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PLASTOME REPORT

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The complete chloroplast genome of *Clematis serratifolia* (Ranunculaceae) from Jilin province, China

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ABSTRACT

Clematis serratifolia has high medicinal and ornamental value. In this study, we characterize and report, for the first time, the complete chloroplast genome sequence of *C. serratifolia* based on high-throughput sequence dates. The whole chloroplast genome of *C. serratifolia* is a circular molecule of 159,648 bp in length, consisting of a large single-copy (LSC) region of 79,394 bp, a small single-copy (SSC) region of 18,112 bp, and two inverted repeat (IR) regions of 31,071 bp. The overall GC content of the chloroplast genome is 38%, while that in the LSC, SSC, and IR regions is 36.3%, 31.3%, and 42.1%, respectively. The chloroplast genome of *C. serratifolia* contains 133 genes, including 89 coding genes, 8 ribosomal RNAs, and 36 transfer RNAs. Among them, 14 protein-coding genes have a single intron, and 2 genes have two introns. The phylogenetic analysis showed a close relationship between *C. serratifolia* and *C. heracleifolia*.

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Introduction

Clematis serratifolia Rehd (1910) is a herb that belongs to the Ranunculaceae family and grows in mountain forests at an altitude of 400 m, as well as on roadsides and cobbled fields. It is distributed in the east and central regions of Liaoning and the east region of Jilin (Wang et al. 1980). The seed of this plant species has high economic value because of its seed oil with a high iodine content (Wang et al. 1980). The chloroplast is a semi-autonomous organelle with circular DNA, whose genome has been the focus of molecular evolution and phylogenetic relationships (Clegg et al. 1994; Jansen et al. 2007; Moore et al. 2010). In recent years, the complete chloroplast genomes of Ranunculaceae have been reported to investigate their

Figure 1. The image of *C. serratifolia*. It was taken by Zeliang Lü and holotype specimen was collected from the Fusong County, Baishan, China in may 2021.

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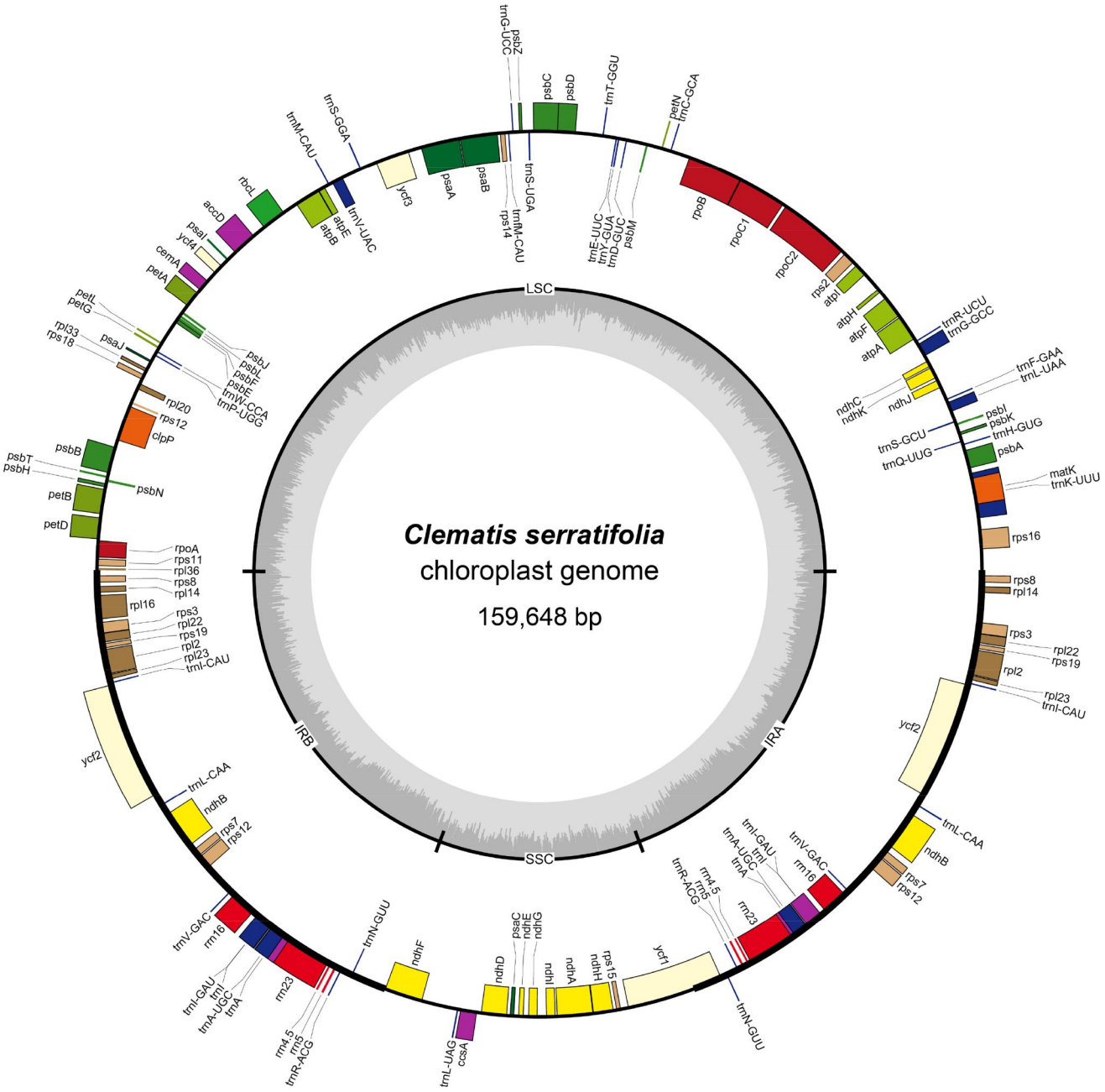

