INTEGRATING DNA SEQUENCES WITH MORPHOLOGICAL ANALYSIS CLARIFIES PHYLOGENETIC POSITION OF *SALVIA GRANDIFOLIA* (LAMIACEAE): AN ENIGMATIC SPECIES ENDEMIC TO SOUTHWESTERN CHINA

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Premise of research. Salvia grandifolia is a narrowly endemic species known only from the Hengduan Mountains of southwestern China. Previous studies have shown that East Asian Salvia species form a clade, Salvia subg. *Glutinaria*, and suggested that *S. grandifolia*, on the basis of geographical distribution, should be included within *Glutinaria*. Calyx and staminal features, however, indicate that *S. grandifolia* may be closely related to taxa of subg. *Sclarea*, subg. *Salvia*, and the informal subg. *"Heterosphace,"* which are primarily found in central and southwest Asia, Europe, and the Mediterranean region. Since *S. grandifolia* is a rare species and has never been included in any molecular phylogenetic studies, the phylogenetic position of this species remains uncertain.

Methodology. The phylogenetic position of *S. grandifolia* was inferred using DNA sequences as well as morphological and cytological evidence. We used two nuclear ribosomal DNA regions (internal transcribed spacers) and one chloroplast marker (*rpl32-trnL*) in concert with maximum likelihood and Bayesian inference approaches to examine phylogenetic relationships.

Pivotal results. The chromosome number of somatic cells within *S. grandifolia* was 2n = 4x = 40. Rather than being grouped with taxa of the East Asian subg. *Glutinaria* as expected, *S. grandifolia* was embedded within subg. *Sclarea* in both nrDNA and cpDNA phylogenetic analyses.

Conclusions. Phylogenetic and morphological evidence indicates that *S. grandifolia* is a member of subg. *Sclarea*, and thus East Asian *Salvia* is nonmonophyletic in a geographic sense. Dispersal of *Salvia* from southwest Asia to East Asia has ostensibly occurred at least twice.

Keywords: Salvia grandifolia, subg. Glutinaria, subg. Sclarea, sect. Aethiopis, sect. Drymosphace.

Introduction

Traditionally delimited *Salvia* L. has been demonstrated to be nonmonophyletic because of the insertion of five genera (*Dorystaechas* Boiss. & Heldr. ex Benth., *Meriandra* Benth., *Perovskia* Kar., *Rosmarinus* L., and *Zhumeria* Rech. f. & Wendelbo; Walker et al. 2004, 2015; Walker and Sytsma 2007; Drew and Sytsma 2012; Will and Claßen-Bockhoff 2014, 2017; Drew et al. 2017; Hu et al. 2018; Kriebel et al. 2019). The nonmonophyly of *Salvia* has generated some controversy with regard to taxonomic alternatives. Will et al. (2015) and Will and Claßen-Bockhoff (2017) advocated splitting traditionally defined *Salvia* into six genera and retaining the generic status of the five embedded genera. However, on the basis of phylogenetic, morphological, taxonomic, and practical considerations, Drew et al. (2017) reduced the five embedded genera to subgenera of *Salvia* and recognized 10 main clades within a broadly defined *Salvia* (seven subgenera and three clades without specific taxonomic categories). Later, Hu et al. (2018) formally named the "*S. glutinosa* clade" subg. *Glutinaria* G.X. Hu, C.L. Xiang & B.T. Drew. Recently, Kriebel et al. (2019) informally included the "*S. aegyptiaca* clade" in subg. *Zhumeria* and named (or redefined) three additional subgenera (subg. *Salvia*, subg. *Sclarea* (Moench) Benth., and the informal subg. "*Heterosphace*"). Currently, 11 subgenera within a broadly defined *Salvia* are recognized. In this article, we use nomenclature consistent with a broadly defined *Salvia*.

Salvia is the largest genus within the mint family (Lamiaceae), with a nearly cosmopolitan distribution and approximately 1000 species (Drew et al. 2017). *Salvia* likely originated in southwest Asia around 32 Ma and then dispersed to other regions of the world, including East Asia, once in the late Oligocene or the early Miocene (Kriebel et al. 2019). The East

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Asian (EA) crown group began to diversify at about 18 Ma in the mid-Miocene (Kriebel et al. 2019).

With approximately 100 species, 83 of which are native to China (Li and Hedge 1994; Hu et al. 2014, 2017; Hu and Peng 2015; Xiang et al. 2016; Ding et al. 2019), East Asia is one of the three diversity centers of Salvia. Additionally, three species native to the New World (S. coccinea Buc'hoz ex Etl., S. reflexa Hornem., and S. tiliifolia Vahl) have become naturalized or invasive in China (Li and Hedge 1994; Hu et al. 2013; Shao et al. 2019). On the basis of staminal morphology, EA Salvia was historically classified into three subgenera: subg. Salvia, subg. Sclarea, and subg. Allagospadonopsis Briq. (Wu 1977; Murata and Yamazaki 1993). However, molecular phylogenetic analyses indicate that all Salvia species native to East Asia form a clade, and the monophyly of the three subgenera was not supported (Takano and Okada 2011; Li et al. 2013; Will and Claßen-Bockhoff 2017; Hu et al. 2018; Kriebel et al. 2019). On the basis of molecular phylogenetic and morphological evidence, Hu et al. (2018) recognized EA Salvia as a subgenus (subg. Glutinaria) and described eight sections within the subgenus.

