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PLASTOME REPORT

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The complete chloroplast genome of the first registered *Paeonia* Itoh hybrid cv. Hexie in China

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ABSTRACT

The first registered *Paeonia* Itoh hybrid cv. Hexie in China is a naturally occurring intersectional hybrid of Sect. *Paeonia* and Sect. *Moutan*. In this study, we sequenced, assembled, and analyzed the complete chloroplast genome of *Paeonia* Itoh hybrid cv. Hexie. The result showed that the chloroplast genome of Hexie, with a typical circular tetrad structure, is 152,958 bp in length, comprising a large single copy (LSC) region of 84,613 bp, a small single copy (SSC) region of 17,051 bp, and two reverse complementary sequences (IRs) of 25,647 bp. The chloroplast genome encoded 116 genes, including 80 protein-coding genes, 32 tRNA genes, and 4 rRNA genes. Phylogenetic analysis inferred from the shared protein-coding genes showed that the *Paeonia* Itoh hybrid cv. Hexie had the closest phylogenetic relationship with *P. suffruticosa*, followed by *P. ostii*, indicating that *P. suffruticosa* was its maternal parent. This study provides a molecular resource for phylogenetic and maternal parent studies of *Paeonia* Itoh hybrid, contributing to a basis for *Paeonia* Itoh hybrid breeding strategies in the future.

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Introduction

Paeonia Itoh hybrid, first bred in 1948 by Japanese horticulturist Dr. Toichi Itoh with herbaceous peony (*Paeonia lactiflora*) and tree peony (*P. suffruticosa*), combines the best features of both parents (Yang et al. 2020). Hexie was first registered and identified as *Paeonia* Itoh hybrid from the aspects of morphology and molecular biology. It was speculated that Hexie was an intersectional cross with *P. lactiflora* as the maternal parent and *P. suffruticosa* as the paternal parent (Hao et al. 2008).

Although several chloroplast genomes of Family Paeoniaceae have been sequenced and analyzed (Wu et al. 2020; Wu et al. 2021), the chloroplast genome of the *Paeonia* Itoh hybrid has not yet been reported. Genomes in chloroplasts of plant cells are usually inherited from the maternal parent, with rare exceptions (Park et al. 2021). In this study, we sequenced, assembled, and analyzed the complete chloroplast genome of the first *Paeonia* Itoh hybrid cv. Hexie and discovered its most likely maternal parent.

Materials and methods

Plant material, DNA extraction, and sequencing

Fresh Hexie leaves were obtained from the National Tree Peony and Herbaceous Peony Germplasm Resource Bank, Luoyang Academy of Agricultural and Forestry Sciences (N34°22′48″, E112°17′24″) (Figure 1). The voucher specimen was deposited in the Herbarium of Luoyang Key Laboratory of Peony Biology, Henan University of Science and Technology with voucher number LKLPB202202 (contact: Bingyou Fan, bingyou.fan@haust.edu.cn). Genomic DNA was extracted from leaves using TaKaRa MiniBEST Plant Genomic DNA Extraction Kit according to the manufacturer's instructions. The DNA was determined to be of high quality, based on visual DNA integrity check using agarose gel electrophoresis, and the concentration was quantified with a NanoDrop™ spectrophotometer ND2000CLAPTOP (Thermo Fisher Scientific, Waltham, Massachusetts, United States). The genomic DNA was fragmented randomly to construct shotgun libraries, and Paired-End (PE) sequencing was performed using the MGISEQ-200 platform (Wuhan, China). Low-

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Figure 1. *Paeonia* Itoh hybrid cv. Hexie (A) The leaves of Hexie resemble those of *P. lactiflora*, instead of those of *P. suffruticosa*. (B) The flowers of Hexie have purple spots at the base of the petals, which was a typical characteristic of *P. rockii*. Photos were taken by Kai Gao in Luoyang Academy of Agricultural and Forestry Sciences (N34°22′48″, E112°17′24″).

quality reads were removed with SOAPnuke v.2.0 (Chen et al. 2018).

Genome assembly and annotation

The genome was assembled using GetOrganelle (Jin et al. 2020), with default parameters to obtain a circular chloroplast genome based on clean data. Chloroplast genome annotation was performed using the CpGAVAS2 online annotation tool (Shi et al. 2019) with *P. suffruticosa* as the reference (ON243820). The Chloroplast genome was mapped using CPGView (Liu et al. 2023).

