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The complete chloroplast genome of *Scutellaria barbata* D. Don 1825 revealed the phylogenetic relationships of the *Scutellaria* genus

Shouhui Pan*, Xiquan Li*, Li Zhang and Quan Zhang

Guizhou Tobacco Corporation Anshun Company, Anshun, People's Republic of China

ABSTRACT

Scutellaria barbata D. Don 1825 is an important medicinal plant distributed in wetlands about 2000 m above sea level and used to treat various diseases. The complete chloroplast genome of *S. barbata* is 152,050 bp with four subregions consisting of a large single-copy region (84,053 bp), a small single-copy region (17,517 bp), and a pair of inverted repeats (25,240 bp). In the chloroplast genome of *S. barbata*, 131 genes were detected, comprising 87 protein-encoding genes, eight ribosomal RNA (rRNA) genes, and 36 transfer RNA (tRNA) genes. Phylogenetic analysis based on the complete chloroplast genome and protein-coding DNA sequences of 27 related taxa of the genus (out group included *Holmskioldia sanguinea* and *Tinnea aethiopica*) indicates that *S. barbata* was made a clade with *S. orthocalyx*, and *S. meehanioides* was a sister to them. The first chloroplast genome of *S. barbata* was reported in this work, serving as a potential reference for important medicinal plants within the *Scutellaria* genus.

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Introduction

Scutellaria barbata D. Don is a perennial herb that often grows on paddy fields, streams, or wet grasslands, with an altitude below 2000 m. The genus *Scutellaria* was classified into five distinct sections, namely *Scutellaria* (Rech.) Paton, *Anaspis* (Rech.) Paton, *Salazaria* (Torrey) Paton, *Perilomia* (Kunth) Epling emend. Paton, and *Salviifoliae* (Boiss.) Edmondson (Ranjbar and Mahmoudi 2013). There are more than 300 species of plants of *Scutellaria* Linn. of Labiatae in angiosperms (Bruno et al. 2002), distributed worldwide. The Irano-Turanian region, specifically Central Asia and Afghanistan, represents the primary hub of maximal diversity for the genus *Scutellaria*. Additionally, the Eastern Mediterranean and the Andes serve as secondary centers for its speciation. In China, *Scutellaria* plants have been used for clearing away heat and toxic materials, inducing diuresis, and treating hepatitis, appendicitis, and traumatic injury, which has been used since 2000 years ago (Shen et al. 2021). *S. barbata* is a traditional Chinese medicine, and its chloroplast genome has not been published, which leads to its systematic genetic location being unclear. For this reason, we constructed a high-quality assembled chloroplast to enhance the molecular investigation of germplasm, genetic diversity, and phylogenetic relationships.

Materials and methods

Fresh leaf samples of *S. barbata* D. Don were collected from Mountain Jinyun, Chongqing (Geospatial coordinates: N29.83, E106.39). The original plant was identified as *S. barbata* by Professor Quan Zhang and the pictures of the original plants were taken by ourselves (Figure 1). Total DNA was isolated from fresh leaves of the species using a Plant Genomic DNA kit (Tiangen Biotech, Beijing, China). The genome sequence was performed on the Hiseq 2500 platform (Illumina, San Diego, CA). A specimen was deposited at the Guizhou Tobacco Company Anshun Tobacco Company in Guizhou (Prof. Quan Zhang, E-mail: zqtobacco2023@163.com) under the voucher number 202210122.

The raw data, consisting of 5.80 GB of raw data, comprised a total of 19,200,779 reads. These reads were subsequently utilized in the assembly of a chloroplast genome, employing the NOVOPlasty (version 2.7.2) (Dierckxsens et al. 2017). Annotation was performed using CPGAVAS2 (Shi et al. 2019). The genome sequence was confirmed by aligning all raw reads against the assembled genome using BWA v0.7.17 and SAMtools v1.9 (WGS500 Consortium 2014) in the environment of Genome Information System (GeIS; https://geis.infoboss.co.kr/).

To explore the phylogenomic relationship of *Scutellaria*, 26 complete chloroplast genome sequences were downloaded,

CONTACT Quan Zhang zqtobacco2023@163.com Guizhou Tobacco Company Anshun Tobacco Company, No. 31 Zhonghua South Road, Anshun, Guizhou 561000, People's Republic of China
*Both authors contributed equally to this work.