In East Asia, S. grandifolia W.W. Smith is a morphologically enigmatic taxon (Li and Hedge 1994). This species was described by British botanist William Wright Smith in 1916 on the basis of a single collection from Yunnan Province in southwest China (G. Forrest 11150; type specimens at K, E, and BM), and it was subsequently found in the neighboring Sichuan Province in 1959 (S.G. Wu 3507; specimens at KUN and PE). Since then, no specimens have been documented. Salvia grandifolia is distinct from other species of EA Salvia in having large leaves, up to 40 cm \times 80 cm (fig. 1C, 1D); a tridentate upper calyx lip (fig. 1G); and abruptly dilated irregular rhomboidal lower arms of the staminal connectives (fig. 11, 1]). On the basis of the unique calyx and stamen characters, Wu (1977) established the monotypic series Grandifoliae C.Y. Wu within subg. Sclarea sect. Aethiopis Benth. to accommodate S. grandifolia. Geographically, S. grandifolia is known only from Yunnan and Sichuan Provinces in southwestern China, and it is very narrowly distributed (fig. 2). On the basis of the assumption that EA Salvia is monophyletic and the lack of a trimucronate or subentire upper calyx lip (as is typical in most species of EA Salvia), Hu (2015) speculated that S. grandifolia might be most closely related to two Chinese-endemic species, S. substolonifera E. Peter and S. trijuga Diels, which form a distinct clade (sect. Substoloniferae (C.Y. Wu) C.L. Xiang & B.T. Drew) within subg. Glutinaria (Hu et al. 2018). In terms of morphological traits alone, however, S. grandifolia is more similar to taxa of subg. Sclarea, subg. Salvia, and subg. Heterosphace distributed in central and southwest Asia, Europe, and the Mediterranean region.

Because *S. grandifolia* is rare and leaf samples suitable for DNA sequencing were not available, this species was not included in previous phylogenetic analyses. The phylogenetic position of *S. grandifolia*, therefore, remains unclear. Recently, we discovered two wild populations from Yunnan and Sichuan Provinces in southwestern China that allowed us to obtain DNA data and investigate the systematic position of this species. In this study, we clarify the phylogenetic position of *S. grandifolia* on the basis of molecular phylogenetic, cytological, and morphological evidence.

Material and Methods

Taxon Sampling

Two populations of Salvia grandifolia were collected in the field in Yunnan and Sichuan Provinces in southwestern China. As S. grandifolia is most closely related to taxa from the Old World and the goal of this study was to establish the placement of S. grandifolia, we included as many species as possible from the Old World in our phylogenetic analyses. We also included a small number of representatives from the New World (subg. Calosphace and subg. Audibertia). In total, we sampled 63 species (taxa for the nrDNA analysis; 54 taxa for the rpl32-trnL analysis) from subg. Sclarea, 32 (27) from subg. Heterosphace, 43 (43) from subg. Salvia, 1 (1) from subg. Rosmarinus, 2 (1) from subg. Perovskia, 75 (36) from subg. Glutinaria, 10 (9) from subg. Calosphace, 2 (3) from subg. Audibertia, 1 (0) from subg. Meriandra, 1 (1) from subg. Dorystaechas, and 12 (10) from subg. Zhumeria (see appendix). Names of subgenera follow Kriebel et al. (2019). Lepechinia lamiifolia (Benth.) Epling and Melissa axillaris (Benth.) Bakh. f. were selected as outgroups in the nrDNA analysis and Melissa officinalis L. as an outgroup in the cpDNA analysis. Forty-two sequences were newly generated in this study.

DNA Extraction, Amplification, and Sequencing

Total genomic DNA was extracted from silica gel-dried leaf samples using the modified CTAB method of Doyle and Doyle (1987). The nuclear ribosomal internal transcribed spacer (ITS) region was amplified using the primers ITS5 and ITS4 (White et al. 1990), while the external transcribed spacer (ETS) region was amplified using the primers ETS-B (Beardsley and Olmstead 2002) and 18S-E (Baldwin and Markos 1998). The chloroplast DNA marker *rpl32-trnL* was amplified using the primers *rpl32*-F and *trnL*^(UGA) (Shaw et al. 2007).

The standard 25- μ L PCR reaction mixtures contained 12.5 μ L of 2 × Es Tap MasterMix, 1 μ L of each primer (10 μ M, Sangon Biotechnology, Shanghai), 1 μ L of unquantified template DNA, and 9.5 μ L of deionized water. Amplification for all markers was performed using the following thermal cycler conditions: 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-min extension (72°C) and ending with a final extension at 72°C for 7 min. PCR products were checked on 1% TAE agarose gels and purified using the QIAquick PCR Purification Kit (BioTeke, Beijing) following the manufacturer's instructions. Sequencing reactions were performed using the dideoxy chain termination method on an ABI PRISM 3730 automated sequencer (Sangon Biotechnology) with the same primers used for the PCR amplification.

Sequence Alignment and Phylogenetic Analyses

Sequences were edited manually using Sequencher version 4.1.4 (Gene Codes, Ann Arbor, MI) and were aligned using MUSCLE (Edgar 2004) as implemented in MEGA version 6.0 (Tamura et al. 2013). The final alignments were manually adjusted in PhyDE version 0.9971 (Müller et al. 2010). Nuclear data (ITS and ETS) and plastid data (*rpl32-trnL*) were analyzed separately using maximum likelihood (ML) and Bayesian inference (BI).



Fig. 1 Morphological characters of *Salvia grandifolia*. *A*, Habitat. *B*, Root. *C*, Habit. *D*, Leaf, adaxially. *E*, Verticillaster. *F*, Verticillaster, showing bracts and bracteoles. *G*, Calyx; the arrow points to the tridentate upper calyx lip. *H*, Corolla. *I*, Stamens, frontal view. *J*, Stamen, side view. at = anterior thecae; f = filament; la = lower arm; pt = posterior thecae; ua = upper arm. *K*, Mericarp.

ML analyses were performed using RAxML-HPC2 on XSEDE version 8.2.12 (Stamatakis 2014) as implemented on the CIPRES Science Gateway (http://www.phylo.org/; Miller et al. 2010) under the GTRGAMMA model. The partitioned model (-q) was used for nrDNA data, bootstrap iterations (-#|-N)

were set to 1000, and other parameters followed the default settings.

BI analysis was performed using MrBayes version 3.2 (Ronquist et al. 2012) as implemented on the CIPRES Science Gateway (http://www.phylo.org/; Miller et al. 2010). The best-fit



Fig. 2 Geographical distribution of Salvia grandifolia. Alt. = altitude.

models were selected using JModelTest 2.1.1 (Darriba et al. 2012). Under the Akaike information criterion, the GTR+I+ Γ model was selected for the nrDNA data set and GTR+ Γ for the *rpl32-trnL* matrix. Four Markov chain Monte Carlo chains were run simultaneously for 20 million generations. Each run began with one random tree and sampled one tree every one-thousandth generation. A 50% majority-rule consensus tree was

calculated for each data set after the first 25% of the trees were discarded as burn-in.

