic sectional delimitation.

Salvia plebeia has perhaps the widest native geographic distribution of any species within the genus (Sales *et al.*, 2010). It is distributed from Iran and Afghanistan in the west to Japan in the east, from far eastern Russia in the north to Australia in the south (Sales *et al.*, 2010). In Asia, *S. plebeia* usually grows in disturbed habitats. As a weed, it is the most widespread sage in China, where only Qinghai, Xizang and Xinjiang lack records (Wei *et al.*, 2015). However, *S. plebeia* is usually found in natural habitats in Australia. There is no consensus on its origin and dispersal. Froissart (2007) argued that *S. plebeia* originated in Asia, and its colonization in Australia was the result of a recent human introduction. However, because it occurs in non-weedy habitats in Australia, Sales *et al.* (2010) posited (but did not advocate) another scenario in which *S. plebeia* had an Australian origin and subsequently arrived in Asia via long-range dispersal. At any rate, the fact that *S. plebeia* is morphologically distinct, geographically widespread and the only (ostensibly) native species of *Salvia* in Australia is noteworthy. Our results clearly demonstrate that *S. plebeia* is a distinct lineage within EA *Salvia*, but further explanation as to its origin and current distribution pattern need to be evaluated by an in-depth phylogeographic study.

Subclade Substoloniferae (G3)

Only two endemic Chinese species, *S. substolonifera* and *S. trijuga*, were included in this lineage. Since they were described, no one has hypothesized that *S. substolonifera* and *S. trijuga* are closely related species. Geographically, they have markedly disjunct distributions and distinctly different habitats. The distribution of *S. substolonifera* ranges from eastern China to eastern Sichuan and north-eastern Yunnan, belonging to the Sino-Japanese distribution pattern (Wu, 1979, 1991), and it grows in riparian areas, moist rocky crevices and mesic forests. *Salvia trijuga*, however, is usually found in dry habitats

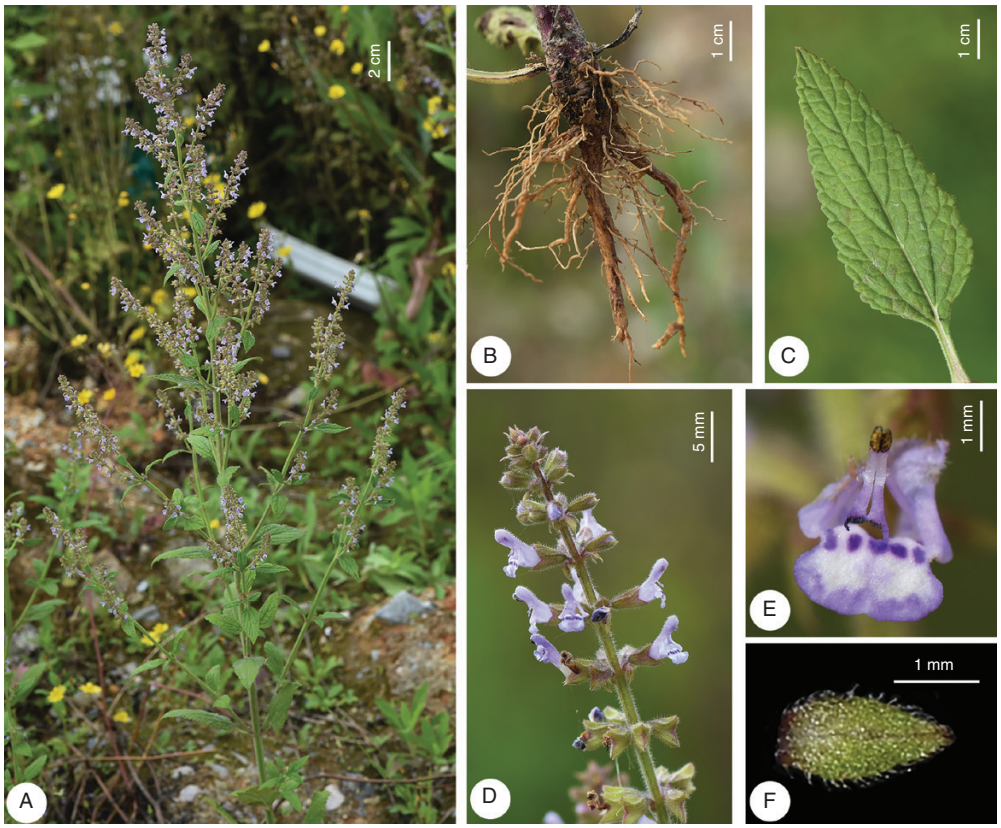

FIG. 5. Morphology of *S. plebeia*. (A) Plant; (B) root; (C) leaf, adaxially; (D) inflorescence; (E) corolla, showing stamen morphology; (F) bract. Photographs by G. X. Hu.

in north-western Yunnan, south-western Sichuan and south-eastern Xizang, and has a Sino-Himalayan distribution pattern (Wu, 1979, 1991).

Morphologically, *S. substolonifera* is an annual ascending or sub-prostrate herb, with small flowers (calyx 3–4 mm, corolla 5–7 mm), but *S. trijuga* is an erect perennial herb, with larger flowers (calyx 10–11 mm, corolla approx. 3 cm). Taxonomically, *S. substolonifera* and *S. trijuga* have been included in different subgenera. Stibal (1934) placed *S. substolonifera* and *S. plebeia* in sect. *Notiosphace* (*sensu* subg. *Eusalvia* Stib.), as they both possess an annual habit, a campanulate calyx and a small corolla (0.4–0.6 cm). Wu (1977), however, placed *S. substolonifera* into subg. *Allagospadonopsis* based on the separated sterile posterior thecae. On the basis of falcate upper corolla lips and fused sterile posterior thecae, both Stibal (1934) and Wu (1977) treated *S. trijuga* as a member of sect. *Drymosphace* of subg. *Sclarea*. Our phylogenetic analyses, however, showed that *S. substolonifera* and *S. trijuga* are sister species, forming a distinct clade (Fig. 2). While both *S. substolonifera* and *S. trijuga* were sampled in the study of Li *et al.* (2013), these two species did not group together. In the study of Li *et al.* (2013), *S. substolonifera* grouped with taxa of subg. *Allagospadonopsis*, and *S. trijuga* was sister to *S. pauciflora* E. Peter, an illegitimate name now replaced by *S. wuana* C.L. Xiang (Xiang *et al.*, 2016*b*). A recent phylogenetic study by Will and Claßen-Bockhoff (2017) showed that these two species form a lineage together with *S. evansiana*. In our study, two accessions of *S. substolonifera* and three accessions of *S. trijuga* were sampled from different localities, and all analyses strongly supported them as monophyletic. The different results in the studies by Li *et al.* (2013) and Will and Claßen-Bockhoff (2017) may have been a result of species misidentification. Indeed, some key morphological characters also support *S. trijuga* and *S. substolonifera* as closely related species. For example, these two species can be easily distinguished from the rest of EA *Salvia* by bearing unique truncate apices of the upper calyx lips [Fig. 1 (16)]. Additionally, except for size, they share similar corolla (± galeate upper corolla lips) and stamen morphology (arcuate connectives and sub-equal arms). In the field, we observed fused posterior thecae in *S. substolonifera*. As the fused posterior thecae in *Salvia* species can be easily (and unknowingly) split when dissecting flowers, the 'free stamens' of *S. substolonifera* described in previous studies may not represent its real status (Stibal, 1934; Wu, 1977; Li and Hedge, 1994). Possible synapomorphies for this clade may include ternate and simple leaves, campanulate calyces, truncate upper calyx lips and sub-equal arms.

Subclade Glutinaria (G4)

In both the nrDNA and cpDNA trees, *S. glutinosa*, *S. nubicola*, *S. koyamae*, *S. glabrescens*, *S. sakuensis* and *S. nipponica* formed an independent lineage with high support values. The floral morphology of these species is similar to that of taxa of the *S. miltiorrhiza* group (*sensu* subg. *Sclarea*), including tubular–campanulate calyces, strongly falcate upper corolla

lips, obviously exserted styles and unequal connective arms. However, foliar morphology (simple leaves) and flowering time (August to October) indicate that taxa of this subclade resemble taxa of subg. *Salvia*, which is characterized by simple leaves, arcuate connectives, sub-equal arms and fused posterior thecae, and bloom usually from August to October. Based on the falcate upper corolla lips and unequal connective arms, Wu (1977) placed *S. glutinosa* and *S. nubicola* (two species distributed from western China to Europe) into subg. *Sclarea*. On the basis of simple leaves and larger corollas (2–3 cm long) (vs. the corolla length of other taxa from Japan ranging from 0.4 to 1.2 cm), Murata and Yamazaki (1993) included *S. koyamae*, *S. glabrescens*, *S. sakuensis* and *S. nipponica* (four species endemic to Japan and Taiwan Island) in subg. *Salvia*. In addition to forming a clade in our phylogenetic analyses, these six species are also morphologically similar, indicating that the six species may belong neither to subg. *Salvia* nor to subg. *Sclarea sensu* Murata and Yamazaki (1993) and Wu (1977). Therefore, both morphological and molecular evidence suggests that these six species are a distinct lineage. Simple leaves, tubular–campanulate calyces, falcate upper corolla lips, unequal connective arms and fused deformed posterior thecae may be synapomorphies for this clade.

