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PLASTOME REPORT

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The complete chloroplast genome of *Pulsatilla chinensis* f. *alba* D. K. Zang (Ranunculaceae, *Pulsatilla* Miller)

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ABSTRACT

Pulsatilla chinensis f. *alba* D. K. Zang 1993 is a forma of *Pulsatilla chinensis* (Bge.) Regel, the root of *P. chinensis* is traditional Chinese medicine called Pulsatillae radix. The biggest difference between *P. chinensis* f. *alba* and *P. chinensis* is that *P. chinensis* f. *alba* sepals is white. The complete chloroplast genome of *P. chinensis* f. *alba* was sequenced using the Illumina NovaSeq platform for the first time. The lengths of the genome, large single-copy (LSC), small single-copy (SSC), two inverted repeats (IRs), and GC content were 163,654 bp, 82,355 bp, 19,069 bp, 31,115 bp, and 37.2%, respectively. It had 134 genes, including 90 protein-coding genes, 36 tRNA genes, and eight rRNA genes. The maximum-likelihood tree indicated that *P. chinensis* f. *alba* had a closer relationship with *P. chinensis*. This study would provide a theoretical basis for the further study of *Pulsatilla* plants genetics phylogenetic research.

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Complete chloroplast genome; phylogenetic tree; *Pulsatilla chinensis* f. *alba* D. K. Zang; Ranunculaceae

Introduction

Pulsatilla chinensis f. *alba* D. K. Zang 1993, a perennial herb of Ranunculaceae, is first discovered in Shandong Province, China, which is the forma of *Pulsatilla chinensis* (Bunge) Regel 1861 (Liang et al. 1993). It was also observed in Liaoning Province of China (Figure 1). *P. chinensis* f. *alba* is different from *P. chinensis* in the color of their sepals. The sepals of *P. chinensis* f. *alba* exhibited a white coloration, while the sepals of *P. chinensis* displayed a violet hue. The dry root of *P. chinensis*, named Pulsatillae Radix, is traditional Chinese medicine included in the Chinese Pharmacopeia (National Pharmacopoeia Commissions 2020). Modern pharmacological research demonstrates that *Pulsatilla* has the traditional antipyretic and antidysentery, anti-microorganism, anti-sucrose enzyme, and antitumor effect (Li and Lin 2005). As a forma of *P. chinensis*, *P. chinensis* f. *alba* is an excellent ornamental flower and an important germplasm resource for cultivating new varieties of *P. chinensis*. In this study, the complete chloroplast genome of *P. chinensis* f. *alba* was sequenced and reported for the first time. It would provide research data for the evolutionary relationships of *Pulsatilla* plants and the cultivation and breeding of *P. chinensis.*

Figure 1. *Pulsatilla chinensis* f. *alba* D. K. Zang. Leaves 4 or 5, not fully expanded at anthesis; densely long pilose; leaf blade broadly ovate; 3-foliolate; margin entire or toothed. Sepals white. Wildlife photos were taken by C.B. in the Chinese city of Liaoyang, Liaoning Province. (E 123°33′09.26″, N 41°42′16.12″).

Materials and methods

Plant material and DNA sequence

We have obtained permission from the local forestry department to collect samples. This study was conducted by the laws of the People's Republic of China. Fresh leaves of *P. chinensis* f. *alba* was collected from Liaoyang, China (E 123°33′09.26″, N 41°42′16.12″), and identified by professor Liang Xu in Liaoning University of Traditional Chinese Medicine. The voucher specimen and genomic DNA were deposited at the herbarium of Liaoning University of Chinese Medicine (Liang Xu 861364054@qq.com, *P. chinensis* f. *alba* number: 10162230517007LY) (Supplemental Figure S1). The genomic DNA was stored in the Key Laboratory of Traditional Chinese Medicine at the University (Dalian, China) (Liang Xu 861364054@qq.com).

