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PLASTOME REPORT

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The complete plastome of *Saxifraga giraldiana* Engler and its phylogenetic analysis

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ABSTRACT

Saxifraga giraldiana Engler is a common subalpine and alpine plant belonging to Saxifragaceae. However, the genetic diversity of this species has remained to be explored. In this study, we have assembled and characterized the complete chloroplast genome of *S. giraldiana*, filling this knowledge gap and uncovering its genetic composition. The chloroplast genome is 147,267 bp long and contains 131 genes, including 85 protein-coding genes, 38 tRNA genes, and eight rRNA genes. Furthermore, we have performed a phylogenetic analysis of 19 representative species within *Saxifraga*. As a result, we have found that *S. giraldiana*, together with *S. implicans* and *S. stellariifolia*, forms a monophyletic group. These findings have implications for the conservation and utilization of *S. giraldiana*.

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Introduction

Saxifraga giraldiana Engler, 1901, is a perennial herb within the Saxifragaceae family. It exhibits distinctive characteristics during its flowering phase, notably the withering of basal and lower stem leaves, petals yellow, brown spotted, ovate or elliptic to oblong. This species thrives predominantly in understory habitats, alpine meadows, and mountain crevices at altitudes of 1000–4000 m (Wang et al. 2001). Morphologically, *S. giraldiana* was considered closely related to *Saxifraga stellariifolia*, primarily distinguished by sepals (Wang et al. 2001; Yuan, Li, et al. 2023).

However, relying solely on morphology for species differentiation presents challenges, urging the need for molecular data. Despite the transformative impact of high-throughput sequencing in phylogenetics (Li et al. 2022), the study on the chloroplast genome of *S. giraldiana* has been notably absent. Besides, many *Saxifraga* species are distributed in alpine regions, but genetic data are still lacking for some species, including *S. giraldiana*, hindering a comprehensive understanding of the phylogenetic position of the species, the phylogenetic relationships within the taxon, and the biogeographic history (Tkach et al. 2015). To fill this gap, this study assembled and characterized the complete chloroplast genome of *S. giraldiana*. This work can lay the foundation for future research on the evolution, protection, and potential medicinal applications of *S. giraldiana*.

Materials and methods

Fresh leaves for sequencing were harvested on 22 August 2018, from Xiling Snow Mountain, Dayi County, Chengdu City, Sichuan Province, China (104.716666 E, 30.702516 N) (Figure 1). The specimen, with the identification number QL-SC01-01, has been meticulously preserved in the herbarium of Sichuan University, with Xiao-Ting Xu (xiaotingxu@sc.edu.cn) serving as the contact person. The genomic DNA extraction followed the modified CTAB method (Porebski et al. 1997) and the quality was assessed using the Qubit 2.0 system. Then, the total genomic DNA was fragmented and subjected to paired-end sequencing with fragment length of 150 bps on the Illumina high-throughput sequencing platform NovaSeq 6000. Following quality control procedures, approximately 4.82 GB of clean paired-end reads were assembled using NOVOPlasty (v.2.7.1) (Dierckxsens et al. 2017). The annotation of the complete circular chloroplast genome was conducted by CPGAVAS2 (Shi et al. 2019) with reference to the *S. stellariifolia* chloroplast genome (NC_070511.1). Manual adjustments were performed using Geneious Primer (v2021.1.1.0). Finally, the annotated chloroplast genome was submitted to GenBank using Bankit and was assigned the accession number *OR948604*. The circular genome mapping of the newly sequenced chloroplast genome was illustrated using the CPGview (Liu et al. 2023) online tool. To ascertain the phylogenetic relationships of *S. giraldiana*, the chloroplast genomes of 19 additional

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Figure 1. Field photos of *S. giraldiana* shot the photo at the position of 104.716 E, 30.702 N. These photos were taken by Dr. Lei Zhang with the author's approval for use. It exhibits distinctive characteristics during its flowering phase, notably the withering of basal and lower stem leaves, petals yellow, brown spotted, ovate or elliptic to oblong.