Chromosome Observation

Seeds of *S. grandifolia* were collected in Yanyuan County, Sichuan Province, in southwestern China (voucher: G.X. Hu

& J.X. Yang 427). Seed germination was conducted on wet filter paper in petri dishes. When the roots grew to approximately 1 cm in length, fresh root tip meristems were pretreated in 2 mM 8-hydroxyquinoline at 20°C for 4 h and then were fixed in 45% acetic acid at 2°C for 10 min. After maceration in a mixture of 45% acetic acid and 1 M hydrochloric acid (1:1) at 60°C for 20–23 s, the root tip meristems were stained using 2% aceto-orcein at room temperature in a moist chamber with 45% acetic acid for 30 min, followed by conventional squash. The slides were faintly heated under an alcohol frame for 1–2 s before observation. A minimum of 10 cells at somatic metaphase were observed to determine the chromosome number. Photographs were taken using an Olympus digital camera (DP72SETA2) system (Tokyo).

Results

Chromosome Counts

The chromosome number of *Salvia grandifolia* in mitotic metaphase cells was found to be 2n=4x=40, and the basic number was x=10 (fig. 3).

Phylogenetic Analyses

Nuclear DNA analysis. Apart from collapsed or weakly supported nodes, ML and BI analyses generated similar topologies (fig. 4; the latter topology was not shown). The monophyly of Salvia was well supported, and all 11 subgenera of Salvia sensu Kriebel (2019) were supported (fig. 4). Salvia grandifolia was embedded within subg. Sclarea. Within subg. Sclarea, a small clade of southwest Asian and Mediterranean species (S. canescens var. daghestanica + S. cana + S. hypargeia + S. phlomoides and S. montbretii) was sister to S. grandifolia and the remainder of subg. Sclarea.

Chloroplast DNA analysis. ML and BI analyses for the rpl32-trnL data set generated similar topologies (fig. 5; the lat-

ter topology was not shown). Except for the unsampled subg. *Meriandra*, the other 10 subgenera of *Salvia* were recovered as monophyletic. In contrast to the nrDNA tree, subg. *Sclarea* was sister to subg. *Salvia* rather than subg. *Heterosphace*, and subg. *Zhumeria* was sister to subg. *Glutinaria* as opposed to a clade including subg. *Calosphace*, subg. *Audibertia*, and subg. *Dorystaechas*. Similar to the results from the nrDNA analysis, *S. grandifolia* was embedded within subg. *Sclarea* but in a sister position to the rest of subg. *Sclarea* (fig. 5).

Discussion

Phylogenetic and Morphological Evidence Indicates That Salvia grandifolia Is a Member of Subg. Sclarea

Wu (1977) placed 15 species of Chinese Salvia into four sections (sect. Aethiopis, sect. Notiosphace Benth., sect. Plethiosphace Benth., and sect. Drymosphace Benth.) within subg. Sclarea. However, Hu et al. (2018) demonstrated that subg. Sclarea sensu Wu (1977) was nonmonophyletic and included 13 taxa from sect. Notiosphace and sect. Drymosphace in the newly defined subg. Glutinaria. Instead of being grouped with EA Salvia, S. deserta Schangin, the only representative of subg. Sclarea sect. Plethiosphace in China (native to northwestern Xinjiang in far western China), has been demonstrated to be closely related to the taxa of subg. Sclarea (Li et al. 2013; Hu et al. 2018; Kriebel et al. 2019). From a floristic point of view, S. deserta (northwestern Xinjiang of China, Russia, Kyrgyzstan, and Kazakhstan) is found in the Irano-Turanian region (Takhtajan 1986), as are many other species of subg. Sclarea. Therefore, it is not surprising that S. deserta was included within subg. Sclarea rather than within the EA clade (subg. Glutinaria). Salvia grandifolia is the only representative of Wu's (1977) sect. Aethiopis in China. In this study, S. grandifolia appears as an isolated lineage within subg. Sclarea in both the nrDNA and the cpDNA trees. Salvia grandifolia is endemic to the Hengduan Mountains (fig. 2) and is distributed within the same



Fig. 3 Photographs of somatic mitosis metaphase of Salvia grandifolia.



Fig. 4 Cladogram based on maximum likelihood (ML) analysis of the combined nrDNA (internal transcribed spacers and external transcribed spacers) matrix. *Salvia grandifolia* is highlighted in the box. Bootstrap values of ML are given above the branches, and posterior probabilities (PP) are below. ML bootstrap support of <50% and PP <0.8 are indicated by a dash. A color version of this figure is available online.

eastern Asiatic region as taxa from subg. *Glutinaria* (except for *S. glutinaria* L., *S. nubicola* Wall. ex Sweet, and *S. plebeia* R. Br.). Thus, EA *Salvia* is nonmonophyletic in a geographic sense. While *S. grandifolia* is demonstrated to be a member of subg. *Sclarea*, *S. grandifolia* seems to be a phylogenetically iso-

lated species within subg. *Sclarea*, as no sister species were found within either nrDNA trees or cpDNA trees (figs. 4, 5).

Morphological characters also support the exclusion of *S. grandifolia* from subg. *Glutinaria*. *Salvia grandifolia* has a falcate upper corolla lip, which also occurs in four sections of subg.



Fig. 5 Cladogram based on maximum likelihood (ML) analysis of the *rpl32-trnL* matrix. *Salvia grandifolia* is highlighted in the box. Bootstrap values of ML are given above the branches, and posterior probabilities (PP) are below. ML bootstrap support of <50% and PP <0.9 are indicated by a dash. A color version of this figure is available online.