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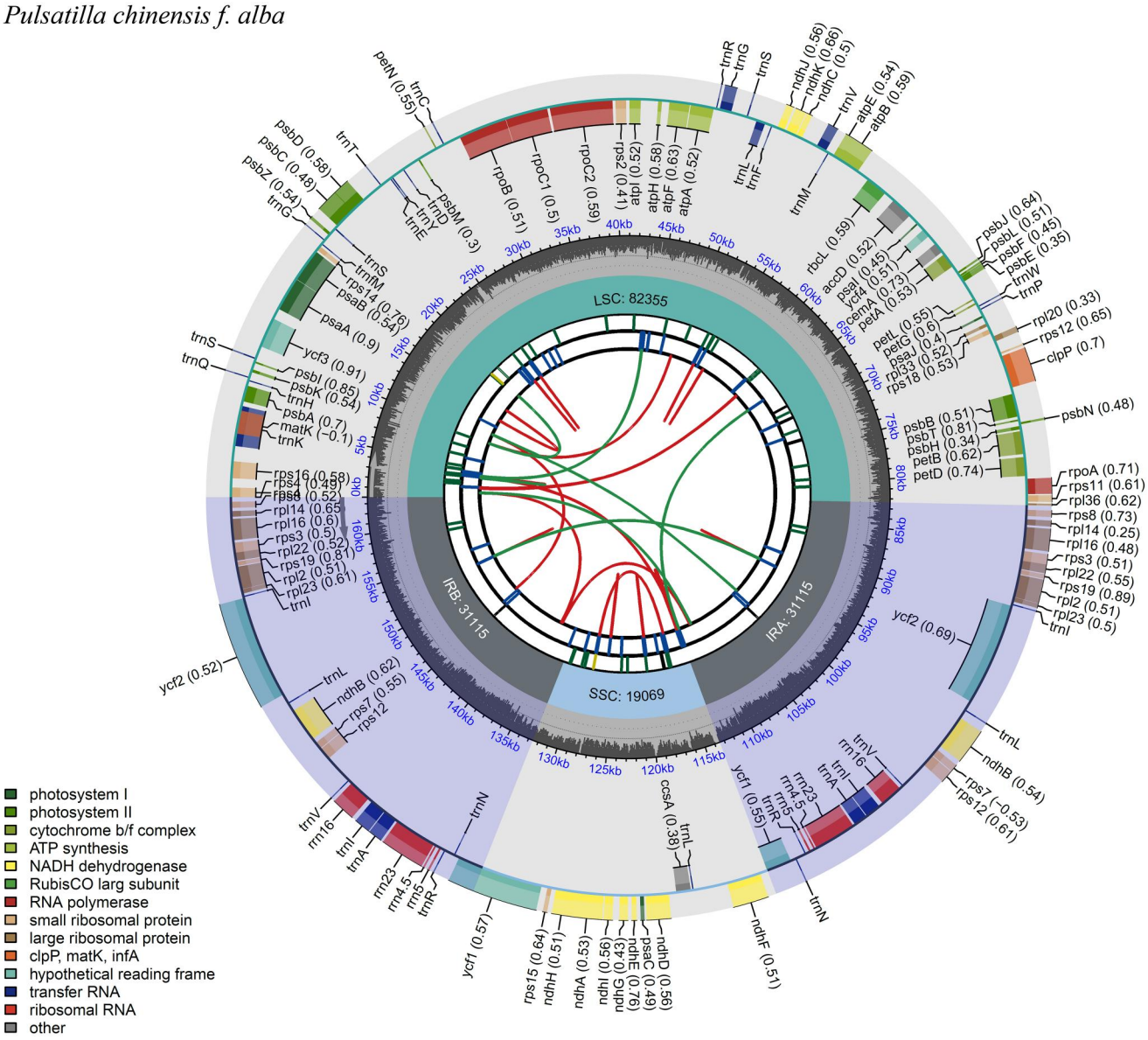

Figure 2. Chloroplast genome map of *Pulsatilla chinensis* f. *alba* D. K. Zang. From the center going outward, the first circle shows the forward and reverse repeats connected with red and green arcs, respectively. The second and third circles show the tandem repeats and microsatellite sequences marked with short bars, respectively. The outer circle shows the gene structure of the chloroplast genome. The genes were colored based on their functional categories, which were shown in the left corner. The map was drawn by cpgview (Liu et al. 2023).