Although we did not obtain DNA sequences for this study, *Salvia chanryoenica*, a species endemic to the Korean Peninsula, should also be included in this lineage based on leaf and flower morphology (Lee, 2004). Species in this clade display a noteworthy distribution pattern in that *S. glutinosa* and *S. nubicola* are distributed from the Himalayan region to Europe, while the other five species are endemic to Japan, the Korean Peninsula or Taiwan Island. Speciation and dispersal patterns within this lineage require additional study.

Subclade Annuae (G5)

This subclade comprises three species (*Salvia roborowskii*, *S. umbratica* and *S. tricuspis*). The distribution of these three species ranges from northern China to south-western China, with *S. roborowskii* extending to Bhutan and Nepal. Morphologically, these species are very similar, with the main diagnostic characters separating the species being corolla colour and length. Zhu *et al.* (2011) described a new speceis (*S. chuanxiensis*) from western Sichuan, China, but it was synonymized with *S. tricuspis* by Xiang *et al.* (2016*a*). Wu (1977) placed the three species in subsect. *Annuae* based on annual or biennial habits, many branched stems and cauline hastate–sagittate leaves. Based on staminal morphology, taxa of this lineage appear to be related to subclade G6, which have arcuate connectives, sub-equal arms and fused posterior thecae with scant pollen, but their hastate–sagittate leaves resemble those of some taxa from subclade G4. Therefore, morphological data are consistent with phylogenetic evidence suggesting that these three species are an independent lineage.

One noteworthy finding is that the corolla tube length of these three species seems to be negatively correlated with elevation. *Salvia umbratica* has the longest corolla (2.3–2.8 cm) and lowest elevation (600–2000 m), while *S. roborowskii* has the shortest corolla (1–1.3 cm) and occurs at the highest elevation (2500–3700 m). Corolla length (2.1–2.3 cm) and elevation (1400–3000 m) of *S. tricuspis* are intermediate between the former two species. In the specimen studies and field surveys, we found morphological characters of some populations intermediate between *S. tricuspis* and *S. roborowskii*, and these two species overlap somewhat in distribution. Thus, we speculate that natural hybridization may occur between these two species.

Subclade Eurysphace (G6)

Taxa within this lineage mirror subsect. *Perennes* of subg. *Salvia sensu* Wu (1977). In Wu's (1977) classification, he divided subg. *Salvia* into two sections. Section *Eusphace* only included *S. officinalis* (introduced from Europe). Our present and previous molecular analyses (Walker *et al.*, 2004; Walker and Sytsma, 2007; Li *et al.*, 2013; Will and Claßen-Bockhoff, 2014, 2017; Drew *et al.*, 2017) indicate that *S. officinalis* and EA *Salvia* reside in two distinct clades. Section *Eurysphace* was divided into two subsections, subsect. *Annuae* and *Perennes*. Subsection *Annuae* comprises three annual or biennial species (*S. roborowskii*, *S. tricuspis* and *S. umbratica*), and both molecular and morphological evidence support subsect. *Annuae* as an independent lineage (see Subclade Annuae above). Subsection *Perennes* includes 42 species, of which 35 were sampled in the present study. Within this subsection, except for *S. sonchifolia* (associated with limestone in south-eastern Yunnan and western Guangxi) and *S. nubicola* (distributed from eastern Afghanistan to western Xizang, China), all other species are distributed in the Hengduan Mountains and adjacent areas, with a clear Sino-Himalayan distribution pattern (Wu, 1979, 1991). Our molecular phylogenetic analyses indicate that 33 of the 35 species sampled from subsect. *Perennes* group into subclade G6, with *S. sonchifolia* and *S. nubicola* embedded into G1 and G4, respectively. While we failed to obtain DNA sequences from *S. alatipetiolata*, *S. dolichantha*, *S. himmelbaurii*, *S. mekongensis*, *S. schizocalyx*, *S. schizochila*, and *S. luteistriata*, these seven species of subsect. *Perennes* should also be included in G6 based on their morphology and Sino-Himalayan distribution pattern. Within subclade G6, interspecific relationships remain unresolved, indicating a potential recent rapid radiation associated with the uplift of the Qinghai–Tibet Plateau (QTP). Possible synapomorphies for this lineage include: perennial herbs, simple leaves, oval–round bracts, campanulate calyces, relatively large corollas (length >1.5 cm), arcuate connectives with sub-equal arms, posterior thecae poorly developed but clearly reduced relative to the anterior thecae, and the posterior thecae fused with sparse pollen grains.

Subclade Drymosphace (G7)

All species of this lineage correspond to sect. *Drymosphace* of subg. *Sclarea sensu* Wu (1977). In Wu's (1977) treatment, he divided sect. *Drymosphace* into three series. Series *Miltiorrhizae* comprises eight species (*S. miltiorrhiza*, *S. bowleyana*, *S. sinica*, *S. trijuga*, *S. yunnanensis, S. nubicola*, *S. cavaleriei* and *S. prionitis*); series *Honaniae* is monotypic; and series *Plectranthoidites* includes three species (*S. plectranthoides*, *S. nanchuanensis* and *S. breviconnectivata*). Based on floral similarities, the subsequently described *S. meiliensis* (Su

et al., 1984) should be included in ser. *Honaniae*, and *S. dabieshanensis*, *S. vasta* and *S. paramiltiorrhiza* (Li and Hedge, 1994) should be placed in ser. *Miltiorrhizae*. In this study, except for *S. vasta* and *S. breviconnectivata*, all other species of sect. *Drymosphace sensu* Wu (1977) were sampled. However, the monophyly of sect. *Drymosphace* was not supported in this study. Phylogenetic and morphological evidence indicates that *S. trijuga* and *S. nubicola* are members of subclades G3 and G4, respectively (see Subclade Substoloniferae and Subclade Glutinaria above), and that the newly described *S. petrophila* arose via an early split in the EA *Salvia* clade (see Subclade Sonchifoliae above). Additionally, our phylogenetic analyses showed that *S. cavaleriei* and *S. prionitis* were embedded in subclade G8. Wu (1977) placed *S. cavaleriei* and *S. prionitis* in ser. *Miltiorrhizae* based on unequal connective arms and fused deformed sterile posterior thecae. However, except for deformed sterile posterior thecae, other morphological characters of these two species implicate them as members of subclade G8. For example, the fibril roots and small corollas (length usually <1 cm) observed in these two species are present in all taxa from subclade G8.

Although subclade G7 is well supported, interspecific relationships remain unresolved. Within the *Salvia miltiorrhiza* group, *S. honania* and *S. meiliensis* are two unique species, with clearly exserted stamens adhering laterally to the corolla wall and styles and two opposite anterior thecae [Figs 1 (28) and 6H, I). According to specimen studies and field observations, we found that there are no diagnostic characters between the two species, and the recently described *S. meiliensis* appears to be conspecific with *S. honania*. Pollen morphology also supports their similarity in that both have sub-oblate pollen, wide muri and large secondary lumina (C. L. Xiang, Kunming Institute of Botany, CAS, China, unpubl. res.). However, molecular phylogenetic results do not follow morphology, as accessions of these two species do not group together. Also, *S. miltiorrhiza* and its morphological allies did not group together, but *S. miltiorrhiza*, *S. bowleyana*, *S. sinica*, *S. dabieshanensis*, *S. paramiltiorrhiza* and *S. vasta* are morphologically similar species. The main diagnostic characters for them are corolla colour, leaf surface trichomes and annulate corolla tubes. However, field investigations found that the corolla colour of these species varies along a continuum (Fig. 6A–G), and leaf trichomes are also variable. Given our current knowledge, it may be more appropriate to regard *S. miltiorrhiza* and its allies as a species complex. At any rate, the identities of these species require further study.

