

The complete chloroplast genome sequence of *Salvia chienii* E.Peter, 1936 (Lamiaceae)

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ABSTRACT

Salvia chienii E.Peter is a medicinal herb mainly distributed in Huangshan Mountain of Anhui province, China. In this study, the first complete chloroplast genome of *S. chienii* was sequenced and assembled. The genome length was 151,530 bp and encoded 143 genes (91 protein-coding genes, eight rRNA genes, and 37 tRNA genes). The phylogenomic analysis showed that *S. chienii* was closely related to *S. miltiorrhiza*. Further evolutionary studies of the genus *Salvia* could benefit from the complete chloroplast genome of *S. chienii* present in this study.

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Introduction

The genus *Salvia* is the largest member of the Lamiaceae family and is widely distributed around the world. Some *Salvia* species have antioxidant, anti-inflammatory and anti-cancer properties (Bonesi et al. 2017). Their main active constituents are phenolic acids and terpenoids. *Salvia chienii* E.Peter is primarily found in Huangshan Mountain of Anhui Province, China. The high content of salvianolic acid B and low levels of tanshinones were recorded in this species. In the 'Flora of China' (Wu and Raven 1999), it was mentioned that *S. chienii* generally grows along roadsides or hillsides and is a perennial plant with upright stems covered with profuse pubescence from the top downward. It has trifoliate or simple leaves and usually blooms in July. Chloroplast genomes of numerous *Salvia* species have been published (Zhou et al. 2020, Hu et al. 2020), but the chloroplast genome of *S. chienii* is not reported yet. In the study, we reported the complete chloroplast genome of *S. chienii* for the first time, which helped to clarify interspecific relationships among the *Salvia* species.

Materials and methods

Fresh samples of *S. chienii* were collected from Huangshan, Anhui, China (GPS: E114°04'03.15", N31°49'46.46") (Figure 1). A specimen was deposited at Herbarium of Shanghai Chenshan Botanical Garden (CSH) (Yukun Wei, ykwei76@hotm.com)


and was given a voucher number S1094. Total genomic DNA was extracted by using DNA Plantzol reagent (Invitrogen, Carlsbad, CA, USA). A 350-bp paired-end library was constructed using Illumina's TruSeq Nano DNA Library Preparation kit following the manufacturer's instructions. About 3.51 G of original data was sequenced using Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA, USA). The chloroplast genome was assembled using *rbcl* gene (genbank number: NC_023209) as a seed sequence by NOVOPlasty (Dierckxsens et al. 2017), and annotated with GeSeq v2.03 and Geneious Prime 2022 (<https://www.geneious.com>). The newly annotated chloroplast genome was submitted to GenBank with accession number OK094518.

To reveal the phylogenetic relationship of *S. chienii*, the sequences of other 23 chloroplast genomes were downloaded from NCBI (<https://www.ncbi.nlm.nih.gov>). *Callicarpa nudiflora* was used as an outgroup. Alignments were performed by MAFFT v7.486 (Kato and Standley 2013), and a maximum likelihood tree was constructed using IQ-TREE (Nguyen et al. 2015) with the best-predicted model TVM + F + R3 and 1000 bootstrap replicates. Bayesian reference were conducted by MrBayes (Ronquist et al. 2012) (v 3.2.7).

Results

The size of the chloroplast genome of *S. chienii* was 151,530 bp with a typical circular structure and a GC content

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Figure 1. Image of *S. chienii* species. Yukun Wei collected the image in Tangkou Town, Huangshan, Anhui, China (GPS: E114°04'03.15", N31°49'46.46"). The leaves of *S. chienii* are long oval with serrated edges. Verticillasters form extended racemes with lavender flowers.

of 38% (Figure 2). It consisted of a large single copy region (LSC) of 82,800 bp, a small single copy region (SSC) of 17,574 bp, and two inverted repeating regions (IR) of 25,578 bp. A total of 143 genes were annotated in the chloroplast genome of *S. chienii*, including 91 protein-coding genes, eight rRNA genes, and 37 tRNA genes. Among these genes, seven protein-coding (*ndhB*, *rpl2*, *rpl23*, *rps12*, *rps7*, *ycf15* and *ycf2*), seven tRNA (*trnA-UgC*, *trnI-Cau*, *trnI-Gau*, *trnL-caa*, *trnN-guu*, *trnR-ACG* and *trnV-GAC*) and four rRNA genes (*rrn5*, *rrn4.5*, *rrn23*, *rrn16*) have two copies. Totally 11 cis-splicing genes including *rps16*, *atpF*, *rpoC1*, *pafl*, *clpP1*, *petB*, *petD*, *rpl16*, *rpl2*, *ndhB* and *ndhA* (Figure S2), and one trans-splicing genes *rps12* (Figure S3) were detected by CPGview (Liu et al. 2023) (<http://www.1kmpg.cn/cpgview/>).