Figure 2. The complete chloroplast genome map of *C. serratifolia*. Color codes represent different functional gene groups. Genes lying inside and outside the outer circle are transcribed clockwise and counterclockwise, respectively. The GC and at content variations are colored darker gray and lighter gray, respectively. The thick lines indicate the extent of the inverted IRa and IRb separated by a large single-copy region (LSC) and small single-copy (SSC) region.

phylogenetic relationships (Liu et al. 2018). However, *Clematis serratifolia's* systematic genetic location is unclear (Yang et al. 2020). For this reason, it is necessary to construct a high-quality assembled chloroplast to understand the relationships between *C. serratifolia* and other Ranunculaceae species at the molecular level. This can provide the foundation for further studies on phylogeny and molecular breeding, as well as offer valuable information regarding the evolution process of *C. serratifolia*.

Materials and methods

Fresh leaves of *C. serratifolia* were collected from Fusong County, Baishan, China (42°20′31.27″ N, 127°16′ 49.30″E) (Figure 1). The voucher specimen was deposited in the Herbarium of the College of Chinese Medicinal Materials, Jilin Agricultural University (https://zhongyao.jlau.edu. cn, Zeliang Lü, lvzeliang@foxmail.com) under voucher number *Y. Cui 2021013* (Figure 1). In this study, genomic DNA was extracted using a plant genome extraction kit (RC1010/301, CONCERT). Then, DNA integrity and concentration were detected using agarose gel electrophoresis and Nanodrop, respectively. High-throughput sequencing was performed using the Illumina Hiseq 2500 (Illumina, USA) with 150 bp paired-end reads. A total of 1.4 Gb raw reads were obtained and assembled into contigs using metaSPAdes (Nurk et al. 2017), taking the reported chloroplast genome of *C. trichotoma*

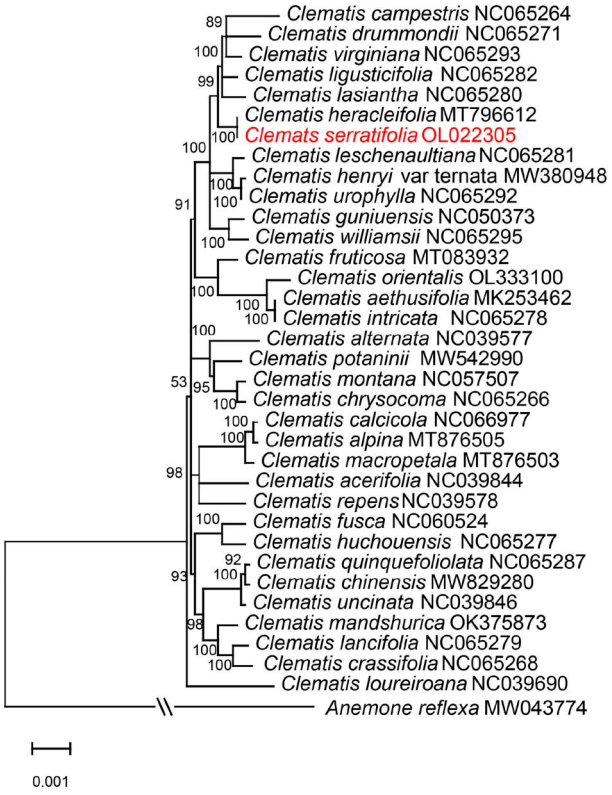

Figure 3. ML phylogenetic tree of the 35 species reconstructed on the basis of the chloroplast genome. Bootstrap support values are shown at the nodes. The following sequence were used: *Clematis serratifolia* (OL022305) (this study), *Clematis campestris* (NC065264), *Clematis drummondii* (NC065271), *Clematis virginiana* (NC065293), *Clematis ligusticifolia* (NC065282), *Clematis lasiantha* (NC065280), *Clematis leschenaultiana* (NC065281), *Clematis urophylla* (NC065292), *Clematis williamsii* (NC065295), *Clematis intricate* (NC065278), *Clematis calcicole* (NC066977), *Clematis chrysocoma* (NC065266), *Clematis alpina* (MT876505), *Clematis macropetala* (MT876503), *Clematis acerifolia* (NC039844), *Clematis fusca* (NC060524), *Clematis huchouensis* (NC065277), *Clematis quinquefoliolate* (NC065287), *Clematis chinensis* (MW829280), *Clematis uncinate* (NC039846), *Clematis lancifolia* (NC065279), *Clematis crassifolia* (NC065268), *Clematis loureiroana* (NC039690) (unpublished), *Clematis heracleifoliab* (MT796612) (Lyu et al. 2021), *Clematis henryi* var *ternate* (MW380948) (Chen et al. 2021), *Clematis guniuensis* (NC050373) (Jiang et al. 2020), *Clematis fruticose* (MT083932) (Yang et al. 2020), *Clematis orientalis* (OL333100) (Cui et al. 2022), *Clematis aethusifolia* (MK253462) (He et al. 2019), *Clematis alternata* (NC039577), *Clematis repens* (NC039578) (Liu et al. 2018), *Clematis potaninii* (MW542990) (Zhang et al. 2022), *Clematis Montana* (NC057507) (Mao et al. 2020), *Clematis mandshurica* (OK375873) (Cui et al. 2022), *Anemone reflexa* (MW043774) (Zhang et al. 2021).