Within subclade G7, *S. plectranthoides*, *S. nanchuanensis* and *S. yunnanensis* form a weakly supported clade (*S. plectranthoides* group), sister to the *S. miltiorrhiza* group. In terms of corolla morphology, *S. yunnanensis* is similar to *S. miltiorrhiza* and its allies, and can be readily distinguished from *S. plectranthoides* and *S. nanchuanensis* by funnelform corolla tubes (vs. tubular corolla tubes) and falcate upper corolla lips (vs. straight upper corolla lips). However, in the present study, *S. yunnanensis* grouped with one accession of *S. plectranthoides* (from Guizhou, south-western China), instead of being embedded within the *S. miltiorrhiza* group. *Salvia plectranthoides*, *S. nanchuanensis* and *S. yunnanensis* share similar staminal morphology (type C1), providing additional possible support for their relationship and distinguishing them from taxa in the *S. miltiorrhiza* group (types C3 and D; see Fig. 3).

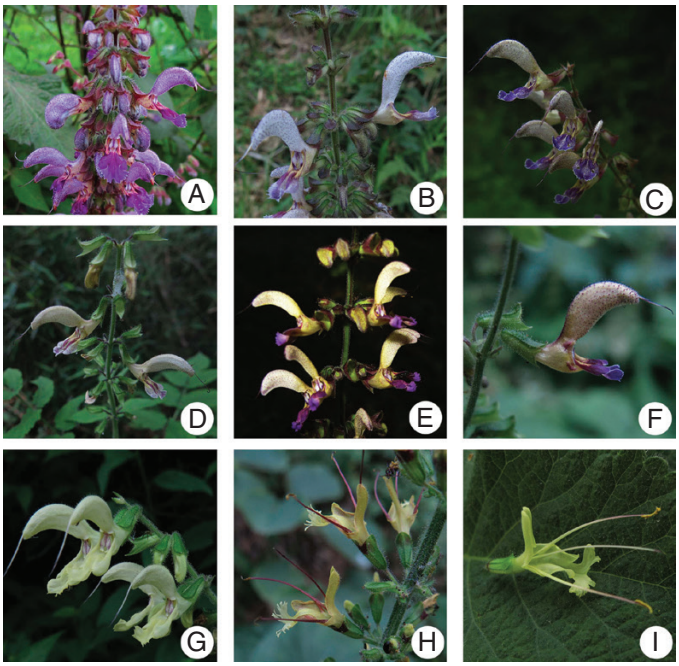

FIG. 6. Variation of corolla colour for *S. miltiorrhiza* group of sect. *Drymosphace sensu* Wu (1977). (A, B) *S. miltiorrhiza*; (C, D) *S. bowleyana*; (E, F) *S. sinica*; (G) *S. dabieshanensis*. (H) *S. honania*; (I) *S. meiliensis*. Photographs by G. X. Hu.

While the *S. plectranthoides* group needs to be confirmed by further molecular studies, this study found both morphological and phylogenetic evidence indicating that the three species and the *S. miltiorrhiza* group form a distinct lineage that is sister to subclade G8. Possible synapomorphies for subclade G7 include robust taproots, pinnate leaves, relatively long corollas (length >2 cm) and fused deformed posterior thecae.

Salvia breviconnectivata was described by Sun (1976) from Lufeng Village, Zhushan Town, Lunan (current name: Yiliang), Kunming, Yunnan, China and was subsequently placed in ser. *Plectranthoidites*, together with *S. plectranthoides* and *S. nanchuanensis* by Wu (1977). Unfortunately, we could not find either specimens or living plants of *S. breviconnectivata* in the field despite repeated efforts (only *S. plectranthoides* was found at the type locality). Based on the original descriptions, except for floral morphology, there are no diagnostic morphological differences separating *S. breviconnectivata* from *S. plectranthoides*. This may be why Wu (1977) placed *S. breviconnectivata* in ser. *Plectranthoidites*. However, within ser. *Plectranthoidites*, the species can be readily distinguished from the other two species by having shorter corolla tubes (0.8 cm vs. 1.4–2.5 cm), sub-equal connective arms (vs. upper arms clearly longer than lower arms) and separated posterior thecae (vs. fused posterior thecae). The small corolla and separated posterior thecae resemble taxa of subclade G8. However, *S. breviconnectivata* lacks fibril roots, one of the putative synapomorphies for subclade G8 (see Subclade Sobiso below). If the species was established based on a population concept, its morphological characters are so unusual in EA *Salvia* that it may have an enigmatic phylogenetic position. Instead, if based on a single specimen, it is probably an abnormal individual of *S. plectranthoides* rather than representing an independent species. Due to a lack

of morphological and molecular evidence, here we tentatively regard *S. breviconnectivata* as a doubtful species.

Subclade Sobiso (G8)

In total, 23 EA *Salvia* species are placed in subg. *Allagospadonopsis* (Wu, 1977; Su *et al.*, 1984; Murata and Yamazaki, 1993; Li and Hedge, 1994; Takano *et al.*, 2014; Hu and Peng, 2015). Except for *S. japonica*, broadly distributed in China, Japan and the Korean peninsula, all other species are endemic either to Japan (six species) or to China (16 species). *Salvia weihaiensis* was first described by Wu and Li (1977) based on collections from Weihai, Shandong, in eastern China and was considered to be a Chinese endemic species. Although Wu (1977) placed this species in subg. *Allagospadonopsis*, it can be readily distinguished from the remainder of EA *Salvia* as characterized by its robust taproots, broadly ovate bracts and three-aristate upper calyx lips. Finally, Hu and Peng (2015) concluded that *S. weihaiensis* is a synonym of *S. verbenaca* (a species widely distributed in Europe) and speculated that its occurrence in China may have resulted from an accidental nursery escape.

Based on the taxa sampled from Japan and Taiwan Island, Takano and Okada (2011) indicated that subg. *Allagospadonopsis* was monophyletic. However, the study of Li *et al.* (2013) showed that subg. *Allagospadonopsis* was non-monophyletic, with intercalation of *S. cavaleriei*, *S. prionitis* and *S. plectranthoides* from subg. *Sclarea*. Here we sampled all species of subg. *Allagospadonopsis* except for *S. fragarioides*, *S. adoxoides* and *S. piasezkii*, three narrowly distributed species endemic to China. Our phylogenetic results showed that *S. substolonifera*, belonging to subg. *Allagospadonopsis*, formed an independent lineage together with *S. trijuga*, and the rest of this subgenus formed a well-supported clade together with *S. cavaleriei* and *S. prionitis sensu* subg. *Sclarea* (Wu, 1977). Therefore, this study confirms that subg. *Allagospadonopsis sensu* Wu (1977) and Murata and Yamazaki (1993) is non-monophyletic. Additionally, no accessions of *S. plectranthoides* were embedded in subclade Sobiso in our nrDNA trees, and the unusual phylogenetic positions of *S. plectranthoides* in previous studies may have resulted from species misidentification (Li *et al.*, 2013; Will and Claßen-Bockhoff, 2017).

Within subclade Sobiso, two lineages were recognized. The *Salvia chinensis* group consisted of 16 species, including one Japanese endemic (*S. pygmaea*), 14 Chinese endemics and the widely distributed *S. japonica* (Japan, China and the Korean peninsula). While DNA sequences of *S. fragarioides*, *S. adoxoides* and *S. piasezkii* were unavailable, these three Chinese endemics should also most probably be placed into this group based on geographic distribution and flower morphology.

Within this group, we observed a distinct stamen movement phenomenon in all sampled taxa: the upper connective arms cling close to the upper corolla lips at early anthesis and then bend downward gradually until anterior fertile thecae reach the middle lobe of the lower corolla lips (Fig. 7A, B). The stamen movement was also mentioned by Huang *et al.* (2014), in which they argued that all taxa of subg. *Allagospadonopsis* and a few taxa of subg. *Sclarea* with small flowers share this similarity. However, we observed that *S. substolonifera* is the only species

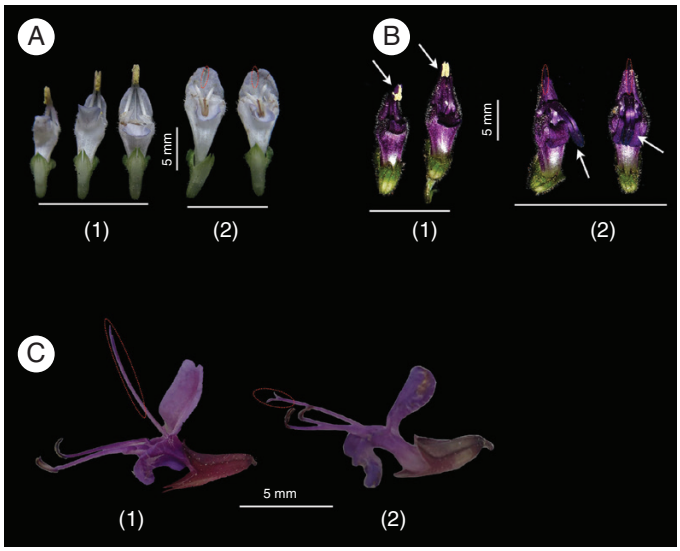

FIG. 7. Stamen and style movement in sect. Sobiso. (A, B) Stamen movement of *S. scapiformis* (A) and *S. cavaleriei* var. *simplicifolia* (B; the arrows point to anterior thecae); (C) style movement of *S. omerocalyx*. 1 = early anthesis, 2 = later anthesis, and the red ovals indicate styles. Photographs: (A) and (B) by G. X. Hu, (C) by A. Takano.

of subg. *Allagospadonopsis* without this type of stamen movement, and our phylogenetic results demonstrate that *S. substolonifera* is a member of subclade G3. Within subg. *Sclarea*, *S. cavaleriei*, *S. prionitis* and *S. plebeia* are small-flowered taxa (corolla length <1 cm). Among these three species, the first two species with the stamen movement phenomenon are embedded in the *S. chinensis* group (G8), while *S. plebeia*, without this phenomenon, represents the distinct subclade Notiosphace (G2).