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Figure 1. Photographs of *Scutellaria barbata* D. Don. (These photographs were taken by Prof. Quan Zhang). The foliage of *S. barbata* exhibits triangular, ovate, or ovate-lanceolate shapes, characterized by a sharp apex and a broad wedge-shaped or nearly truncated base. The raceme of this species is inconspicuous and positioned terminally. The lower bracts are elliptic or narrowly elliptic, while the bracteoles take the form of needle-shaped structures. Furthermore, the corollas of *Scutellaria barbata* display a vivid purple-blue coloration. (A) Plant panorama of *S. barbata* and (B) the flowers of *S. barbata*.

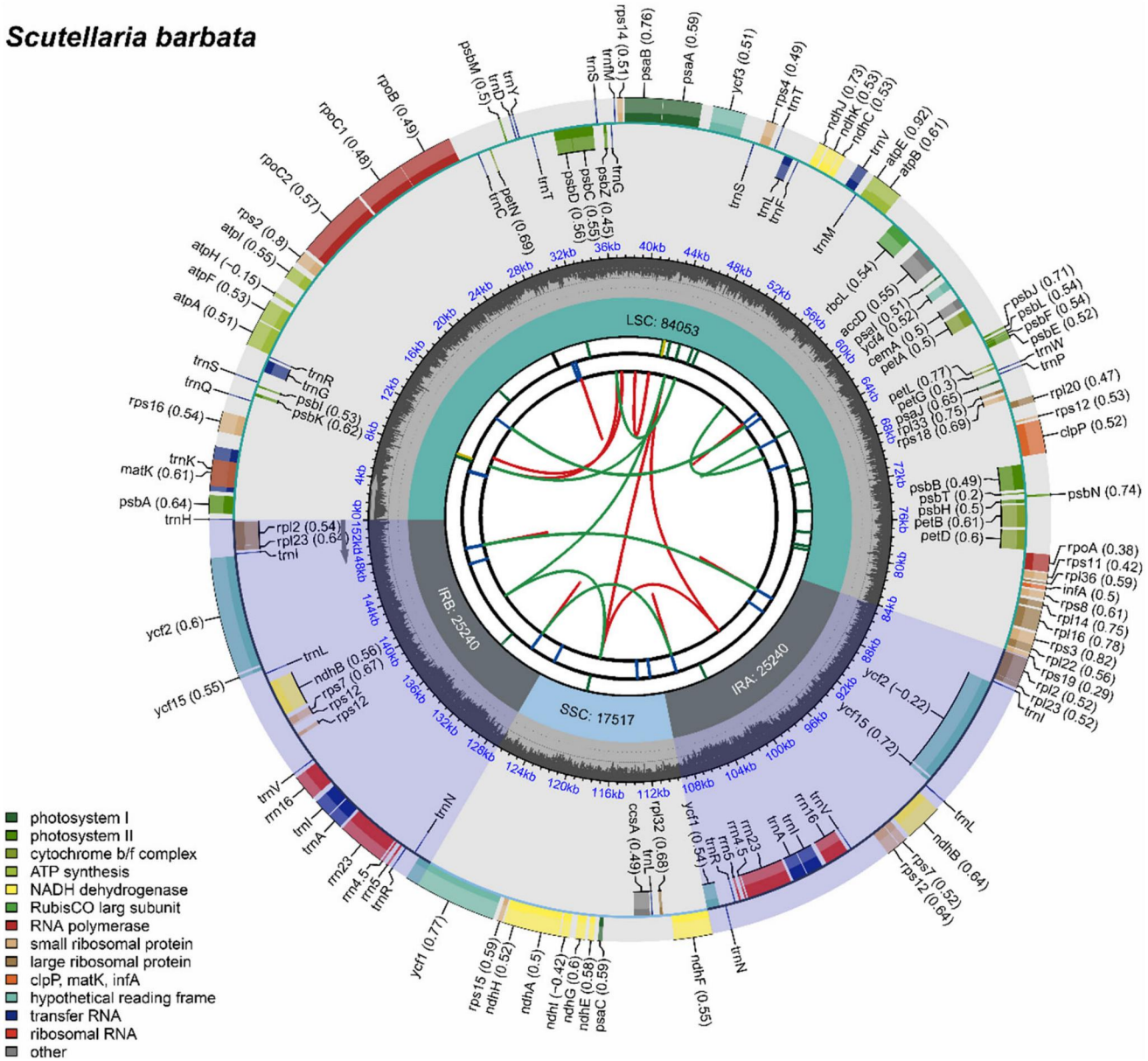

Figure 2. The circle map of chloroplast genome map of *S. barbata*. Distinctive colored boxes encircling the outer circle depict genes, with clockwise and counterclockwise transcribed genes represented inside and outside the circle, respectively. The inner circle features a gray region indicating the GC content, while the quadripartite structure (LSC, SSC, IRA, and IRB) is illustrated on the inner circle accordingly.