Phylogenetic analysis

A phylogenetic tree was constructed based on the shared protein-coding genes of 20 species from Family Paeoniaceae and an additional species *Coptis teeta* as an outgroup from Family Ranunculaceae, using the Maximum likelihood method (ML). The shared protein-coding genes were obtained from the 21 chloroplast genomes using PhyloSuite v1.2.2 (Zhang et al. 2020). The multiple sequences alignment analysis was carried out using MAFFT v7.475 (Nakamura et al. 2018). Then a phylogenetic tree was constructed using IQ-TREE v2.03 (Minh et al. 2020). The best-fitting nucleotide substitution model GTR + F + I + G4 was determined using the Akaike Information Criterion (AIC) by ModelFinder (Kalyaanamoorthy et al. 2017) in the IQ-TREE package and 1,000 bootstrap replicates.

Results

The assembled chloroplast genome of *Paeonia* Itoh hybrid cv. Hexie exhibited a typical quadripartite structure (Figure 2) with152,958 bp in length and comprising a large single copy (LSC) region of 84,613 bp, a small single copy (SSC) region of 17,051 bp, and two reverse complementary sequences (IRs) of 25,647 bp. Coverage was 7581× (Figure S1). The GC content was not uniform in Hexie, with 36.64%, 32.63%, and 43.07% in the LSC, SSC, and IRs, respectively, while the overall GC content of the complete chloroplast genome is 38.34%. A total of 116 genes were predicted, consisting of 80 protein-coding genes, 32 tRNA genes, and 4 rRNA genes. The protein-coding genes were further divided into four categories i.e. photosynthesis-related genes, self-replication-related genes, other genes, and unknown functional genes. Among the predicted genes, 19 genes were duplicated in IRa & IRb, including 7 protein-coding genes (*ycf2*, *ycf15*, *rps7*, *rps12*, *rpl23*, *rpl2*, *ndhB*), 8 tRNA genes (*trnN-GUU*, *trnV-GAC*, *trnR-ACG*, *trnL-CAA*, *trnA-UGC*, *trnT-GGU*, *trnM-CAU*, *trnI-CAU*, *trnI-GAU*) and 4 categories of rRNA genes (*rrn4.5S*, *rrn23S*, *rrn5S*, and *rrn16S*. In addition, 18 genes contain one intron. Among them, 11 genes are protein-coding genes (Figures S2 and S3) and 7 are tRNA genes. Additionally, *clpP* and *ycf3* had two introns (Figure S2).

In total, 21 species were included in phylogenetic analysis. 20 species were from the Family *Paeoniaceae* and *Coptis teeta* was included as an outgroup (Figure 3). The phylogenetic tree inferred from ML was presented with ML bootstrap support (BS) indicated. The phylogenetic tree