(MG952896) as a reference. Annotation was performed using CPGAVAS2 (Shi et al. 2019), followed by manual correction. The complete annotated chloroplast (cp) genome sequence was submitted to GenBank under accession number OL022305. The gene maps of the complete chloroplast genome and the structure of the genes that were difficult to annotate were generated using ogdraw and CPGVIEW (Greiner et al. 2019; Liu et al. 2023).

To identify the phylogenetic position of *C. serratifolia* within the Ranunculaceae family, 35 cp genome sequences (34 species from *Clematis*, along with 1 species from *Anemone* as an outgroup) were downloaded from GenBank to construct the maximum-likelihood (ML) phylogenetic tree. IQ-tree was used to infer the maximum likelihood (ML) tree with 1000 bootstraps under the GTR + I + G model. The IQ-tree was edited in iTOL (Letunic and Bork 2021).

Results

The Cp genome of *C. serratifolia* assembly data is showed in Table S1. The cp genome of *C. serratifolia* is circular, with a length of 159,648bp, a 75×∼890× depth of coverage (Figure S1), and a typical quadripartite structure, containing a pair of inverted repeat regions (IRs) of 31,071 bp each, which is separated by a large single-copy region (LSC) of 79,394 bp and a small single-copy region (SSC) of 18,112 bp. The overall G + C content of the genome is 38% (36.3% in the LSC region, 31.3% in the SSC region, and 42.1% in the IR regions; Figure 2). A total of 133 genes were predicted in the whole chloroplast genome, consisting of 36 tRNAs, 8 rRNAs, and 89 protein-coding genes. In the genome, 12 protein-coding genes (*atpF*, *ndhA*, two *ndhB*, *petB*, *petD*, two *rpl16*, two *rpl2*, *rpoC1*, and *rps16*) contain an intron. Only, *clpP* and *ycf3* contain two introns (Figure S2). A trans-splicing *rps12* gene was identified (Figure S3). The GTG+I + G model was determined to be the best-fit model based on the Bayesian information criterion (BIC) (Table S2). The phylogenetic trees generated using the ML methods showed a closer relationship between *C. serratifolia* and *C. heracleifolia* (Figure 3). In addition, the nucleotide diversity (Pi) of chloroplast genomes of *C. serratifolia* and *C. heracleifolia* was calculated. Moreover, identifying three genes (*trnG-UCC*, *atpB* and *ycf1*) that could be used as a potential barcoding area would assist further research (Figure S4).

Discussion and conclusion

The cp genome of *C. serratifolia* was sequenced and assembled; this structure is identical to the plastids of the previously reported *Clematis* species (Choi et al. 2021). The phylogenetic relationship of *C. serratifolia* was determined based on the chloroplast genome. Despite showing the close relationship between *C. serratifolia* and *C. heracleifolia*, the matrilineal inheritance of the chloroplast genome could limit evolutionary tree analysis. The nuclear genome and chloroplast genome need to be analyzed together to construct a more accurate evolutionary tree. Therefore, the genomes of more *Clematis* species need to be sequenced in order to determine the complex evolutionary relationships of *C. serratifolia*. In conclusion, this study's results and findings not only provide a potential barcoding area between *C. serratifolia* and *C. heracleifolia*, but are also useful for future phylogenetic studies in the Ranunculaceae family.

Ethics statement

This article does not contain any studies with human participants or animals performed by any of the authors. The study has been performed in accordance with guidelines provided by our institutions and national regulations. We strictly comply with the regulations of the People's Republic of China on the Protection of Wild Plants, the International Union for Conservation of Nature, the Convention on Biological Diversity and the Convention on International Trade in Endangered Species of Wild Fauna and Flora. The sampling site is not located in any protected area. The specie used in this paper is not endangered, protected, or personally owned.

Authors' contribution

Zhongming Han and Yunhe Wang conceptualized and designed research; Lihua Yang and Yi Cui analyzed data and wrote original draft of the manuscript; Zeliang Lü and Qian Wang contributed to research materials and to the draft manuscript. All authors read and approved the final manuscript.

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 (https://www.ncbi.nlm.nih.gov/) under the accession numbers OL022305. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA764578, SRR16609053, and SAMN22567489, respectively.

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