The *S. lutescens* group is another lineage of subclade G8. With the exception of *S. hayatana*, endemic to Taiwan Island, the five other species are Japanese endemics. Within this group, most species (*S. isensis*, *S. lutescens*, *S. akiensis* and *S. omerocalyx*) display stylar (as opposed to staminal) movement during anthesis. When anthesis begins, the style clings to the upper corolla lip, and the stigma does not open. Later, the style moves downward slowly, and the stigma begins to open (becomes bilobed). However, the long exserted stamens retain their position throughout anthesis (Fig. 7C). In this group, neither stamens nor styles of *S. ranzaniana* change position during anthesis. Whether *S. hayatana* is dynamic in terms of staminal and/or stylar position during anthesis needs to be evaluated further. Stamen and style movement could be regarded as a diagnostic character to differentiate these two groups. In subclade G8, all taxa have fibril roots, lanceolate–linear bracts, small tubular calyces (4–7 mm), small corollas (length usually 5–10 mm, rarely up to 18 mm) and completely reduced posterior thecae (type F). These characters could be regarded as possible synapomorphies of subclade Sobiso. The crown of subclade G8 began to diversify approx. 4.89 Ma. Of the two lineages, the crown of the *S. lutescens* group diversified earlier than that of the *S. chinensis* group (4.02 vs. 2.89 Ma), and interspecific relationships within the *S. lutescens* group were relatively clear (see Fig. 2). In contrast, interspecific relationships of the *S. chinensis* group remain unresolved, and a recent rapid radiation may have occurred in this group.

Staminal evolution of EA Salvia

Himmelbaur and Stibal (1932–1934) first hypothesized an evolutionary trend in the staminal morphology in *Salvia*, proceeding from a curved connective bearing two fertile thecae to a situation where the lower connective became sterile and was modified in various ways. This evolutionary trend was observed in subg. *Calosphace*, subg. *Audibertia* and the *S. officinalis* clade, and stamens with fertile posterior thecae have been demonstrated to be plesiomorphic (Claßen-Bockhoff *et al.*, 2004*a*; Walker and Sytsma, 2007; Will and Claßen-Bockhoff, 2014; Walker *et al.*, 2015).

In this study, we recognize six stamen types for EA *Salvia* (types A–F; see Fig. 3). Stamen type A is only found in *Salvia sonchifolia* and is distinct from the other five types by bearing fully fertile posterior thecae. In both the nrDNA and cpDNA trees, *S. sonchifolia* was one of the two first diverging lineages of EA *Salvia*, and divergence time analyses showed that this lineage diverged approx. 17.4 Ma (Fig. 4; Supplementary Data Fig. S2). Therefore, phylogenetic and morphological evidence both indicate stamen type A as the ancestral type in EA *Salvia*, and that the other five types may have been derived from it. Stamen types A and B both have curved connectives and subequal upper and lower arms. The differences between them are that the posterior thecae of type B are obviously smaller, vertical to the lower arms, and produce little (B1) or no (B2) pollen. Therefore, we speculate that type B is derived from type A. All of the other four stamen types (C–F) have very unequal arms (the upper arms clearly longer than the lower arms). Posterior thecae within these types gradually degrade (C–E) until total abortion (F) and do not produce pollen. Stamen type F1 resembles type E in that both of them have non-parallel connectives, forming a large gap between the two connectives. Differences between the two types are that stamen type F1 loses posterior thecae completely, with separated lower arms. Both stamen types occur in the *S. chinensis* group, and stamen type F1 may have evolved from stamen type E. Stamen type F2, limited to the *S. lutescens* group, also loses posterior thecae completely, and therefore the stamen type may also have evolved from stamen type E. Within EA *Salvia*, fusion of the posterior thecae restricts access to nectar and forms the lever mechanism (type A–E), which occurs in all subclades (G1–G8) of EA *Salvia*. Complete reduction of the posterior thecae makes species lose the lever mechanism (type F), which only occurs in subclade G8. By studying the pollination mechanism of *S. liguliloba*, a Chinese endemic species of subclade G8 with stamen type F, Huang *et al.* (2015) indicated that the corolla tube of species with stamen type F becomes shorter and narrower, and pollinators are not required to enter the tube to access nectar; this was hypothesized to be an energy-saving and specialized pollination pattern. Given the above, staminal evolutionary trends within EA *Salvia* may present such a scenario: starting from curved connectives with equal (or sub-equal) arms with two fully fertile and fused posterior thecae (Fig. 3, type A), the posterior thecae become smaller and produce little or no pollen (Fig. 3, type B); subsequently, connectives elongate to make the upper arms obviously longer than the lower arms, and posterior thecae gradually degrade, producing no pollen (Fig. 3, types C, D and E); finally, posterior thecae are completely reduced and the lower arms are separated, resulting in a loss of the lever mechanism (Fig. 3, type F). The evolutionary trend is similar to the previous hypotheses (Himmelbaur and Stibal 1932–1934; Claßen-Bockhoff *et al.*, 2004*a*), supporting the independent origins of the lever mechanism in *Salvia*.

It is clear based on our analyses that there is parallel evolution of staminal morphology within EA *Salvia.* For instance, stamen type C occurs mainly in subclade G7, but it is also observed in all six species of subclade G4 and *S. petrophila* of subclade G1. Likewise, stamen type B is found in four distinct subclades (G2, G3, G4 and G6). EA *Salvia* have traditionally been placed into three subgenera on the basis of staminal morphology alone (Wu, 1977; Murata and Yamazaki, 1993), but the three subgenera are not supported by our molecular analyses. Parallel evolution of stamen types may be largely responsible for the previous inaccurate infrageneric classifications of EA *Salvia*. Parallel evolution of staminal morphology has also been noted among other clades of *Salvia* (Himmelbaur and Stibal, 1932–1934; Claßen-Bockhoff *et al.*, 2004; Walker and Sytsma, 2007; Will and Claßen-Bockhoff, 2014). Therefore, stamen structure should not be the only diagnostic character for delimiting infrageneric (or generic) categories.

Diversification of East Asian Salvia

We estimate that *Salvia* diverged from other Salviinae during the early Oligocene (30.15 Ma; Fig. 4, node 1) and then began to diversify during the middle Oligocene (27.79 Ma; Fig. 4, node 2). The EA *Salvia* diverged from other *Salvia* in the early Oligocene (25.32 Ma; Fig. 4, node 6) and extant EA *Salvia* began to diversify during the mid-Miocene (17.4 Ma; Fig. 4, node 7). The divergence time estimates of *Salvia* presented here are mostly consistent with previous studies (Drew and Sytsma, 2012; Walker *et al.*, 2015; Drew *et al.*, 2017). However, the divergence estimate of EA *Salvia* in Drew *et al.* (2017) is younger than that estimated here (approx. 12 Ma vs. 17.4 Ma). Most probably, the failure of Drew *et al.* (2017) to include the early diverging EA *Salvia* subclades G1 and G2 led to the difference in divergence times.

The East Asian flora is a major biodiversity hotspot, and is often divided into two sub-kingdoms: the Sino-Japanese forest sub-kingdom and the Sino-Himalayan forest sub-kingdom (Wu, 1979, 1991). The Sino-Japanese flora includes most paleoendemic taxa while the Sino-Himalayan bears more neoendemic taxa. Therefore, the former has traditionally been considered to be older than the latter (Wu and Wu, 1996; Li and Li, 1997). However, based on molecular and fossil data, Chen *et al.* (2018) argue that the two floras probably have a similar age. Our study supports the findings of Chen *et al.* (2018). Geographically, EA *Salvia* has typical Sino-Japanese and Sino-Himalayan distribution patterns, in which species of the G6 clade are mainly distributed in the Hengduan Mountains and adjacent regions (Sino-Himalayan), while species within the G8 lineage are mainly found in sub-tropical China (Central/South/East), the Korean Peninsula and the Japanese Archipelago (Sino-Japanese). Divergence time analyses estimated that subclades G6 and G8 both began to diversify since the early Pliocene (Fig. 4: nodes G6 and G8).