IQTree conducted the phylogenetic analysis with 24 chloroplast genomes (Figure 3). As shown in the figure, six clades were generated in the phylogenetic tree, consisting of *Salviinae*, *Menthinae*, *Elsholtzieae*, *Ocimeae* and outgroup. Our results showed that *Salviinae* had two subgroups (*S. miltiorrhiza* subgroup and *S. officinalis* subgroup). The separation time of *S. officinalis* subgroup was earlier than *S. miltiorrhiza* subgroup. The clustering results supported that *S. chienii* was

more closely related to *S. miltiorrhiza*, as the two species were clustered together under *S. miltiorrhiza* subgroup.

Discussion

Salvia species belongs to multi-lineage evolution, but the relationship between the lineages is still unclear. The evolutionary relationships of *Salvia* species are frequently investigated using phylogenetic analysis. Our phylogenetic tree showed that there are two subgroups of *Salvia* species; *S. miltiorrhiza* subgroup which comprises *Salvia* species in East Asia and *S. officinalis* subgroup which comprises *Salvia* species in Europe. The divergence between the two subgroups is consistent with what was previously reported (Hu et al. 2018). *S. miltiorrhiza* group has two core subgroups, which suggests the evolutionary relationships of *Salvia* species in Eastern Asia. However, relationships within *S. miltiorrhiza* group remain unclear (Hu et al. 2018). *S. chienii* is clustered with *S. miltiorrhiza*, which will provide valuable information to explore the lineage of *Salvia* species in Eastern Asia.

Ethical approval

Research and collection of plant material were conducted according to the guidelines provided by Zhejiang Sci-Tech University. The plant was collected with special permission from Shanghai Chenshan Botanical Garden. This article does not contain any studies with endangered or protected species performed by any of the authors. Experiments were performed following the recommendations of the Ethics Committee of Zhejiang Sci-Tech University.

Authors' contributions

The experiments were conceived and organized by Dongfeng Yang and Yukun Wei. Ying Cheng was involved in the design, data analysis, and paper drafting. Fuhai Yuan and Yukun Wei contributed to the collection of plant material. The paper was written by Ying Cheng and Dongfeng Yang, and revised by Fuhai Yuan and Yelong Sheng, Dongfeng Yang, Ann Abozeid and Yanbo Huang. All authors agree to be accountable for all aspects of the final manuscript.

Disclosure statement

The authors report no conflicts of interest. Fuhai Yuan and Yelong Sheng are the employee of Changzhou Menghe Shuangfeng Chinese Herbal Medicine Technology, and they provided the funding of Science and Technology Support Program (Agriculture) of Changzhou Jiangsu Province (CE20212032) for the study. The authors alone are responsible for the content and writing of the paper.