Glutinaria (sect. *Sonchifoliae* (C.Y. Wu) G.X. Hu, C.L. Xiang & H. Peng, sect. *Substoloniferae*, sect. *Glutinaria*, and sect. *Drymosphace*; Hu et al. 2018). However, it can be readily differentiated from these four sections of EA *Salvia* by its ovate bracts (fig. 1*F*) and tridentate upper calyx (fig. 1*G*). Additionally, Hu

et al. (2018) recognized six distinct stamen types within subg. *Glutinaria*, but the staminal architecture of *S. grandifolia* is clearly different from the stamen types within subg. *Glutinaria*; *S. grandifolia* possesses abruptly dilated irregular rhomboidal lower arms (fig. 1*I*, 1*J*). Furthermore, we observe that all taxa

of subg. Glutinaria have exserted corolla tubes that are obviously longer than the calyx. However, the corolla tube of S. grandifolia is shorter than or subequal to the calvx (fig. 1E). The unique characters of S. grandifolia (ovate bracts, tridentate upper calyx lips, falcate upper corolla lips, stamens with abruptly dilated irregular rhomboidal lower arms, and a nearly included corolla tube) are also found in taxa with a Mediterranean region/southwest Asian distribution, such as S. sclarea L. and S. aethiopis L., which suggests that S. grandifolia is more closely related to subg. Sclarea than to subg. Glutinaria. A morphometric analysis of corolla, anther connective, and style shapes across Salvia clearly demonstrates that the "Sclarea"-type flower has evolved elsewhere in a convergent fashion (Kriebel et al. 2020); thus, it is not surprising that the similarity in the floral morphologies of S. grandifolia and subg. Sclarea was previously overlooked.

Finally, cytological evidence indicates that S. grandifolia is more closely related to subg. Sclarea than to subg. Glutinaria. In this study, we found that S. grandifolia was tetraploid, with a basic chromosome number of x = 10. However, all species of subg. Glutinaria that have been tested have had the same basic chromosome number of x=8 (Gill 1971; Yang et al. 2004; Zhao et al. 2006; Wang et al. 2009; Hu et al. 2016); this could be regarded as a diagnostic character for the subgenus (Hu et al. 2018). Solely on the basis of the difference in basic chromosome number, S. grandifolia should be excluded from subg. Glutinaria. As mentioned above, S. grandifolia is morphologically similar to taxa of subg. Salvia, subg. Heterosphace, and especially subg. Sclarea, which have overlapping distributions in central and southwest Asia, Europe, and the Mediterranean region. For the three related subgenera, we found 16 species with the basic chromosome number of 10 in subg. Sclarea (S. amplexicaulis Lam., S. atropatana Bunge, S. frigida Boiss., S. hierosolymitana Boiss., S. karabachensis Pobed. (Will), S. macrosiphon Boiss., S. moorcroftiana Wall. ex Benth., S. palaestina Benth., S. persepolitana Boiss., S. reuteriana Boiss., S. saccardiana (Pamp.) D. Carr. & Garbari, S. sarawschanica Regel & Schmalh., S. sclareoides Brot., S. sharifii Rech. f. & Esfand., S. spinosa L., and S. syriaca L.), one species in subg. Heterosphace (S. lanceolata Brouss.), and no taxa in subg. Salvia (Scheel 1931; Patudin et al. 1975; Afzal-rafii 1980, 1981; Bir and Saggoo 1981; Strid and Franzen 1981; Markova and Ivanova 1982; Fernandes and Leitão 1984; Baltisberger 1991; Kharazian 2011). Therefore, on the basis of the basic chromosome number, S. grandifolia fits nicely as a member of subg. Sclarea.

Salvia Dispersed Twice to East Asia

Kriebel et al. (2019) showed that *Salvia* most likely originated in southwest Asia at about 32 Ma in the early Oligocene and subsequently dispersed nearly worldwide. The dispersal to East Asia at about 18 Ma (Hu et al. 2018; Kriebel et al. 2019) would have followed the initial uplift of the Kunlun, Tianshan, and Altay mountain ranges on the west edge of the Qinghai-Tibetan Plateau, with subsequent uplift events presumably blocking additional dispersals to East Asia (Favre et al. 2015; Kriebel et al. 2019). Kriebel et al. (2019) indicated that the crown of subg. *Sclarea* radiated at about 13 Ma, probably in concert with central Asian aridification (Favre et al. 2015). By demonstrating that *S. grandifolia* is embedded within subg. *Sclarea*, we have uncovered a second colonization event of Salvia in East Asia, which ostensibly occurred during or after the mid-Miocene. On the basis of our phylogenetic results, S. grandifolia diverged relatively early within subg. Sclarea, and so it appears that this second dispersal event occurred sometime during the mid- to late Miocene, before the final uplifts of the Kunlun, Tianshan, and Altay mountain ranges at the western edge of the Qinghai-Tibetan Plateau (Sun et al. 2004; Vassallo et al. 2007). In their present form, these mountains present a formidable challenge to dispersal for most plant species (Yu et al. 2014). In general, members of subg. Sclarea are adapted to open xeric environments, while species within subg. Glutinaria are found in more mesic environments-either broad-leaved or coniferous forests (Kriebel et al. 2019). Since it is now established that subg. Sclarea has dispersed into China, it will be interesting to formally examine the reasons why subg. Glutinaria has flourished in East Asia while subg. Sclarea has barely hung on.

Supplementary Description of S. grandifolia

Because of limited herbarium specimens and a lack of field observations, previous descriptions of *S. grandifolia* were deficient (Li and Hedge 1994). On the basis of field observations and specimen studies, we provide a supplementary description for *S. grandifolia*.

Salvia grandifolia W.W. Smith, Notes Roy. Bot. Gard. Edinburgh 9:123, 1916

Type. China. Yunnan: open pastures on the margins of thickets in the mountains in the northeast of the Yangtze bend, September 1913, G. Forrest 11150 (holotype: E; isotype: K, BM).

Plants perennial, with most aboveground parts dying in winter. Taproot thickened, subsucculent, scarlet inside. Stems erect, up to 2 m tall, robust, stem diameter up to 4 cm, densely hispid, interspersed with glandular hairs, with many branches. Leaves sessile or with petioles up to 7 cm long, densely tomentosevillous; leaf blade obovate, up to 40 cm × 80 cm, papery, sparsely hispidulous adaxially, tomentose or villous abaxially, base rounded to broadly cuneate, margin remotely deep, undulate-lobulate; lobes rounded, 4-15 cm × 4-12 cm, apex rounded. Inflorescences densely glandular, pilose; verticillasters six flowered, widely spaced, forming many-branched terminal panicles; bracts ovate, persistent in fruit, acuminate, 8-11 mm × 8-11 mm, bracteoles similar to bracts, 5-8 mm × 2-4 mm. Pedicel 5-7 mm long. Calyx campanulate, 1.3-1.8 cm \times 0.9–1.2 cm, 13 veined, with upper lip five veined, glandular pubescent, sparsely red-brown glandular, tube 8-12 mm long; upper lip tridentate; lower lip slightly longer than upper, deeply two toothed, teeth triangular. Corolla 2.4-3 cm long, tube yellowish, imperfectly pilose, annulate inside, 1.3-1.7 cm long, nearly included, 3-4 mm wide at base, slightly constricted at hairy annulus, gradually dilated above hairy annulus, 9-11 mm wide at throat; lips purple red with two yellowish stripes and spots on upper lip; upper lip slightly foldedfalcate, apex emarginate, 10-13 mm × 10-12 mm, densely glandular, pilose; lower lip 11-12 mm × 9-11 mm; middle lobe obcordate, 3-4 mm × 4-5 mm; lateral lobes elliptical, revolute,