The uplift of the QTP and the initiation of the East Asia monsoon around the early Miocene greatly influenced the East

Asian flora (Yin and Harrison, 2000; Decelles *et al.*, 2007; Chen *et al.*, 2018). The initiation of EA *Salvia* diversification approx. 17.4 Ma may have been spurred by these geological and/or climatic events. Subclade G6 is a well-supported subclade in the nrDNA tree, but interspecific relationships are mostly unresolved. As all species of this subclade are distributed in the Hengduan Mountains and adjacent regions, a rapid radiation may have occurred in this lineage in association with Pliocene/Pleistocene QTP uplift and Pleistocene glaciation events. Indeed, several taxa in this region (e.g. *Isodon*, *Saussurea*, *Aconitum* and *Gentiana*) are hypothesized to have experienced rapid radiations in association with QTP uplift episodes since the late Miocene (Yu *et al.*, 2014; Favre *et al.*, 2016). As a consequence of QTP uplift events, environmental heterogeneity was increased in association with increasingly high mountains and deep valleys, which probably triggered bursts of speciation (Yu *et al*., 2014).

Stamen and style movement in EA Salvia

Stamen and style movement have been reported in many angiosperm plants, and their hypothesized adaptive significance includes avoidance of self-pollination, promotion of outcrossing, delayed autonomous self-pollination and reduction in intrafloral male–female interference (Ruan and Teixeira da Silva, 2011). A lever-like staminal mechanism, which features pollinator-induced movement of the stamens, was the main trait that traditionally defined *Salvia*. In this study, we observed active staminal and stylar movements that were not pollinator induced, with both movements being irreversible compared with such movement induced by pollinators.

Staminal movement has been considered to be a key factor affecting male reproductive success, and can directly determine the contact frequency and precision of anther/pollen with pollinators (Schlindwein and Wittmann, 1997; Taylor *et al.*, 2006). To date, four main types of stamen movement have been described: (1) stimulated movement is found in Cactaceae (Schlindwein and Wittmann, 1997), Berberidaceae (Lechowski and Białczyk, 1992) and Ericaceae (Nagy *et al.*, 1999); (2) simultaneous and slow movement is found in Calycanthaceae (Azuma *et al.*, 2005; Du *et al.*, 2012); (3) quick and explosive movement is found in *Morus alba* and *Cornus canadensis* (Taylor *et al.*, 2006; Whitaker *et al.*, 2007); and (4) cascade (successive) movement is found in *Nasa macrothyrsa* and *Ruta graveolens* (Weigend *et al.*, 2010; Ren and Tang, 2012). In EA *Salvia*, stamen movement corresponds to the simultaneous and slow type, and seems to be restricted to the *S. chinensis* group of subclade Sobiso (G8). At the beginning of anthesis, both the styles and the upper connective arms cling to the upper corolla lips, with the styles behind the upper connective arms and anterior thecae (Fig. 7A, B). The configuration prevents the style from receiving pollen from pollinators or self-pollinating. When the upper connective arms move downward to the middle lobes of lower corolla lips, the styles remain fixed in position or move downwards slightly. This separation ensures that the style of one flower will receive pollen from a different flower. Although we did not test stylar receptivity at different flowering stages, we hypothesized that taxa of G8 are protandrous based on positional changes at full anthesis. If protogynous, the styles will logically waste some opportunities to receive pollen because of blocking from the upper arms and anterior thecae at the onset of anthesis. This type of stamen movement is also observed in *Chimonanthus praecox* (Azuma *et al.*, 2005; Du *et al.*, 2012). In contrast, *Chimonanthus praecox* is protogynous, in which stamens with immature pollen first recurve outward (the stigmas are receptive), then the stamens gradually become upright and ultimately enclose the carpels. Except for promotion of outcrossing and avoidance of self-pollination, as suggested by previous studies (Lloyd and Yates, 1982; Barrett, 2002), stamen movement of *Salvia* can separate male and female functions well spatially (herkogamy) and temporally (dichogamy), which will reduce sexual interference between female and male function within a flower.

In contrast to stamen movement, we observed obvious style movement in most species of the *S. lutescens* group of subclade G8, in which the style moves downward slowly and the stigma lobes cluster together first then bifurcate. The obvious style movements were also observed in another two morphologically similar species (*S. honania* and *S. meiliensis*) of subclade G7. Based on the available data (personal field photos), we speculate that all EA *Salvia* except few for taxa of the *S. chinensis* group of subclade G8 may have an apparent or cryptic style movement phenomenon. Similar movement (although not described) has been observed in *S. hierosolymitana*, which is native to the eastern Mediterranean and belongs to the *S. officinalis* clade. *Salvia hierosolymitana* has been demonstrated to be protandrous, which allows it to reduce the risk of geitonogamy and promote outcrossing (Leshem *et al.*, 2011). Based on this observation, all EA *Salvia* may be protandrous and its adaptive significance needs further study.

Taxonomic treatment

Based on staminal morphology, EA *Salvia* have been placed in three subgenera: subg. *Salvia*, subg. *Sclarea* and subg. *Allagospadonopsis* (Wu, 1977; Murata and Yamazaki, 1993). However, our molecular phylogenetic results do not support these delimitations. Based on their phylogenetic results, Will and Claßen-Bockhoff (2017) suggested treating EA *Salvia* either as three distinct genera or as three sections of a new genus. In this study, we recognized eight distinct lineages that should be given equal taxonomic weight. Following the philosophy of Will and Claßen-Bockhoff (2017), this would mean that EA *Salvia* should be treated as either eight genera or eight sections of a single genus. Treating EA *Salvia* as eight separate genera would be confusing to say the least; furthermore, it seems untenable to treat EA *Salvia* as a single genus because we were unable to find any single morphological character that distinguishes EA *Salvia* from *Salvia* in the other centres of diversity, particularly in south-western Asia and the Mediterranean region. Therefore, following the suggestion of Drew *et al.* (2017), and based on our molecular analysis and morphological investigation, we formally treat the EA *Salvia* clade as a subgenus, including eight sections, below. Morphologically, all species of EA *Salvia* are herbaceous and have the same basic chromosome number, $x = 8$ (Gill, 1971; Yang *et al.*, 2004; Zhao *et al.*, 2006; Wang *et al.*, 2009; Hu *et al.*, 2016). These two characters distinguish this group and could be regarded as diagnostic characters of the EA *Salvia* clade.

Salvia subg. ***Glutinaria*** (Raf.) G.X. Hu, C.L. Xiang & B.T. Drew, **comb. & stat. nov.**
Basionym: ***Glutinaria*** Raf. in Fl. Tellur. 3: 93. 1836.
Type: *Salvia glutinosa* L. in Sp. Pl. 1: 26. 1753.

Herbs perennial, rarely biennial or annual. Leaves simple or pinnately compound. Verticillasters two- to many flowered. Calyx tubular to campanulate, two-lipped; upper lip entire or three-mucronate, rarely truncate or three-dentate; lower lip two-toothed. Corolla two-lipped; tube straight or curved, annulate or not; upper lip straight or falcate; lower lip three-lobed, the middle lobe largest. Stamens two; connectives elongated, usually articulating with the filament; anterior thecae fertile, connivent or separated; posterior thecae developed, reduced or completely lost, sterile, rarely fully fertile (*S. sonchifolia*), fused or separated. Style two-lobed. Nutlets triquetrous, ovoid or oblong, glabrous.