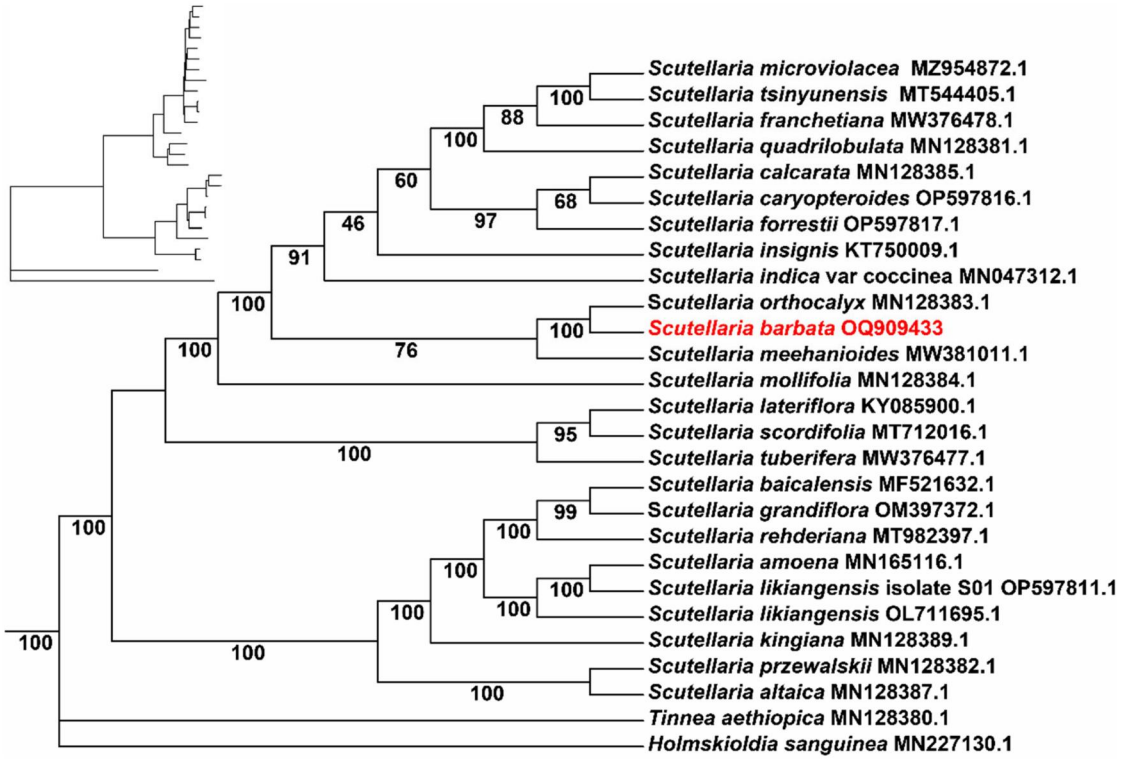

Figure 3. The maximum-likelihood phylogenetic tree of 25 *Scutellaria* species was constructed based on the CDS sequences extracted by IQ-TREE, with *Tinnea aethiopica* and *Holmskioldia sanguinea* added as outgroup. The phylogenetic tree was constructed using the maximum-likelihood method (ML) and bootstrap was performed 1000 times. The number on each branch indicates the boot support value. The following sequences were used: *S. microviolacea* MZ954872.1 (Wang et al. 2022), *S. tsinyunensis* MT544405.1 (Shan et al. 2021), *S. franchetiana* MW376478.1, *S. calcarata* MN128385.1 (Zhao et al. 2020), *S. quadrilobulata* MN128381.1 (Zhao et al. 2020), *S. caryopteroides* OP597816.1, *S. forrestii* OP597817.1, *S. insignis* KT750009.1, *S. indica* var coccinea MN047312.1 (Lee and Kim 2019), *S. orthocalyx* MN128383.1 (Zhao et al. 2020), *S. meehanioides* MW381011.1 (Zhang et al. 2021), *S. mollifolia* MN128384.1 (Zhao et al. 2020), *S. lateriflora* KY085900.1, *S. scordifolia* MT712016.1, *S. tuberifera* MW376477.1 (Shan et al. 2021), *S. baicalensis* MF521632.1 (Jiang et al. 2017), *S. grandiflora* OM397372.1, *S. rehderiana* MT982397.1, *S. amoena* MN165116.1 (Chen and Zhang 2019), *S. likiangensis* isolate S01 OP597811.1, *S. likiangensis* OL711695.1, *S. kingiana* MN128389.1 (Zhao et al. 2020), *S. przewalskii* MN128382.1 (Zhao et al. 2020), *S. altaica* MN128387.1 (Zhao et al. 2020), *T. aethiopica* MN128380.1 (Zhao et al. 2020), and *H. sanguinea* MN227130.1 (Lee and Kim 2020).