 $9-10 \text{ mm} \times 3-4 \text{ mm}$. Filaments 4-5 mm long; connectives nearly straight, 1.5-1.7 cm, lower arms 4-5 mm, upper arms 1.1-1.2 cm. Upper arms ca. four times as long as lower arms, lower arms abruptly dilated, irregular rhomboidal; anthers bithecous, anterior thecae elongate, yellowish, 4-5 mm long, fertile, posterior thecae reduced and sterile, connivent. Mericarp brown, ovoid, $4-5 \text{ mm} \times 3-4 \text{ mm}$.

Phenology. Flowering from June to September, fruiting from July to October.

Distribution. Salvia grandifolia is endemic to northwestern Yunnan and southwestern Sichuan in China. It grows at the edges of dry thickets or pasturelands at elevations between 2000 and 3000 m asl. Common associated species include *Quercus rehderiana* Hand.-Mazz., *Isodon phyllostachys* (Diels) Kudo, *Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult., *Arthraxon lanceolatus* (Roxb.) Hochst, and *Incarvillea arguta* (Royle) Royle.

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Appendix

Voucher Information and GenBank Accession Numbers for Taxa Used in This Study

New sequences are indicated by an asterisk. The following information is provided for each taxon: taxon name and authority, collector name(s) and collection number, and GenBank accession numbers (from left to right; dashes indicate no sequence available: ETS, ITS, *rpl32-trnL*).