1. Sect. ***Sonchifoliae*** (C.Y. Wu) G.X. Hu, C.L. Xiang & H. Peng, **stat. nov.** ≡ Ser. *Sonchifoliae* C.Y. Wu in Fl. Reipubl. Popularis Sin. 66: 581. 1977 – **Type**: *Salvia sonchifolia* C.Y. Wu. in Fl. Yunnan. 1: 679. 1977.
2. Sect. ***Notiosphace*** Benth. in Labiat. Gen. Spec. 309. 1833, p.p. – **Type**: *Salvia plebeia* R. Br. in Prodr. Fl. Nov. Holland. 501. 1810.
3. Sect. ***Substoloniferae*** (C.Y. Wu) C.L. Xiang & B.T. Drew **stat. nov.** ≡ Ser. *Substoloniferae* C.Y. Wu in Fl. Reipubl. Popularis Sin. 66: 583. 1977 – **Type**: *Salvia substolonifera* E. Peter. in Acta Horti Gothob. 9: 138. 1934.
4. Sect. ***Glutinaria*** – **Type**: *Salvia glutinosa* L. in Sp. Pl. 1: 26. 1753.
5. Sect. ***Annuae*** (C.Y. Wu) C.L. Xiang & H. Peng **stat. nov.** ≡ Subsect. *Annuae* C.Y. Wu in Fl. Reipubl. Popularis Sin. 66: 581. 1977 – **Type**: *Salvia roborowskii* Maxim. in Bull. Acad. Imp. Sci. Saint-Pétersbourg xxvii: 527. 1881.
6. Sect. ***Eurysphace*** Stib. in Act. Hort. Gothob. 9: 105, 112. 1934, p.p. – **Type**: *Salvia przewalskii* Maxim. in Bull. Acad. Imp. Sci. Saint-Pétersbourg xxvii: 527. 1881.
7. Sect. ***Drymosphace*** Benth. in Labiat. Gen. Spec. 195, 218. 1833, p.p. – **Type**: *Salvia miltiorrhiza* Bunge in Enum. Pl. China Bor. 50. 1833.
8. Sect. ***Sobiso*** (Raf.) G.X. Hu, A. Takano & B.T. Drew, **comb. & stat. nov.** ≡ *Sobiso* Raf. in Fl. Tellur. 3: 94. 1837. – **Type**: *Salvia japonica* Thunb. in Syst. Veg., ed. 14. 72. 1784.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Figure S1: cladogram based on BEAST analysis of the combined cpDNA (*psbA–trnH*, *ycf1–rps15*, *trnL–trnF* and *rbcL*) matrix. Figure S2: divergence time estimation of Salviinae based on the nrDNA matrix (ITS and ETS). Appendix: voucher information and GenBank accession numbers for taxa used in this study.

ACKNOWLEDGEMENTS

We thank Mr Fei Zhao, Mr Wei Fang, Mr Fazhi Shangguan, Miss Li Chen, Dr Hongjin Dong, Dr Yaping Chen, Dr Ying Tang and Dr Zhikun Wu for help with field work; Dr Zehuan Wang, Dr Wenzhang Ma, Dr Zhuo Zhou, Dr Zhiwei Wang, Dr Xinxin Zhu, Dr Jie Cai, Dr Ting Zhang, Dr Yousheng Chen, Dr Zhixi Fu, Dr Yi Tong, Dr Yan Liu, Dr Weibin Xu, Dr Longfei Fu, Dr Renbin Zhu, Dr Xiaojun Shi, Mr Sheng Huang, Mr Hui Tang and Mr Sirong Yi for collecting plant materials; Mr Xiaoyu Wang for his line drawing of stamen types; the curators of KUN and PE herbaria for access to herbarium materials; and Dr Petra Wester and three anonymous reviewers for helpful comments to improve the manuscript. This work was supported by the Natural Science Foundation of Yunnan Province (2015FB169); the National Natural Science Foundation of China (31370229, 31600164); the Japan Society for the Promotion of Science [26440227]; the united fund of the Natural Science Foundation of Guizhou Province and Guizhou University (LH [2017]7278); and Construction Program of Biology First-class Discipline in Guizhou (GNYL [2017] 009).

LITERATURE CITED

Albaladejo RG, **Aguilar JF**, **Aparicio A**, **Feliner GN. 2005**. Contrasting nuclear–plastidial phylogenetic patterns in the recently diverged Iberian *Phlomis crinita* and *P. lychnitis* lineages (Lamiaceae). *Taxon* **54**: 987–998.
Ali NM, **Yeap SK**, **Ho WY**, **Beh BK**, **Tan SW**, **Tan SG. 2012**. The promising future of chia, *Salvia hispanica* L. *Journal of Biomedicine and Biotechnology* **2012**: 171956. doi: 10.1155/2012/171956.
Alziar G. 1988–1993. Catalogue synonymique des *Salvia* L. du monde (Lamiaceae). I–VI. *Biocosme Mesogéen* **5**: 87–136, 6: 79–115, 163–204, 7: 59–109, 9: 413–497, 10: 33–117.
Azuma H, **Toyota M**, **Asakawa Y. 2005**. Floral scent chemistry and stamen movement of *Chimonanthus praecox* (L.) Link (Calycanthaceae). *Acta Phytotaxonomica et Geobotanica* **56**: 197–201.
Baldwin BG, **Markos S. 1998**. Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* **10**: 449–463.
Barrett SCH. 2002. Sexual interference of the floral kinds. *Heredity* **88**: 154–159.
Beardsley PM, **Olmstead RG. 2002**. Redefining Phrymaceae: the placement of *Mimulus*, tribe Mimuleae, and *Phryma*. *American Journal of Botany* **89**: 1093–1102.
Bentham G. 1832–1836. *Labiatarum Genera et Species*. London: Ridgeway.
Bentham G. 1848. Labiatae. In: De Candolle A, ed. *Prodromus systematis naturalis regni vegetabilis*, Vol. **12**. Treuttel et Wurtz: Paris, 27–603.
Bentham G. 1876. Labiatae. In: Bentham G, Hooker JD, eds. *Genera Plantarum*, Vol. **2**. London, UK: Reeve and Co., 1160–1196.
Blattner F. 1999. Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. *Biotechniques* **27**: 1180–1186.
Briquet J. 1897. *Salvia*. In: Engler A, Prantl K, eds. *Die naturlichen Pflanzenfamilien nebst ihrer Gattungen und wichtigeren Arten*, Vol. **IV**. Leipzig: Wilhelm Engelmann, 183–287.
Brown R. 1810. *Prodromus Florae Novae Hollandiae, et Insulae van Diemen*. London.
Chen YS, **Deng T**, **Zhou Z**, **Sun H. 2018**. Is the East Asian flora ancient or not? *National Science Review* (in press).
Claßen-Bockhoff R, **Wester P**, **Tweraser E. 2003**. The staminal lever mechanism in *Salvia* L. (Lamiaceae) – a review. *Plant Biology* **5**: 33–41.
Claßen-Bockhoff R, **Crone M**, **Baikova E. 2004*a***. Stamen development in *Salvia*: homology reinvestigated. *International Journal of Plant Science* **165**: 475–498.
Claßen-Bockhoff R, **Speck T**, **Tweraser E**, **Wester P**, **Thimm S**, **Reith M. 2004*b***. The staminal lever mechanism in *Salvia* L. (Lamiaceae): a key innovation for adaptive radiation? *Organisms, Diversity & Evolution* **4**: 189–205.
Clebsch B. 2008. *The new book of salvias: sages for every garden*. Portland, ME: Timber Press.
Cunningham CW. 1997. Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* **14**: 733–740.