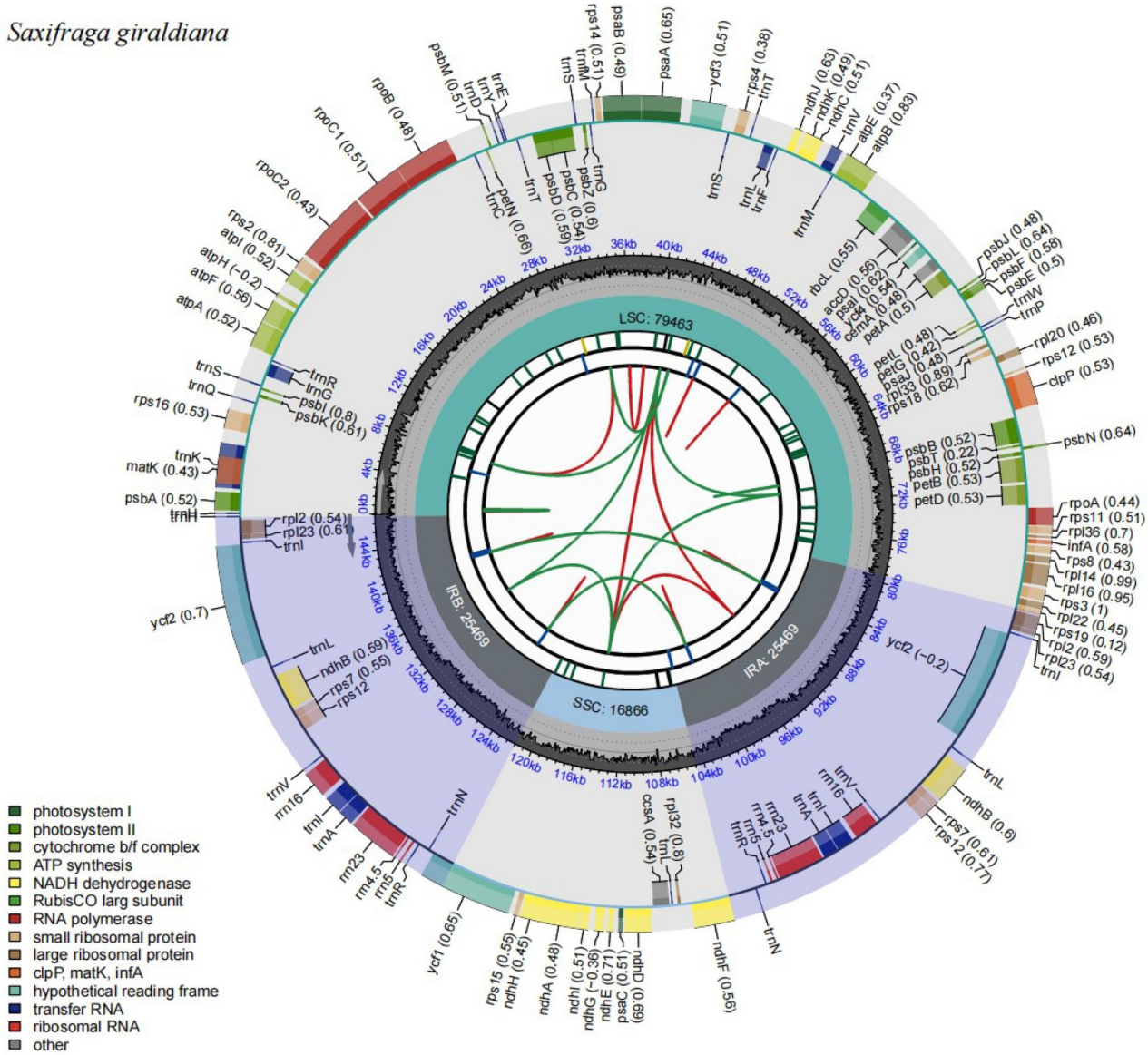

Figure 2. The complete chloroplast genome map of *S. giraldiana* generated using CPGview. The map comprises six tracks. Dispersed repeats at the center, then short blue bars for long tandem repeats. Various-colored bars for microsatellite sequences in the third track. The fourth track is for small single-copy (SSC), inverted repeat (IRa and IRb), and large single-copy (LSC) regions. The fifth track illustrates GC. The sixth showcases genes, genes with inner ones in a clockwise and outer ones in an anticlockwise direction. Functional gene classifications are shown in the bottom left corner.