Lepechinia lamiifolia (Benth.) Epling, B. Drew 178, JF301320, JF301348, -; Melissa axillaris (Benth.) Bakh. f., D.E. Boufford et al. 24526, JQ669189, JQ669114, -; Melissa officinalis L., M. Will 64, -, -, KJ747325; Salvia adenocaulon P.H. Davis, F. Celep 1251, -, KU563828, KU578149; Salvia adenophylla Hedge & Hub.-Mor., F. Celep 1500, -, KU563789, KU578218; Salvia aegyptiaca L., M. Kuschewitz s.n., KJ584254, DQ667285, KJ747314; Salvia aerea H. Lév., Liu et al. 2898, MG824294, MG824166, MN080449*; Salvia aethiopis L., J. Hellwig s.n., -, KJ584163, KU578150; Salvia africana L., P. Wester 319, -, -, KJ747271; Salvia africana L., E. Eifler 443870, MK425851, MK425906, -; Salvia africana-lutea L., P. Wester 342, KJ584256, -, -; Salvia akiensis A. Takano, T. Sera & Kurosaki, M. Sakoda et al. 1, LC060826, LC060728, -; Salvia albicaulis Benth., P. Wester 340, KJ584257, KJ584206, KJ747274; Salvia albimaculata Hedge & Hub.-Mor., F. Celep 1032, -, KU563790, KU578219; Salvia amplexicaulis Lam., D. Podlech 28360, -, KU563829, KU578151; Salvia amplexicaulis Lam., C756, MG824296, -, -; Salvia apiana Jeps., P. Wester 411, -, -, KJ747321; Salvia apiana Jeps., JBW 2509, -, DQ667214, -; Salvia apiana Jeps., JBW 3081, KP852936, -, -; Salvia appendiculata E. Peter, Hu & Zhao 0063, MG824297, MG824169, -; Salvia aramiensis Rech.f., F. Celep 1400, -, KU563791, KU578247; Salvia areysiana Deflers, Thulin 8472, KJ584259, -, KJ747315; Salvia argentea L., R. Claßen-Bockhoff s.n., -, KJ584164, KJ747299; Salvia ariana Hedge, D. Podlech & K. Jarmal 30029, -, KU563843, KU578152; Salvia aristata Aucher ex Benth., Y. Ajani 1569, -, -, KJ747264; Salvia aristata Aucher ex Benth., Wedelbo & Assadi s.n., JF301336, DQ667280, -; Salvia atropatana Bunge, A. Kahraman 1570, -, KU563841, KU578153; Salvia atropurpurea C.Y. Wu, Liu et al. 3428, MG824298, MG824170, MN080450*; Salvia atrorubra C.Y. Wu, s.n., MG824299, MG824171, -; Salvia aucheri Benth., F. Celep 1245, -, KJ584193, -; Salvia aurita L. f., P. Wester 324, -, KJ584218, KJ747276; Salvia aurita L. f., M.Will 26, KJ584260, -, -; Salvia austriaca Jacq., R. Claßen-Bockhoff s.n., -, -, KJ747261; Salvia austriaca Jacq., Walker s.n., MK425853, MK425907, -; Salvia axillaris Moc. & Sessé ex Benth., J. Walker 3038, -, DQ667294, JQ669366; Salvia axillaris Moc. & Sessé ex Benth., N.I. Cacho 22, MK425854, MK425908, -; Salvia aytachii Vural & Adigüzel, S. Bagherpour 412, -, -, KU578220; Salvia baimaensis S.W. Su & Z.A. Shen, Hu & Shangguan Hu0097, MG824300, G824172, -; Salvia ballsiana (Rech.f.) Hedge, A. Kahraman 1505, -, KU563792, KU578215; Salvia bariensis Thulin, M.Thulin, A.Dahir & A.Osman 9429, KJ584262, -, KJ747316; Salvia bifidocalyx C.Y.Wu & Y.C. Huang, Liu et al. FW11076, MG824301, MG824173, -; Salvia blepharochlaena Hedge & Hub.-Mor., F. Celep 1217, -, KU563793, KU578210; Salvia bowleyana Dunn, Yin & Dong 0105, MG824302, MG824174, MN080451*; Salvia brachyantha (Bordz.) Pobed., A. Kahraman 1572, -, KU563844, KU578154; Salvia brachyloma E. Peter, Q.Q. Li, HLQ201002, -, [Q934108, -; Salvia brevilabra Franch., China: Sichuan, -, EF373638, -; Salvia broussonetii Benth., M.Will 33, KJ584263, -, KJ747293; Salvia broussonetii Benth., R. Claßen-Bockhoff. 2/10, -, KJ584225, -; Salvia bucharica Popov, M.G. Pimenov, E.V. Kljuykov, I. Mukumov 113, -, KU563794, KU578222; Salvia bulleyana Diels, Sino-Amer. Bot. Exped. No 412, -, KU563780, KU578203; Salvia bulleyana Diels, Yin et al. 1314, MG824307, -, -; Salvia cabulica Benth., H.Freitag 4683, -, KJ584189, -; Salvia cadmica Boiss., F. Celep 1426, -, -, KU578223; Salvia cadmica Boiss., Celep, F. 916, MK425857, MK425910, -; Salvia caespitosa Montbret & Aucher ex Benth., F. Celep 1544, -, KU563796, KU578224; Salvia campanulata Wall. ex Benth., Hu & Dong D990, MG824309, MG824181, MN080452*; Salvia cana Wall. ex Benth., Rechinger 30.587, -, KU563878, -; Salvia canariensis L., M. Will 46, -, -, KJ747295; Salvia canariensis L., UCB 79.0235, MK425859, MK425912, -; Salvia candelabrum Boiss., M. Will 42, -, KJ584190, KJ747255; Salvia candidissima Vahl, Royal Bot. Gard. Edinburg #1999-2202A, -, DQ667261, -; Salvia canescens var. daghestanica (Sosn.) Menitsky, M. Will 34, -, KJ584187, KJ747308; Salvia cassia Sam. ex Rech.f., F. Celep 1411, -, KU563845, KU578190; Salvia castanea Diels, Hu et al. D585, MG824312, MG824184, MN080453*; Salvia castaleriei H. Lév., Hu & Shangguan Hu0051, MG824316, MG824188, MN080454*; Salvia cedronella Boiss., F. Celep 1455, -, KU563797, -; Salvia ceratophylla L., A. Kahraman 1378, -, -, KU578146; Salvia cerinopruinosa Rech. f., Sivas, -, KM519758, -; Salvia chamelaeagnea Berg., P. Wester 314, -, -, KJ747289; Salvia chamelaeagnea Berg., M. Will 47, -, KJ584212, -; Salvia chamelaeagnea Berg., P. Wester, R. Claßen-Bockhoff & E.van Jaarsveld 313, KJ584267, -, -; Salvia chienii E. Peter, Hu & Shangguan Hu0071, MG824321, MG824193, MN080455*; Salvia chinensis Benth., UCB 99.0544, MK425861, MK425914, -; Salvia chionantha Boiss., F. Celep 1464, -, KU563846, KU578155; Salvia chloroleuca Rech. f. & Aellen, TUH: 7917, -, -, KX641671; Salvia chloroleuca Rech. f. & Aellen, H. Akhani 11027, -, KU563847, -; Salvia chrysophylla Stapf, F. Celep 1464, -, KU563848, KU578157; Salvia chrysophylla Stapf, F. Celep 1464, -, KU563848, KU578157; Salvia chrysophylla Stapf, F. Celep 1464, -, KU563848, KU578157; Salvia chrysophylla Stapf, F. Celep 1464, -, KU563848, KU578157; Salvia chrysophylla Stapf, F. Celep 1464, -, KU563848, KU578157; Salvia chrysophylla Stapf, F. Celep 1464, -, KU563848, KU578157; Salvia chrysophylla Stapf, F. Celep 1464, -, KU563848, KU578157; Salvia chrysophylla Stapf, F. Celep 1464, -, KU563848, KU578157; Salvia chrysophylla Stapf, F. Celep 1464, -, KU563848, KU578157; Salvia chrysophylla Stapf, F. 