Total genomic DNA was extracted from 150 mg fresh leaves using the cetyltrimethylammonium bromide method (Doyle and Doyle 1987). An aliquot of purified DNA (1 μg) was then fragmented to construct a short-insert (350 bp) library using the Nextera XT DNA library preparation kit (Illumina, San Diego, CA). The library was sequenced using the Illumina NovaSeq 6000 platform, and the coverage was measured using samtools depth.

Genome assembly and annotation

The raw data was edited using the NGS QC Tool Kit v2.3.3 (https://nipgr.ac.in/ngsqctoolkit.html) (Patel and Jain 2015). High-quality sequence data (4.09 G) were then selected for the de novo assembly of the complete chloroplast genome using the assembler SPAdes v3.14.1 (http://cab.spbu.ru/software/spades/) (Bankevich et al. 2012). Finally, the complete chloroplast genome was annotated using PGA (Qu et al. 2019) with the chloroplast genome of *Pulsatilla chinensis* var. *kissii* (MK860683) as a reference. The maps of the chloroplast genome, cis-splicing genes, and trans-splicing genes of *P. chinensis* f. *alba* were processed by CPGview (Liu et al. 2023). The mVista (http://genome.lbl.gov/vista/mvista/submit.shtml) was used to analyze the similarities of *P. chinensis* f. *alba* and four other published *P. chinensis* chloroplast genomes (NC039452, MK860682, MK569491, MK860683) in Shuffle-LAGAN mode with *P. chinensis* (MK860682) as a reference.

Phylogenetic analysis

Phylogenetic tree can be represented by branching diagrams, which illustrate the relationships between similar organisms in a tree-like structure (Feng 2009). To analyze the phylogenetic relationship of *Pulsatilla* genus, 30 other complete