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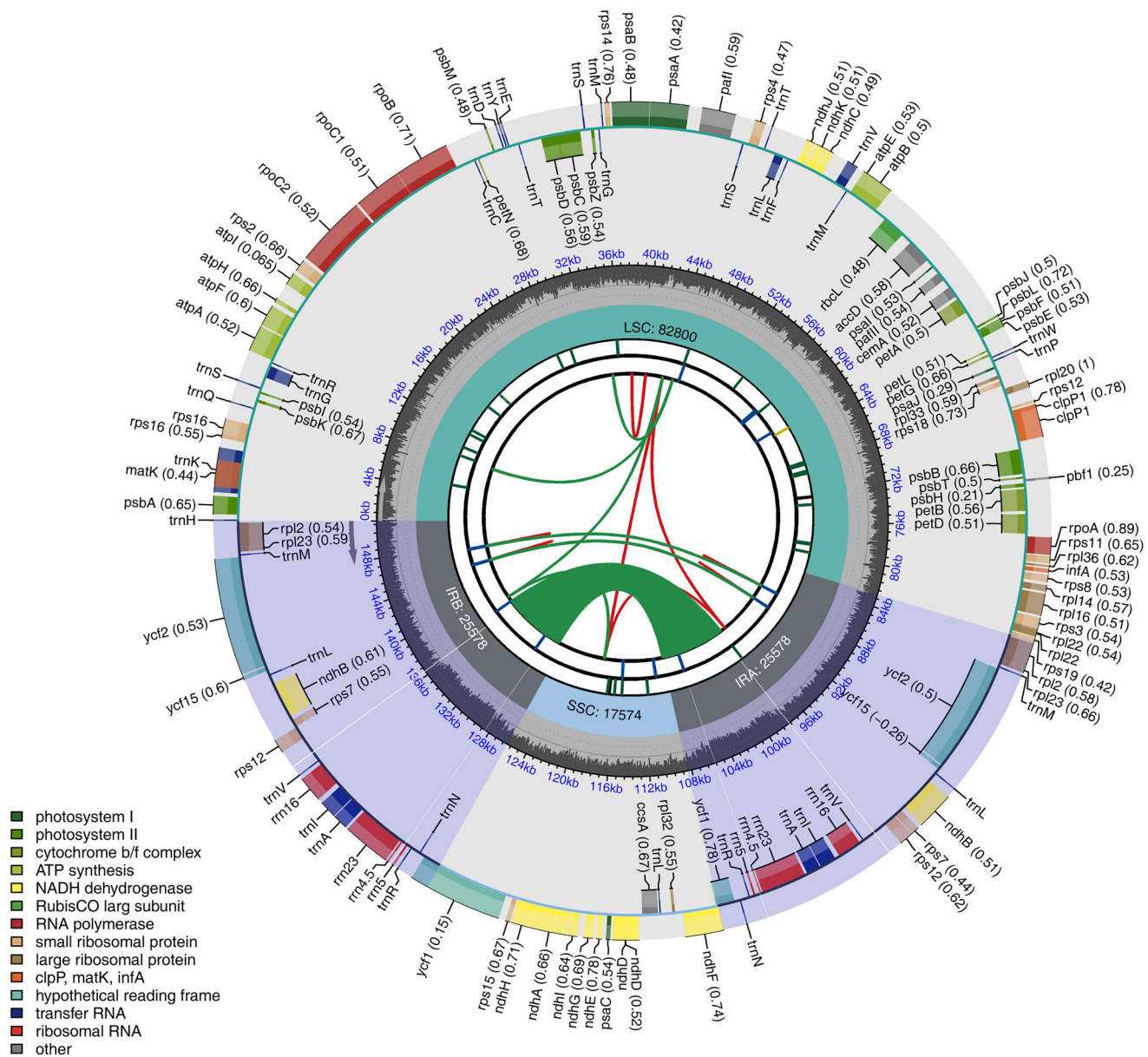


Figure 2. The chloroplast genome map of *S. chienii* chloroplast genome. The length of genome is presented in the inner circle. Large single copy (LSC), Single short copy (SSC) and inverted repeat regions (IRA and IRB) are marked. A total of 143 annotated genes are presented in the outer circle, consisting of 91 protein-coding genes, eight rRNA genes, and 37 tRNA genes. Genes were classified by their function in colors. From the center outward, the first track shows the dispersed repeats. The dispersed repeats consist of direct (D) and Palindromic (P) repeats, connected with red and green arcs. The second track shows the long tandem repeats as short blue bars. The third track shows the short tandem repeats or microsatellite sequences as short bars with different colors. The colors, the type of repeat they represent, and the description of the repeat types are as follows. Black: c (complex repeat); Green: p1 (repeat unit size = 1); Yellow: p2 (repeat unit size = 2); Purple: p3 (repeat unit size = 3); Blue: p4 (repeat unit size = 4); Orange: p5 (repeat unit size = 5); Red: p6 (repeat unit size = 6). The small single-copy (SSC), inverted repeat (IRA and IRB), and large single-copy (LSC) regions are shown on the fourth track. The GC content along the genome is plotted on the fifth track. The genes are shown on the sixth track. Genes are color-coded by their functional classification. The functional classification of the genes is shown in the bottom left corner.

Data availability statement

The genome sequence data supporting this study's findings of this study are openly available in GenBank of NCBI (<https://www.ncbi.nlm.nih.gov>) under accession no. OK094518. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA763313, SRR15901094, and SAMN21437815, respectively.