including two outgroup plants. The common genes of 27 chloroplast genomes (including *S. barbata*, and out group were *H. sanguinea* and *T. aethiopica*) were extracted and concatenated with Phylosuite (Zhang et al. 2020). These sequences were aligned using MAFFT (v7.450) (Rozewicki et al. 2019), and a phylogenetic tree was constructed using the alignment and ML method implemented in IQtree (Trifinopoulos et al. 2016).

Results

The average and minimum read mapping depths of the assembled genome were 2099× and 540×, respectively (Figure S1). A circular map of the chloroplast genome and a schematic map of the cis- and trans-splicing genes (Figure 2 and FigureS2) were visualized by CPGView (Liu et al. 2023). The total length of the chloroplast genome of *S. barbata* (GenBank accession number: NC_059814.1) is 152,050 bp with 38.37% GC content (Figure 2). The chloroplast genome of *S. barbata* is a typical quadratic structure containing a pair of inverted repeats (IRs) of 25,240 bp separated by a large single-copy (LSC) region of 84,053 bp and a small single-copy (SSC) region of 17,517 bp. The chloroplast genome included 131 genes, of which 112 are unique genes, including 87 protein-coding genes, eight ribosomal RNA (rRNA), and 36 transfer RNA (tRNA) genes. Nineteen protein-coding genes had one intron, and four had two introns. The phylogenetic analysis revealed that *S. barbata* was taxonomically classified within the genus *Scutellaria*. Furthermore, it was observed that *S. barbata* formed a monophyletic clade together with *S. orthocalyx*, while *S. meehanioides* was found to be their sister taxon (Figure 3). The chloroplast genome sequence of *S. barbata* is a valuable resource for genome evolution and taxonomy research of the genus *Scutellaria*.

Discussion and conclusions

The complete chloroplast genome of *S. barbata* was first sequenced and found to exhibit a total length of 152,050 bp. The genome size and gene content of *S. barbata* are not significantly different from those of most chloroplast genomes or plastomes in the genus *Scutellaria* (Chen 2019; Shan et al. 2021). Our research results could be used for authenticating *S. barbata* and analyzing the genetic diversity and phylogenetic relationships in the genus *Scutellaria*. Although the species of the genus *Scutellaria* are very rich, the *Scutellaria* plant sequence is still relatively few in NCBI (latest date: 25 May 2023). Therefore, to further study the evolutionary history of *S. barbata*, a more complete *Scutellaria* species chloroplast sequence is required. The unveiling of the chloroplast

genome sequence of *S. barbata* will provide meaningful information for the phylogeny and plant molecular identification of *Scutellaria* species.

Author contributions

Shouhui Pan: drafting the work and revising it critically for important intellectual content. Xiquan Li and Li Zhang: analyzed and interpretated the data for the work. Quan Zhang: final check, revision, and approval of the version to be published. Authors agree to be accountable for all aspects of the work.

Ethical approval

The authors declare no ethical or legal violations when obtaining the study materials and performing the research. The species used in this study is not listed on the IUCN Red List, and the sample was legally collected by guidelines stipulated in national and international regulations. The materials were collected in a location not designated as a protected area in China.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The genome sequence data supporting the findings of this study can be publicly obtained at NCBI GenBank in https://www.ncbi.nlm.nih.gov with the accession number OQ909433. Associated BioProject, SRA, and BioSample numbers are PRJNA947595, SRR23952789, and SAMN33861697.

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