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Shi QT002, MG824331, MG824203, MN080457*; Salvia deserti Decne., E.Gamal Eldin s.n., KJ584270, -, KJ747312; Salvia dianthera (Roth ex Roem. & Schult.) Briq., Lavranus & Newton 15796, JF301326, DQ667329, -; Salvia dichroantha Stapf, F. Celep 1330, -, KU563830, KU578159; Salvia digitaloides Diels, Walker s.n., MK425866, MK425917, -; Salvia disermas L., P. Wester 326, KJ584271, KJ584179, KJ747296; Salvia disjuncta Fernald, P. Wester 296, -, -, KU578197; Salvia divaricata Montbret & Auch. ex Benth., A. Kahraman 1591, -, -, KU578226; Salvia dolomitica Codd, P. Wester 321, KJ584273, -, KJ747290; Salvia dolomitica Codd, F. Brusse 5610, -, KJ584213, -; Salvia dominica L., M.Kuschewitz s.n., -, -, KJ747262; Salvia dominica L., isolate 1604, -, KJ864961, -; Salvia dorystaechas B.T. Drew, Cult. RBGE1972-0177D, -, -, JQ669302; Salvia dorystaechas B.T. Drew, Walker s.n., MK425867, MK425918, -; Salvia elegans Vahl, C767, MG824334, MG824206, -; Salvia engelmannii A. Gray, P. Wester 360, -, KU563870, KU578163; Salvia eriophora Boiss. & Kotschy, A. Kahraman 1581, -, KU563850, KU578167; Salvia euphratica Montbret & Aucher ex Benth., A. Kahraman 1585A, -, KU563819, KU578227; Salvia evansiana Hand.-Mazz., M. Will 55, -, -, KJ747323; Salvia evansiana Hand.-Mazz., Hu et al. D557, MG824335, MG824207, -; Salvia farinacea Benth., Liu et al. FW11205, MG824337, MG824209, -; Salvia flava Forrestex Diels, Hu et al. D484, MG824339, MG824210, MN080458*; Salvia freyniana Bornm., S. Bagherpour 493, -, -, KJ747266; Salvia frigida Boiss., F. Celep 1082, -, KU563851, KU578168; Salvia fruticosa Mill., F. Celep 1373, -, KJ584195, KJ747256; Salvia garipensis E. Mey. ex Benth., Strohbach 149, -, DQ667281, -; Salvia geminata Thulin, Thulin, Beier & Hussein 9629, KJ584276, -, -; Salvia glabrescens Makino, Sudarmono et al. Jap03/68 BO, -, AB295104, -; Salvia glutinosa L., F. Celep 1196, -, KJ584253, -; Salvia glutinosa L., J. Walker 2568, KF307496, -, -; Salvia grandifolia W.W. Smith, NL, G.X. Hu & J.X. Yang 430, MN080488*, MN080448*, MN080460*; Salvia grandifolia W.W. Smith, YY, G.X. Hu & J.X. Yang 427, MN080487*, MN080447*, MN080459*; Salvia greatae Brandegee, JBW 2511, -, -, JQ669367; Salvia handelii E. Peter, Hu et al. D586, MG824340, MG824211, -; Salvia hayatana Makino ex Hayata, Okada H. et al. 5677, LC060827, AB295100, -; Salvia hedgeana Dönmez, A. Kahraman 1516, -, -, KU578216; Salvia heldreichiana Boiss., F. Celep 1427, -, KU563799, KU578246; Salvia henryi A. Gray, P. Wester 389, -, -, KU578165; Salvia henryi A. Gray, J. Walker 2516, JF301337, DQ667216, -; Salvia herbanica A. Santos & M. Fernández, R. Claßen-Bockhoff 1/05, KJ584278, KJ584246, KJ747313; Salvia heterochroa E. Peter, Boufford et al. 35205, -, -, KJ747324; Salvia heterochroa E. Peter, Liu et al. 3198, MG824341, MG824212, -; Salvia hians Royle ex Benth., Walker, J. B. 2577, MK425870, MK425919, -; Salvia hierosolymitana Boiss., C770, MG824342, -, -; Salvia hispanica L., I. Fragoso-Martinez 4, -, MF664593, -; Salvia honania L.H. Bailey, Hu & Zhao 0168, MG824343, MG824213, MN080461*; Salvia huberi Hedge, A. Kahraman 1295, -, KU563800, KU578228; Salvia hupehensis E. Peter, R.B. Zhu QT006, MG824345, MG824215, -; Salvia hydrangea DC. ex Benth., A. Kahraman 1468, -, -, KJ747257; Salvia hydrangea DC. ex Benth., hy.26, -, KP294355, -; Salvia hylocharis Diels, Hu et al. D554, MG824346, MG824216, -; Salvia hypargeia Fisch. & C.A. Mey., A. Kahraman 1354, -, KU563877, -; Salvia hypoleuca Benth., Y. Ajani 1600, -, KU563852, KU578171; Salvia indica L., A. Kahraman 1539, -, KU563853, KU578172; Salvia interrupta Schousb., M. Will 30, -, KJ584191, KJ747265; Salvia isensis Nakai ex H. Hara, A-200933, LC060831, LC060730, -; Salvia japonica Thunb., Hu & Shangguan Hu0042, MG824347, MG824217, MN080462*; Salvia judaica Boiss., M. Will 57, -, KJ584241, KU578160; Salvia judaica Boiss., C769, MG824348, -, -; Salvia jurisicii Kosanin, M. Kuschewitz s.n, -, KU563831, KU578173; Salvia kiangsiensis C.Y. Wu, Hu & Shangguan Hu0062, MG824349, MG824219, -; Salvia kiaometiensis H. Lév., Xiang et al. C376, MG824350, MG824220, -; Salvia korolkovii Regel & Schmalh., O. Neustrueva-Knorring 4857, -, KU563801, -; Salvia koyamae Makino, UCB 86.1153, MK425872, MK425922, -; Salvia kronenburgii Rech. f., A. Kahraman 1575, -, KM519759, KU578245; Salvia kurdica Boiss. & Hohen. ex Benth., Rechinger 11637, -, KU563821, KU578212; Salvia lanceolata Lam., P. Wester 316, KJ584280, KJ584201, KJ747278; Salvia lanigera Poir., A.El-Banhawy 11, -, KJ584185, -; Salvia lankongensis C.Y. Wu, D. Grey & R. Brown 128, MG824351, MG824221, -; Salvia leucantha Cav., G.X. Hu OT003, MG824352, MG824222, MN080463*; Salvia leucodermis Baker, Clement, Phillipson & Rafamantanantsoa 2137, -, K[584220, K]747280; Salvia leucodermis Baker, B. Bytebier 3193, K[584283, -, -; Salvia liguliloba Y.Z. Sun, Z. Li Liz0100, MG824353, MG824223, MN080464*; Salvia limbata C.A. Mey., A. Kahraman 1571, -, KU563854, KU578174; Salvia lutescens (Koidz.) Koidz., Jap04/73 BO, LC060837, AB266243, -; Salvia lyrata L., G.X. Hu QT011, MG824355, MG824225, MN080465*; Salvia macrochlamys Boiss. & Kotschy, A. Kahraman 1540, -, -, KU578229; Salvia macrosiphon Boiss., Podlech & Jarmal 29.817, -, -, KU578191; Salvia mairei H. Lév., Peng et al. PH9268, MG824356, MG824226, -; Salvia majdae Ramamoorthy, Wendelbo 15793, -, -, JQ669381; Salvia majdae Ramamoorthy, Terme 14573, MK425875, MK425924, -; Salvia marashica Ilçim, Celep & Dogan, F. Celep 1020, -, KU563802, KU578230; Salvia margaritae Botsch., M.G. Pimenov et al. 127-1, -, KU563880, KU578201; Salvia maximowicziana Hemsl., SNJ Exped. 20110719092, KM886717, KM886750, -; Salvia maymanica Hedge,