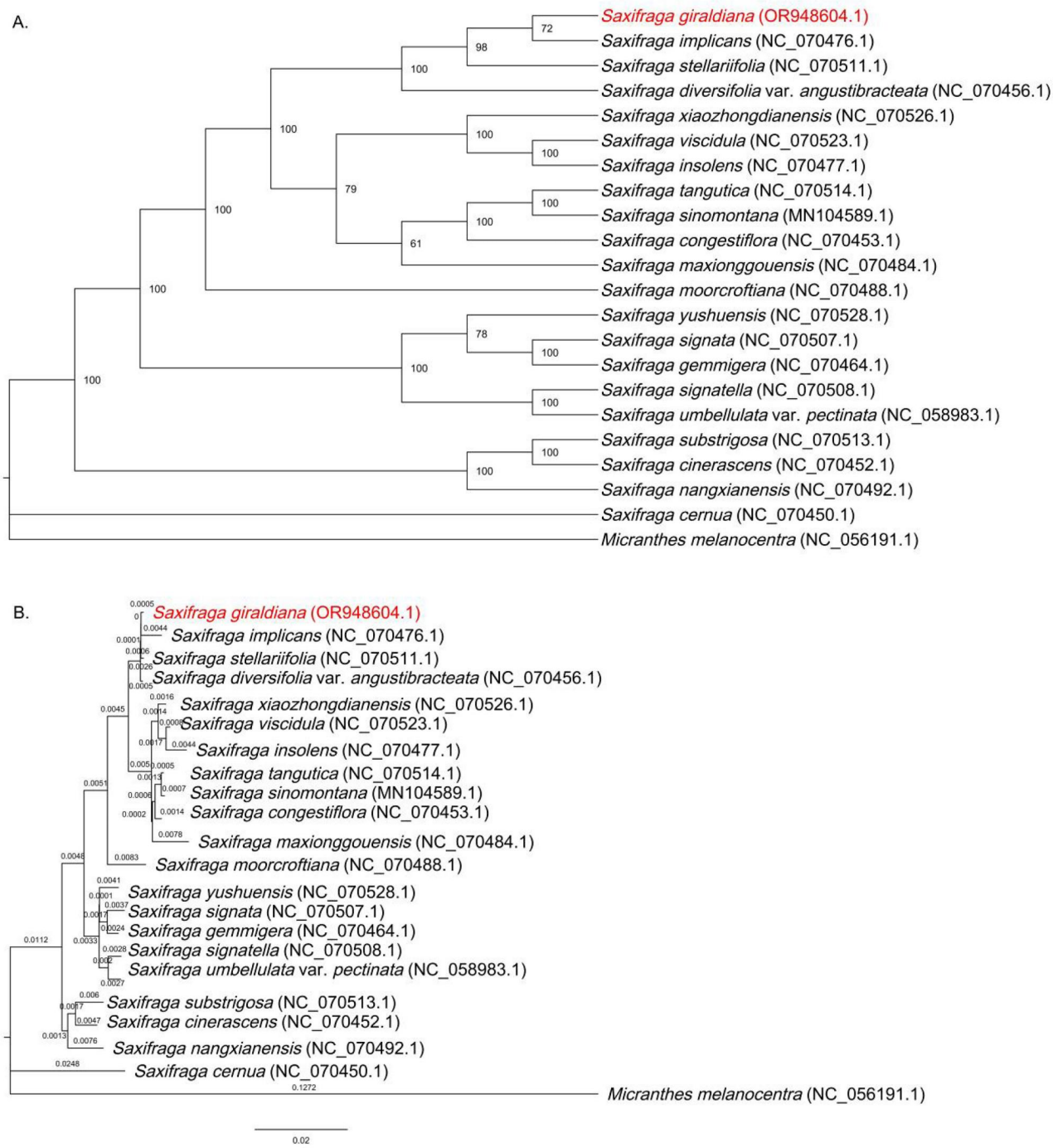

Figure 3. Maximum-likelihood (ML) phylogenetic tree of *S. giraldiana* (red font) and 19 *Saxifraga* species. *Micranthes melanocentra* was used as outgroup taxa. The bootstrap support values are shown at the branches in the cladogram tree (A). The corresponding phylogram tree is shown in panel B. GenBank accession number of each species was shown in brackets. The corresponding phylogram tree is shown in panel B. Sequences used for tree construction were as follows: *S. giraldiana* (OR948604.1, this study), *Saxifraga implicans* (NC_070476.1, Yuan, Ma, et al. 2023), *Saxifraga stellariifolia* (NC_070511.1, Yuan, Ma, et al. 2023), *Saxifraga diversifolia* var. *angustibracteata* (NC_070456.1, Yuan, Ma, et al. 2023), *Saxifraga xiaozhongdianensis* (NC_070526.1, Yuan, Ma, et al. 2023), *Saxifraga viscidula* (NC_070523.1, Yuan, Ma, et al. 2023), *Saxifraga insolens* (NC_070477.1, Yuan, Ma, et al. 2023), *Saxifraga tangutica* (NC_070514.1, Yuan, Ma, et al. 2023), *Saxifraga sinomontana* (MN104589.1, Li et al. 2019), *Saxifraga congestiflora* (NC_070453.1, Yuan, Ma, et al. 2023), *Saxifraga maxionggouensis* (NC_070484.1, Yuan, Ma, et al. 2023), *Saxifraga moorcroftiana* (NC_070488.1, Yuan, Ma, et al. 2023), *Saxifraga