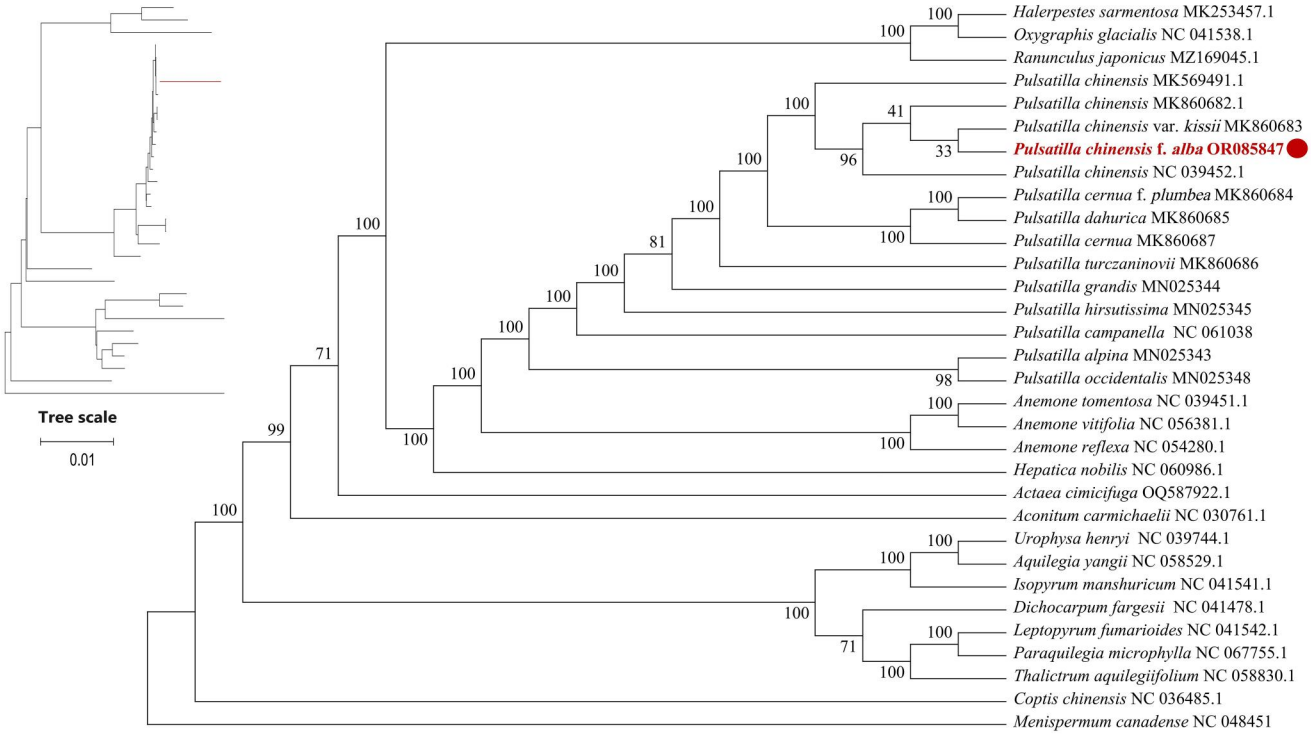

Figure 3. Maximum-likelihood (ML) phylogenetic tree of *P. chinensis* f. *alba* and 31 other complete chloroplast genome sequences. The numbers above the branches indicate the bootstrap values from ML analyses. The best evolutionary model was chosen as JTT + F+R2, which was selected using ModelFinder. The scale bar in the lower left corner of the figure represents the evolutionary distance, with a unit length of 0.01. The following sequences were used: *Halerpestes sarmentosa* (MK253457) (He et al. 2019), *Oxygraphis glacialis* NC_041538 (Zhai et al. 2019), *Ranunculus japonicus* MZ169045, *P. chinensis* MK569491, *P. chinensis* MK860682 (Zhang et al. 2019), *Pulsatilla chinensis* var. *kissii* MK860683 (Zhang et al. 2019), *P. chinensis* NC_039452 (Liu et al. 2018), *P. cernua* f. *plumbea* MK860684 (Zhang et al. 2019), *P. dahurica* MK860685 (Zhang et al. 2019), *P. turczaninovii* MK860686, *P. cernua* MK860687 (Zhang et al. 2019). *P. grandis* MN025344 (Li et al. 2020), *P. hirsutissima* MN025345 (Li et al. 2020), *P. campanella* NC_061038 (Xue et al. 2022), *P. alpina* MN025343 (Zhang et al. 2019), *P. occidentalis* MN025348 (Li et al. 2020). *Anemone. tomentosa* NC_039451 (Liu et al. 2018), *A. vitifolia* NC_056381, *A. reflexa* NC_054280 (Zhang et al. 2021), *Hepatica nobilis* NC_060986, *Actaea cimicifuga* OQ587922, *Aconitum carmichaelii* NC_030761, *Urophysa henryi* NC_039744, *Aquilegia yangii* NC_058529, *Isopyrum manshuricum* NC_041541 (Zhang et al. 2019), *Dichocarpum fargesii* NC_041478 (Zhang et al. 2019), *Leptopyrum fumarioides* NC_041542 (Zhang et al. 2019), *Paraquilegia microphylla* NC_067755, *Thalictrum aquilegiifolium* NC_058830, *Coptis chinensis* NC_036485, *Menispermum canadense* NC_048451.

chloroplast genomes from Ranunculaceae plants (14 from the *Pulsatilla* genus), one outgroup taxon from Menispermaceae (*Menispermum canadense*), were obtained from NCBI. The MAFFT version 7.037 (Katoh and Standley 2013) was used to identify common protein-coding genes of 32 chloroplast genomes and compare the *P. chinensis* f. *alba* chloroplast genome with 31 other complete chloroplast genomes by the FFT-NS-2 strategy. The gaps in the alignment were trimmed using Gblocks (Version 0.91b, http://molevol.cmima.csic.es). A phylogenetic tree of the 32 chloroplast genomes was then constructed using IQ-TREE-1.6.12, (http://www.iqtree.org/) based on the maximum-likelihood method with 1000 bootstrap replications and the JTT + F+R2 model, which was selected using ModelFinder (Kalyaanamoorthy et al. 2017).