Moh. Amin 154, -, KU563805, KU578231; Salvia meiliensis S.W. Su, Hu & Shangguan Hu0089, MG824359, MG824229, MN080466*; Salvia mellifera Greene, JBW 255, -, -, JQ669368; Salvia merjamie Forssk., M. Will 83, KJ584286, KJ584184, KJ747297; Salvia microstegia Boiss. & Balansa, F. Celep 1539, -, KU563855, KU578169; Salvia miltiorrhiza Bunge, Hu & Shangguan Hu0088, MG824362, MG824232, MN080467*; Salvia modesta Boiss., F. Celep 1543, -, KU563856, KU578175; Salvia moniliformis Fern., A. Espejo & M. Crone 21, -, -, KU578198; Salvia montbretii Benth., A. Kahraman, -, KU563869, KU578195; Salvia moorcroftiana Wall. ex Benth., Anders 3627, -, KU563857, -; Salvia muirii L. Bolus, P. Wester & R. Claßen-Bockhoff 318, KJ584287, KJ584208, KJ747283; Salvia multicaulis Vahl, S. Bagherpour 282, -, -, KU578232; Salvia multicaulis Vahl, mu.44, -, KP294353, -; Salvia namaensis Schinz, P. Wester 330, -, -, KJ747281; Salvia namaensis Schinz, M.Will 28, -, KJ584217, -; Salvia namaensis Schinz, W.Giess & M.Mueller 14319, KJ584289, -, -; Salvia nanchuanensis H.T. Sun, Xu & Liu 09397, MG824364, -, MN080468*; Salvia napifolia Jacq., M. Will 49, -, KJ584229, -; Salvia nilotica Juss. ex Jacq., M. Will 49, -, KJ584229, KJ747258; Salvia nipponica Miq., Okada H. et al 5665, -, AB295103, -; Salvia nipponica Miq., Y. Ibaragi s.n., LC060848, -, -; Salvia nubicola Wall. ex Sweet, FLPH 12-124, MG824367, MG824236, MN080469*; Salvia nutans L., M. Will s.n., -, -, KU578176; Salvia nutans L., A. Suchorukow s.n. VII. 2001, -, KU563833, -; Salvia nydeggeri Hub.-Mor., F. Celep 1491, -, KU563803, KU578233; Salvia oaxacana Fernald., P. Wester 259, -, -, KU578199; Salvia officinalis L., M. Palma s.n. UCBG 7.0083, -, -, JQ669369; Salvia officinalis L., Walker, J. B. 2580, MK425881, MK425928, -; Salvia oligophylla Auch. ex Benth., Y. Ajani 1569, -, -, KU578177; Salvia omeiana E. Peter, Liu et al. 3025, MG824369, EF373642, -; Salvia omerocalyx Hayata, A. Takahashi 4601, LC060852, -, -; Salvia omerocalyx Hayata, HYO:A. Takahashi 4601, -, AB353205, -; Salvia pachystachya Trautv., A. Kahraman 1443, -, KU563804, -; Salvia palaestina Benth., F. Celep 1083, -, KJ584175, KJ747304; Salvia paobsingensis C.Y. Wu, Liu et al. 3195, MG824370, MG824239, -; Salvia paramiltiorrhiza H.W. Li & X.L. Huang, SPAR_1, -, JQ934140, -; Salvia patens Cav., Cult RBGE 1973-9197, -, -, JQ669370; Salvia patens Cav., Walker s.n., MK425882, MK425929, -; Salvia pentstemonoides Kunth & C.D. Bouché, P. Wester 386, -, KU563871, KU578162; Salvia persepolitana Boiss., Sh. Zarre et al. 35213, -, KU563858, KU578178; Salvia petrophila Rydb., Y. S. Huang Y1344, MG824372, MG824241, MN080470*; Salvia phlomoides Asso, R. Vogt 10336, -, KJ584186, KJ747309; Salvia pilifera Benth., A. Kahraman 1506, -, -, KU578208; Salvia pinnata L., F. Celep 1416, -, KU563798, KU578217; Salvia pisidica Boiss. & Heldr. ex Benth., F. Celep 1457, -, KU563806, KU578234; Salvia plebeia R.Br., G.X. Hu 607, -, -, MN080471*; Salvia plebeia R.Br., Sudarmono et al. Jap04/75, LC060858, AB295107, -; Salvia plectranthoides Griff., Liu et al. 2849, MG824378, MG824247, MN080472*; Salvia poculata Nöbelek, A. Kahraman 1560, -, KU563859, KU578179; Salvia pogonochila Diels ex Limpr., Liu et al. 3224, MG824381, MG824250, -; Salvia polystachya Cav., Breedlove & Mahoney 72286, -, -, JQ669371; Salvia polystachya Cav., Breedlove & Mahoney 72286, JF301334, -, -; Salvia polystachya Cav., JBW 3035, -, DQ667292, -; Salvia potaninii Krylov, F.T. Wang 21529, MG824382, MG824251, -; Salvia potantillifolia Boiss. & Heldr. ex Benth., F. Celep 1457, -, KU563807, KU578235; Salvia pratensis L., A. Suchorukow s.n.VII 2003, -, KJ584178, KJ747291; Salvia prattii Hemsl., Liu et al. FW11271, MG824383, MG824252, MN080473*; Salvia prionitis Hance, Yin & Dong 0172, MG824386, MG824255, MN080474*; Salvia przewalskii Maxim., Liu et al. FW11116, -, -, MN080475*; Salvia przewalskii Maxim., Walker s.n., MK425885, MK425931, -; Salvia pseudeuphratica Rech. f., -, KM519762, -; Salvia pterocalyx Hedge, P. Wendelbo et al. W8550, -, -, KU578200; Salvia pygmaea Matsum., H.Okada Jap03/71(TNS), LC060854, -, -; Salvia pygmaea Matsum., Okada H. et al. 5671, -, AB295098, -; Salvia qimenensis S.W. Su & J.Q. He, Hu & Shangguan Hu0082, MG824391, MG824260, -; Salvia quezelii Hedge & Afzal-Rafii, F. Celep 1626, -, KU563808, KU578249; Salvia radula Benth., Germishuizen 3950, KJ584293, KJ584180, -; Salvia ranzaniana Makino, Okada 5698, LC060856, AB287375, -; Salvia recognita Fisch. & C.A. Mey., S. Bagherpour 380, -, KU563808, KU578236; Salvia repens Burch. ex Benth., M. Will 50, KJ584295, KJ584232, KJ747282; Salvia reuteriana Boiss., D. Podlech & Sh. 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