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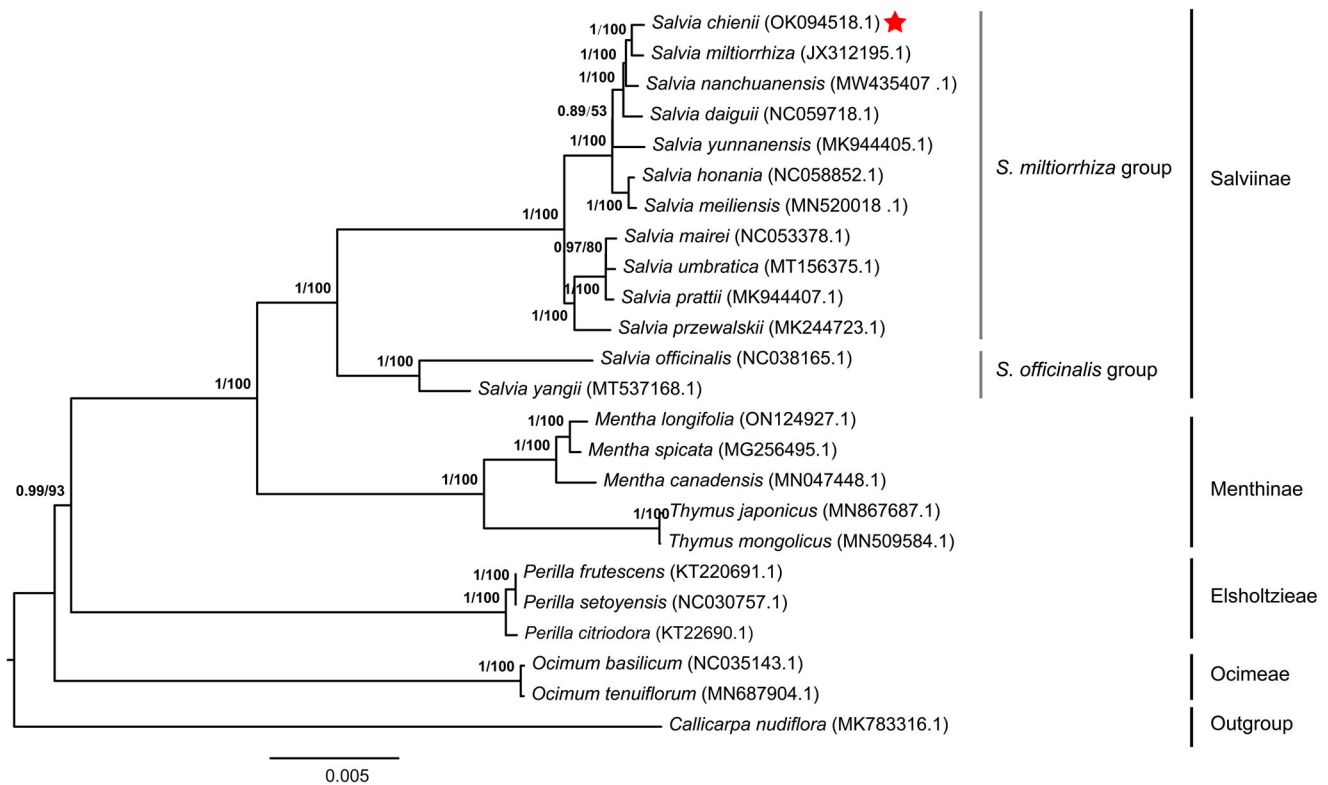


Figure 3. Phylogeny of Lamiaceae based on complete chloroplast genomes, accession numbers were listed behind each taxon. Statistical support values were shown on nodes. The complete chloroplast genomes used for constructing the phylogenetic tree contains *S. miltiorrhiza* (Hu et al. 2020) (JX312195.1), *Callicarpa nudiflora* (Wang et al. 2019) (MK783316.1), *Mentha canadensis* (Huaizhu et al. 2019) (MN047448.1), *Mentha longifolia* (Zubair Filimban et al. 2022) (ON124927.1), *Mentha spicata* (Wang et al. 2017) (MG256495.1), *Ocimum basilicum* (Rabah et al. 2017) (NC035143.1), *Ocimum tenuiflorum* (Kavya et al. 2021) (MN687904.1), *Perilla citriodora* (Mo et al. 2017) (KT22690.1), *Perilla frutescens* (Shen et al. 2016) (KT220691.1), *Perilla setoyensis* (NC030757.1; Unpublished), *Salvia daiguii* (Zhou et al. 2020) (NC059718.1), *Salvia honania* (Wang et al. 2022) (NC058852.1), *Salvia mairei* (NC053378.1; Unpublished), *Salvia meliensi* (Su et al. 2022) (MN520018.1), *Salvia nanchuanensis* (Su et al. 2022) (MW435407.1), *S. officinalis* (Du et al. 2022) (NC038165.1), *Salvia prattii* (MK944407.1; Unpublished), *Salvia przewalskii* (Du et al. 2019) (MK344723.1), *Salvia umbratica* (MT156375.1; Unpublished), *Salvia yangjii* (Cao et al. 2020) (MT537168.1), *Salvia yunnanensis* (Tao et al. 2019) (MK944405.1), *Thymus japonicus* (Kim et al. 2020) (MN867687.1), *Thymus mongolicus* (Huaizhu et al. 2020) (MN509584.1). The type of sequences used for building the phylogenetic tree are the complete chloroplast genomes downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/>), and built with IQTree with the best predicted model TVM + F + R3 and 1000 bootstrap replicates. Bayesian posterior probabilities and bootstrap scores were present on the tree nodes. Bayesian posterior probabilities were calculated by MrBayes (v3.2.7). *S. chienii* is close to *S. miltiorrhiza*.

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