signata* (NC_070507.1, Yuan, Ma, et al. 2023), *Saxifraga gemmigera* (NC_070464.1, Yuan, Ma, et al. 2023), *Saxifraga signatella* (NC_070508.1, Yuan, Ma, et al. 2023), *Saxifraga umbellulata* var. *pectinata* (NC_058983.1), *Saxifraga substrigosa* (NC_070513.1, Yuan, Ma, et al. 2023), *Saxifraga cinerascens* (NC_070452.1, Yuan, Ma, et al. 2023), *Saxifraga nangxianensis* (NC_070492.1, Yuan, Ma, et al. 2023), *Saxifraga cernua* (NC_070450.1, Yuan, Ma, et al. 2023), and *Micranthes melanocentra* (NC_056191.1).

Saxifraga species were downloaded from GenBank, including the morphological closely related species, *Saxifraga stellariifolia*, *Saxifraga implicans*, and *Saxifraga insolens* (Yuan, Li, et al. 2023). Another species in the sister branches of *Saxifraga*: *Micranthes melanocentra* was chosen as outgroup (Tkach et al. 2015; Yuan, Ma, et al. 2023). The entire chloroplast genome sequence was aligned using MAFFT (v7.487) (Katoh et al. 2002; Katoh and Standley 2013) under default parameters settings. Subsequently, a maximum-likelihood (ML) phylogenetic tree was constructed using IQ-TREE (v2.2.0)

(Minh et al. 2020) using the best-fit model determined by ModelFinder (Kalyaanamoorthy et al. 2017) and 1000 ultrafast bootstrap replicates (Hoang et al. 2018).