Results

Genome structure analysis

The chloroplast genome of *P. chinensis* f. *alba* was 163,654 bp, including a large single-copy (LSC) region with a length of 82, 355 bp, a small single-copy (SSC) region with a length of 19,069 bp, and two inverted repeat regions (IRs) with a length of 31,115 bp (Figure 2). The genome had 134 genes, including 90 protein-coding genes, 36 tRNA genes, and eight rRNA genes, with a GC content of 37.2%. The *rps*16, *trn*K-UUU, *rpo*C1, *atp*F, *trn*G-UCC, *trn*L-UAA, *trn*V-UAC, *pet*B, *pet*D, *rpl*16, *rpl*2, *ndh*B, *trn*I-GAU, *trn*A-UGC and *ndh*A genes contained one intron; the *clp*P and *ycf*3 genes contained two introns (Figure S2). The *rps*12 gene was a trans-spliced gene (Figure S3). The chloroplast genomes of *P. chinensis* f. *alba* and four other published *P. chinensis* (NC039452, MK860682, MK569491, MK860683) had high similarity (Figure S4). The chloroplast genome of *P. chinensis* f. *alba* was correctly assembled according to the coverage depth (Average sequencing depth was 389.92X, Maximal sequencing depth was 616X, Minimal sequencing depth was 33X) (Figure S5) (Li et al. 2009; Li 2013).

Phylogenetic analysis

Menispermum canadense was the outgroup, which was distant from the other species. *P. chinensis* f. *alba*, three *P. chinensis*, and *P. chinensis* var. *kissii* formed a single branch and there was a relatively short genetic distance between *P. cernua* and *P. dahurica*. The phylogenetic tree analysis revealed that *Anemone* is the closest relative to *Pulsatilla* within the Ranunculaceae (Figure 3).

Discussion and conclusion

The chloroplast genome of *P. chinensis* f. *alba* was reported in this study. The structure, size, and genetic composition of this genome were similar to those of *P. chinensis* and other plants in the *Pulsatilla* genus (Zhang et al. 2019). Based on the phylogenetic analysis results, *P. chinensis* f. *alba* had very close relation to *P. chinensis*. The above results support traditional morphological classification, and this study also provides data for the study of evolutionary relationships in the Ranunculaceae plants, the identification of Pulsatillae radix and development of germplasm resources.

Ethics statement

According to the Wild Plants Protection Regulations of the People's Republic of China, *P. chinensis* f. *alba* was not included on the list of national protected wild plants. Article five also encourages scientific research on wild plants. Therefore, the sample collection does not require any permission. This study protocol has been approved by the School of Pharmacy, Liaoning University of Traditional Chinese Medicine. All operation was conducted in compliance with the guidelines in Specification on Good Agriculture and Collection Practices for Medicinal Plants (GACP; number: T/CCCMHPIE 2.1-2018).

Author contributions

C.B. and Y.P.X.: Analysis of data, conception and drafting for the work. Y.Y.Y. and L.X.: Identification and collection of the plant, preservation of plant specimens and conceiving the work. T.G.K.: Revising critically important intellectual content and was involved in validation and supervision. H.F.X. and W.J.H: Acquisition and analysis of data. W.J.H. and Y.P.X.: Contributed to the writing and revising. All authors agree to be accountable for all aspects of the work.

Disclosure statement

No potential conflict of interest was reported by the authors. Che Bian and Yan-ping Xing contributed equally to this research. It is worth noting that Che Bian and Yan-ping Xing are co-first authors.

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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 accession NO. OR085847. The associated BioProject, SRA, and BioSample numbers are PRJNA978389, SRX20587082 (Illumina), and SAMN35555254, respectively.

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