Darriba D, **Taboada GL**, **Doallo R**, **Posada D. 2012**. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.
Decelles PG, **Quade J**, **Kapp P**, **Fan M**, **Dettman DL**, **Ding L. 2007**. High and dry in central Tibet during the Late Oligocene. *Earth & Planetary Science Letters* **253**: 389–401.
Deng T, **Nie ZL**, **Drew BT**, ***et al.*** **2015**. Does the arcto-tertiary biogeographic hypothesis explain the disjunct distribution of northern hemisphere herbaceous plants? The case of *Meehania* (Lamiaceae). *PLoS One* **10**: e0117171. doi: 10.1371/journal.pone.0117171.
Doyle JJ, **Doyle JL. 1987**. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11–15.
Drew BT, **Sytsma KJ. 2011**. Testing the monophyly and placement of *Lepechinia* in the tribe Mentheae (Lamiaceae). *Systematic Botany* **36**: 1038–1049.
Drew BT, **Sytsma KJ. 2012**. Phylogenetics, biogeography, and staminal evolution in the tribe Mentheae (Lamiaceae). *American Journal of Botany* **99**: 933–953.
Drew BT, **Sytsma KJ. 2013**. The South American radiation of *Lepechinia* (Lamiaceae): phylogenetics, divergence times and evolution of dioecy. *Botanical Journal of the Linnean Society* **171**: 171–190.
Drew BT, **Cacho NI**, **Sytsma KJ. 2014**. The transfer of two rare monotypic genera, *Neoeplingia* and *Chaunostoma*, to *Lepechinia* (Lamiaceae), and notes on their conservation. *Taxon* **63**: 831–842.
Drew BT, **González-Gallegos JG**, **Xiang CL**, ***et al.*** **2017**. *Salvia* united: the greatest good for the greatest number. *Taxon* **66**: 133–145.
Drummond AJ, **Suchard MA**, **Xie D**, **Rambaut A. 2012**. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**: 1969–1973.
Du W, **Qin KZ**, **Wang XF. 2012**. The mechanism of stamen movement in *Chimonanthus praecox* (Calycanthaceae): differential cell growth rates on the adaxial and abaxial surfaces of filaments after flower opening. *Plant Systematics and Evolution* **298**: 561–567.
Edgar R. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.
Enard W, **Paabo S. 2004**. Comparative primate genomics. *Annual Review of Genomics and Human Genetics* **5**: 351–378.
Epling C. 1938. The California salvias. A review of *Salvia*, section *Audibertia*. *Annals of the Missouri Botanical Garden* **25**: 95–188.
Epling C. 1939. A revision of *Salvia*, subgenus *Calosphace*. *Beihefte Feddes Repertorium Specierum Novarum Regni Vegetabilis* **110**: 1–383.
Farris JS, **Källersjö M**, **Kluge AG**, **Bult C. 1994**. Testing significance of incongruence. *Cladistics* **10**: 315–319.
Favre A, **Michalak I**, **Chen C**, **Wang J**, **Pringle JS**, **Matuszak S**, ***et al.*** **2016**. Out-of-Tibet: the spatio-temporal evolution of *Gentiana* (Gentianaceae). *Journal of Biogeography* **43**: 1967–1978.
Fragoso-Martínez I, **Martínez-Gordillo M**, **Salazar GA**, ***et al.*** **2018**. Phylogeny of the Neotropical sages (*Salvia* subg. *Calosphace*; Lamiaceae) and insights into pollinator and area shifts. *Plant Systematics and Evolution* **304**: 43–55.
Froissart C. 2007. *Salvia plebeia*, les grandes voyages d'une petite plante. *Mémoires Académie d'Orleans* **6**: 55–65.
Funamoto T, **Zushi M**, **Harana T**, **Nakamura T. 2000**. Comparative karyomorphology of the Japanese species of *Salvia* L. (Lamiaceae). *Journal of Phytogeography & Taxonomy* **48**: 11–18.
Gill LS. 1971. Chromosome studies in *Salvia* (Labiatae): West Himalayan species. *Experientia* **27**: 596–598.
González-Gallegos JG. 2015. Two new *Salvia* species (Lamiaceae) from the Sierra Madre Occidental, Durango, Mexico. *Systematic Botany* **40**: 1093–1101.
Hedge IC. 1974. A revision of *Salvia* in Africa and the Canary Islands. *Notes from the Royal Botanic Garden, Edinburgh* **33**: 1–121.
Hedge IC. 1982*a*. *Salvia*. In: Davis PH, ed. *Flora of Turkey and the East Aegean Islands*, Vol. **7**. Edinburgh: Edinburgh University Press, 400–461.
Hedge IC. 1982*b*. *Salvia*. In: Rechinger KH, ed. *Flora Iranica*, Vol. **150**. Graz: Akademische Druck und Verlags-Anstalt, 403–476.
Himmelbaur W, **Stibal E. 1932–1934**. Entwicklungsrichtungen in der Blutenregion der Gattung *Salvia* L. I–III. *Biologia Generalis* **8**: 449–474; **9**: 129–150; **10**: 17–48.
Hipp AL, **Hall JC**, **Sytsma KJ. 2004**. Congruence versus phylogenetic accuracy: revisiting the incongruence length difference test. *Systematic Biology* **53**: 81–89.
Hu GX. 2015. *Phylogeny of Salvia (Lamiaceae) in East Asia*. PhD Thesis, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.
Hu GX, **Peng H. 2015**. Identity of *Salvia weihaiensis* (Lamiaceae) from China. *Phytotaxa* **202**: 298–300.
Hu GX, **Xiang CL**, **Liu ED. 2013**. Invasion status and risk assessment for *Salvia tiliifolia*, a recently recognised introduction to China. *Weed Research* **53**: 355–361.
Hu GX, **Liu Y**, **Xu WB**, **Liu ED. 2014**. *Salvia petrophila* sp. nov. (Lamiaceae) from north Guangxi and south Guizhou, China. *Nordic Journal of Botany* **32**: 190–195.
Hu GX, **Xiang CL**, **Liu ED**, **Dong HJ**, **Funamoto T. 2016**. Karyotypic study of eighteen taxa of *Salvia* (Lamiaceae) from China. *Caryologia* **69**: 50–57.
Hu GX, **Liu ED**, **Zhang T**, **Cai J**, **Xiang CL. 2017**. *Salvia luteistriata* (Lamiaceae), a new species from northeastern Sichuan, China. *Phytotaxa* **314**: 123–128.
Huang YB, **Wei YK**, **Ge BJ**, **Wang Q. 2014**. Pollination mechanisms of genus *Salvia* (Lamiaceae) in East Asia (China). *Acta Ecologica Sinica* **34**: 2282–2289.
Huang YB, **Wei YK**, **Wang Q**, **Xiao YE**, **Yue XY. 2015**. Floral morphology and pollination mechanism of *Salvia liguliloba,* a narrow endemic species with degraded lever-like stamens. *Chinese Journal of Plant Ecology* **39**: 753–761.
Jenks AA, **Walker JB**, **Kim SC. 2012**. Phylogeny of New World *Salvia* subgenus *Calosphace* (Lamiaceae) based on cpDNA (*psbA–trnH*) and nrDNA (ITS) sequence data. *Journal of Plant Research* **126**: 483–496.
Lechowski Z, **Białczyk J. 1992**. Effect of external calcium on the control of stamen movement in *Berberis ulgaris* L. *Biologia Plantarum* **34**: 121–130.
Lee YN. 2004. *Flora of Korea*. Seoul: Kyohak Publishing Co., Ltd.
Leshem L, **Keasar T**, **Shmida A. 2011**. Female-biased nectar production in the protandrous, hermaphroditic shrub *Salvia hierosolymitana* (Lamiaceae). *Australian Journal of Botany* **59**: 18–25.
Li HW, **Hedge IC. 1994**. *Salvia*. In: Wu CY, Raven PH, Hong DY, eds. *Flora of China*, Vol. **13**. Beijing and St. Louis: Science Press and Missouri Botanical Garden Press, 196–224.
Li HW, **Li J. 1997**. The Tanaka–Kaiyong line: an important floristic line for the study of the flora of East Asia. *Annals of the Missouri Botanical Garden* **84**: 888–892.
Li QQ, **Li MH**, **Yuan QJ**, **Cui ZH**, **Huang LQ**, **Xiao PG. 2013**. Phylogenetic relationships of *Salvia* (Lamiaceae) in China: evidence from DNA sequence datasets. *Journal of Systematics and Evolution* **51**: 184–195.
Lloyd DG, **Yates JMA. 1982**. Intrasexual selection and the segregation of pollen and stigma in hermaphrodite plants, exemplified by *Wahlenbergia albomarginata* (Campanulaceae). *Evolution* **36**: 903–913.
Miller MA, **Pfeiffer W**, **Schwartz T. 2010**. *Creating the CIPRES Science Gateway for inference of large phylogenetic trees*. Gateway Computing Environments Workshop (GCE), 2010, IEEE.
Müller J, **Müller K**, **Quandt D**, **Neinhuis C. 2010**. *PhyDE – phylogenetic data editor, version 0.995*. Available from: www.phyde.de.
Murata G, **Yamazaki T. 1993**. *Salvia*. In: Iwatsuki K, Yamazaki T, Boufford D, Ohba H, eds. *Flora of Japan*, Vol **IIIa**. Tokyo: Kodansha, 302–307.
Nagy ES, **Strong L**, **Galloway LF. 1999**. Contribution of delayed autogamous selfing to reproductive success in Mountain Laurel, *Kalmia latifolia* (Ericaceae). *American Midland Naturalist* **142**: 39–46.
Pobedimova EG. 1954. *Salvia*. In: Shishkin BK, ed. *Flora of the U.S.S.R.*, Vol. **21**. Moscow: Izdatel'stvo Akademii Nauk SSR, 154–255.
Pollard DA, **Iyer VN**, **Moses AM**, **Eisen MB. 2006**. Widespread discordance of gene trees with species tree in *Drosophila*: evidence for incomplete lineage sorting. *PLoS Genetics* **2**: e173.
Rambaut A. 2014. *FigTree, v.1.4.2*. http://tree.bio.ed.ac.uk/software/figtree/.
Rambaut A, **Suchard MA**, **Drummond AJ. 2014**. *Tracer v1.6*. http://beast.bio.ed.ac.uk/Tracer.
Ren MX, **Tang JY. 2012**. Up and down: stamen movements in *Ruta graveolens* (Rutaceae) enhance both outcrossing and delayed selfing. *Annals of Botany* **110**: 1017–1025.
Ruan CJ, **Teixeira da Silva JA. 2011**. Adaptive significance of floral movement. *Critical Reviews in Plant Sciences* **30**: 293–328.
Sales F, **Hedge I**, **Christie F. 2010**. *Salvia plebeia* R. Br.: taxonomy, phytogeography, autogamy and myxospermy. *Pakistan Journal of Botany* **42**: 99–110.
Sang T, **Crawford D**, **Stuessy T. 1997**. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* **84**: 1120–1136.