Results

The *S. giraldiana* complete chloroplast genome is 147,267 bp long with 38.1% GC content (Figure 2). The genome assembly is highly reliable with an average mapping depth of ×393 (Supplementary Figure 1). The genome has four subregions: a large single-copy (LSC) region (79,463 bp), a small single-copy (SSC) region (16,866 bp) regions, and two inverted repeat (IR) regions of 25,469 bp long each, showing a conserved quadripartite structure. A total of 131 genes are predicted, including 85 protein-coding genes, 38 tRNA genes, and eight rRNA genes (Figure 2). In the *S. giraldiana* chloroplast genome, six unique protein-coding genes (*ndhB*, *rpl*2, *rpl*23, *rps*12, *rps*7, and *ycf*2), six unique tRNA genes (*trnA*, *tnrI*, *trnL*, *trnN*, *trnR*, and *trnV*), and four unique rRNA genes (*rrn*16, *rrn*23, *rrn*4.5, and *rrn*5) are located at the IR regions. Seventeen genes possess two exons each (*rps*16, *atpF*, *ndh*A, two copies of *ndhB*, *petB*, *petD*, *rpl*16, *ropC*1, two copies of *trnA*, *trnG*, two *trnI*, *trnK*, *trnL*, *trnV*), while four genes (*clpP*, two copies of copies of *rps*12, and *ycf*3) comprise three exons. Eleven genes (*rps*16, *atpF*, *rpoC*1, *ycf*3, *clpP*, *petB*, *petD*, *rpl*16, two copies of *ndhB*, and *ndhA*) are cis-splicing genes (Supplementary Figure 2), and the *rps*12 gene is trans-splicing gene (Supplementary Figure 3).

Our phylogenetic analysis, involving representative *Saxifraga* species, reveals that *S. giraldiana* forms a monophyletic group alongside *S. implicans* and *S. stellariifolia* (Figure 3). Notably, *S. giraldiana* and *S. stellariifolia* exhibit extreme morphological similarity (Wang et al. 2001). The bootstrap value for the sister species of *S. girladiana* and *S. implicans* is only 72, and the branch length is significantly shorter compared to other species. These findings contribute valuable insights to the conservation and utilization of *S. giraldiana* and enhance our understanding of the evolution and biogeography of the genus *Saxifraga*.

Discussion and conclusions

This study represents the first sequencing and characterization of the chloroplast genome of *S. giraldiana*, revealing a conserved quadripartite structure with a size of 147,267 bp and 131 predicted genes. Phylogenetic analysis positions *S. giraldiana* in close relation to *S. implicans* and *S. stellariifolia*, forming a monophyletic group with notably short branch lengths. This consistent with previous studies that identify *S. stellariifolia* as a sister species to the clade housing *S. implicans*, supported by RAD sequences, albeit with limited support (Yuan, Li, et al. 2023). The bootstrap value between *S. girladiana* and *S. implicans* for each other's sister species is low, suggesting a minimal level of differentiation in the chloroplast genome's CDS region. This low variation between species may necessitate a more extensive sampling of nuclear genes for accurate phylogenetic analysis. Increasing the density of nuclear gene data could help elucidate phylogenetic relationships between species and facilitate the detection of hybrid introgression within the evolutionary network. Overall, this study contributes valuable insights into the genomics and evolutionary relationships of *S. giraldiana*, emphasizing the need for further research, especially involving nuclear genomes, to enhance understanding of speciation processes and intricate phylogenetic associations among *Saxifraga* species.

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Author contributions

Xiaoting Xu and Xiulian Chi conceived the project, Xin Liang and Luxuan Yang carried out experiments and data analysis. Xin Liang and Xiaoting Xu wrote the manuscript. Xiulian Chi revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethical approval

This study includes no human, animal, or endangered plant samples, and the sampling site was not in the natural reserve. No permissions are needed during the collection of samples.

Disclosure statement

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

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/ under the accession number OR948604. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1051492, SRX22864841, and SAMN38773424, respectively.

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