Schlindwein C, **Wittmann D. 1997**. Stamen movements in flowers of Opuntia (Cactaceae) favour oligolectic pollinators. *Plant Systematics and Evolution* **204**: 179–193.

Soltis PS, **Soltis DE**, **Smiley CJ. 1992**. An *rbcL* sequence from a Miocene *Taxodium* (bald cypress). *Proceedings of the National Academy of Sciences, USA* **89**: 449–451.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.

Stibal E. 1934. A revision of Chinese and East Burmese species of *Salvia*. *Acta Horti Gotoburgensis* **9**: 101–165.

Stibal E. 1935. Revision der Gruppe der *Salvia japonica*. *Acta Horti Gotoburgensis* **10**: 55–69.

Stöver BC, **Müller KF. 2010**. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* **11**: 7. doi: 10.1186/1471-2105-11-7.

Su SW, **Shen ZA**, **He JQ. 1984**. New species of the genus *Salvia* from Anhui. *Acta Botanica Yunnanica* **6**: 55–62.

Sun XC. 1976. *Salvia breviconnectivata*. In: Wu CY, ed. *Flora Yunnanica*, Vol. **1**. Beijing: Science Press, 686–687.

Swofford DL. 2003. *PAUP*. Phylogenetic analysis using parsimony (* and other methods), version 4.0b10*. Sunderland, MA: Sinauer Associates.

Taberlet P, **Gielly L**, **Pautou G**, **Bouvet J. 1991**. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105–1109.

Takano A, **Okada H. 2011**. Phylogenetic relationships among subgenera, species, and varieties of Japanese *Salvia* L. (Lamiaceae). *Journal of Plant Research* **124**: 245–252.

Takano A, **Sera T**, **Kurosaki N. 2014**. A new species of *Salvia* (Lamiaceae) from Chugoku District, Japan, *Salvia akiensis* sp. nov. *Acta Phytotaxonomica et Geobotanica* **65**: 99–104.

Tamura K, **Stecher G**, **Peterson D**, **Filipski A**, **Kumar S. 2013**. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725–2729.

Taylor PE, **Card G**, **House J**, **Dickinson MH**, **Flagan RC. 2006**. High-speed pollen release in the white mulberry tree, *Morus alba* L. *Sexual Plant Reproduction* **19**: 19–24.

Thunberg CP. 1784. *Salvia japonica*. In: Murray JA, ed. *Systema Vegetabilium,* 14th edn. Göttingen: Dietrich, 72.

Walker JB, **Sytsma KJ. 2007**. Staminal evolution in the genus *Salvia* (Lamiaceae): molecular phylogenetic evidence for multiple origins of the staminal lever. *Annals of Botany* **100**: 375–391.

Walker JB, **Sytsma KJ**, **Treutlein J**, **Wink M. 2004**. *Salvia* (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of *Salvia* and tribe Mentheae. *American Journal of Botany* **91**: 1115–1125.

Walker JB, **Drew BT**, **Sytsma KJ. 2015**. Unravelling species relationships and diversification within the iconic California Floristic Province sages (*Salvia* subgenus *Audibertia*, Lamiaceae). *Systematic Botany* **40**: 826–844.

Wang BQ. 2010. *Salvia miltiorrhiza*: chemical and pharmacological review of a medicinal plant. *Journal of Medicinal Plant Research* **4**: 2813–2820.

Wang M, **Zhang L**, **Ding CB**, ***et al.* 2009**. Meiotic observations of eight taxa in the genus *Salvia*. *Caryologia* **62**: 334–340.

Wei YK, **Wang Q**, **Huang YB. 2015**. Species diversity and distribution of *Salvia* (Lamiaceae). *Biodiversity Science* **23**: 3–10.

Weigend M, **Ackermann M**, **Henning T. 2010**. Reloading the revolver-male fitness as a simple explanation for complex reward partitioning in *Nasa macrothyrsa* (Loasaceae, Cornales). *Biological Journal of the Linnean Society* **100**: 124–131.

Whitaker DL, **Webster LA**, **Edwards J. 2007**. The biomechanics of *Cornus canadensis* stamens are ideal for catapulting pollen vertically. *Functional Ecology* **21**: 219–225.

White TJ, **Bruns T**, **Lee S**, **Taylor J. 1990**. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T, eds. *PCR protocols: a guide to methods and applications*. San Diego: Academic Press, 315–322.

Will M, **Claßen-Bockhoff R. 2014**. Why Africa matters: evolution of Old World *Salvia* (Lamiaceae) in Africa. *Annals of Botany* **114**: 61–83.

Will M, **Claßen-Bockhoff R. 2017**. Time to split *Salvia* s.l. (Lamiaceae) – new insights from Old World *Salvia* phylogeny. *Molecular Phylogenetics and Evolution* **114**: 33–58.

Will M, **Schmalz N**, **Claßen-Bockhoff R. 2015**. Towards a new classification of *Salvia* s.l.: (re) establishing the genus *Pleudia* Raf. *Turkish Journal of Botany* **39**: 693–707.

Wu CY. 1976. *Salvia sonchifolia*. In: Wu CY, ed. *Flora Yunnanica*, Vol. **1**. Beijing: Science Press, 679–681.

Wu CY. 1977. *Salvia*. In: Wu CY, ed. *Flora Reipublicae Popularis Sinicae*, Vol. **66**. Beijing: Science Press, 70–196.

Wu CY. 1979. The regionalization of Chinese flora. *Acta Botanica Yunnanica* **1**: 1–22.

Wu CY. 1991. The areal-types of Chinese genera of seed plants. *Acta Botanica Yunnanica* **S4**: 1–139.

Wu CY, **Li HW. 1977**. *Salvia weihaiensis*. In: Wu CY, ed. *Flora Reipublicae Popularis Sinicae*, Vol. **66**. Beijing: Science Press, 185–193.

Wu CY, **Wu SG. 1996**. A proposal for a new floristic kingdom (realm): the E. Asiatic Kingdom, its delineation and characteristics. In: Zhang AL and Wu SG, eds. *Proceedings of the First International Symposium on Floristic Characteristics and Diversity of East Asian Plants*. Beijing: Chinese Higher Education Press, 3–24.

Xiang CL. 2016. Lamiaceae. In: Xiang CL, Liu QX, Peng H, eds. *Species catalogue of China, plants*, Vol. **1(IX)**. Beijing: Sciences Press, 1–85.

Xiang CL, **Zhang Q**, **Scheen AC**, **Cantino PD**, **Funamoto T**, **Peng H. 2013**. Molecular phylogenetics of *Chelonopsis* (Lamiaceae: Gomphostemmateae) as inferred from nuclear and plastid DNA and morphology. *Taxon* **62**: 375–386.

Xiang CL, **Hu GX**, **Peng H. 2016*a***. New combinations and new synonyms in Lamiaceae from China. *Biodiversity Science* **24**: 719–722.

Xiang CL, **Hu GX**, **Peng H. 2016*b***. *Salvia wuana* (Lamiaceae), a new name for *S. pauciflora* E. Peter. *Phytotaxa* **255**: 99–100.

Yang ZY, **Gong X**, **Pan YZ. 2004**. Cytological study of six *Salvia* species (Lamiaceae) from Hengduanshan Mountains region of China. *Caryologia* **57**: 360–366.

Yao G, **Drew BT**, **Yi TS**, **Yan HF**, **Yuan YM**, **Ge XJ. 2016**. Phylogenetic relationships, character evolution and biogeographic diversification of *Pogostemon* s.l. (Lamiaceae). *Molecular Phylogenetics and Evolution* **98**: 184–200.

Yin A, **Harrison TM. 2000**. Geologic evolution of the Himalayan–Tibetan orogen. *Annual Review of Earth & Planetary Sciences* **28**: 211–280.

Yoder AD, **Irwin JA**, **Payseur BA. 2001**. Failure of the ILD to determine data combinability for slow loris phylogeny. *Systematic Biology* **50**: 408–424.

Yu XQ, **Maki M**, **Drew BT**, ***et al.* 2014**. Phylogeny and historical biogeography of *Isodon* (Lamiaceae): rapid radiation in south-west China and Miocene overland dispersal into Africa. *Molecular Phylogenetics and Evolution* **77**: 183–194.

Zhao HX, **Zhang L**, **Fang X**, **Yang RW**, **Ding CB**, **Zhou YH. 2006**. Studies on chromosome numbers of *Salvia miltiorrhiza*, *S. flava* and *S. evansiana*. *China Journal of Chinese Materia Medica* **31**: 1847–1849.

Zhu ZY, **Min BQ**, **Wang QL. 2011**. Taxa Nova *Salviorum labiatarum*. *Bulletin of Botanical Research* **31**: 1–3.