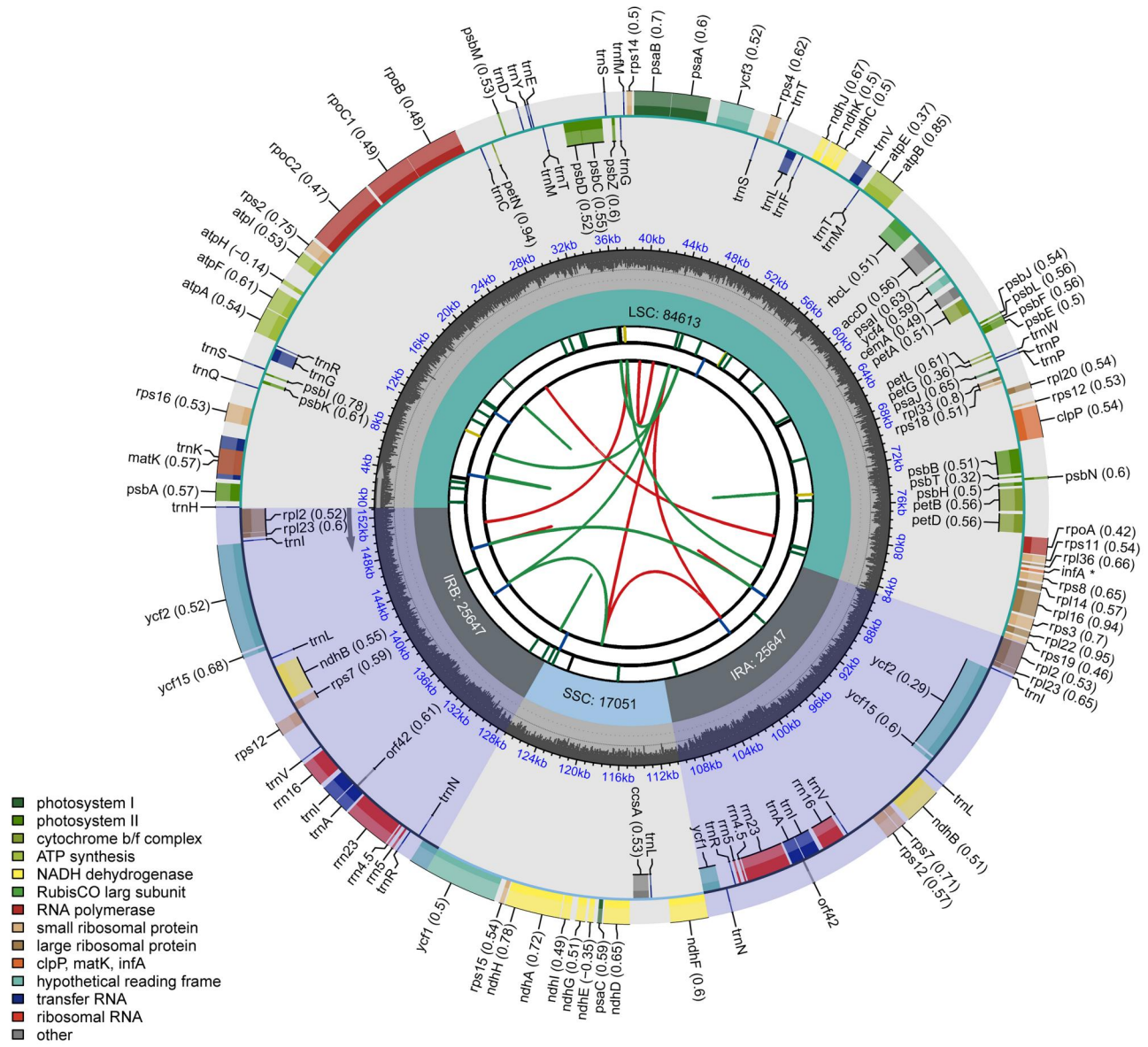

Figure 2. The chloroplast genome map of *Paeonia* Itoh hybrid cv. Hexie. From the center outward, the map consists of six tracks. The first track is a scattered repeating sequence, with a red arc indicating forward repetition and a green arc indicating reverse repetition. The second track displays the tandem repeats marked. The third track presents the microsatellite sequences. The fourth track shows the sizes of feature regions, including LSC, IRa, SSC, and IRb. The fifth track illustrates the GC content along the genome. The sixth track presents the genes.

shows that species of Sect. *Moutan*, Sect. *Onaepia* and Sect. *Paeonia* were located on different branches and *Paeonia* Itoh hybrid cv. Hexie was closest related to *P. suffruticosa*.

Discussion and conclusion

Chloroplast genomes have been widely studied because of their high conservation and mode of inheritance, which can help determine the evolutionary relationships of related plant species (Xie et al. 2020). In this study, the phylogenetic tree demonstrated that the Sect. *Moutan* and Sect. *Paeonia* plants formed branches independently, whereas Sect. *Onaepia* plants formed a third branch alone. Moreover, the Subsect. *Vaginatae* plants and Subsect. *Delavayan* plants formed two sister branches. Thus, comparing the complete chloroplast genome of Family *Paeoniaceae* species helped identify their corresponding Sections.

Recent research on 10 newly assembled plastomes and phylogenetic analysis of 63 plastomes of 16 *Paenoia* species, speculated the maternal parents of *P. suffruticosa* and *P. lactiflora* accessions (Chen et al. 2023). In this study, the *Paeonia* Itoh hybrid cv. Hexie was distributed within Subsect. *Vaginatae*, indicating it has the closest phylogenetic relationship with *P. suffruticosa*, followed by *P. ostii*. Consequently, it is speculated that the maternal parent of *Paeonia* Itoh hybrid cv. Hexie is probably *P. suffruticosa* cultivar with purple spots instead of *P. lactiflora*, which contradicts a previous hypothesis (Hao et al. 2008).

Overall, our study provides a molecular resource for phylogenetic and maternal parent studies of *Paeonia* Itoh hybrid cv. Hexie, which can support *Paeonia* Itoh hybrid breeding strategies in the future.

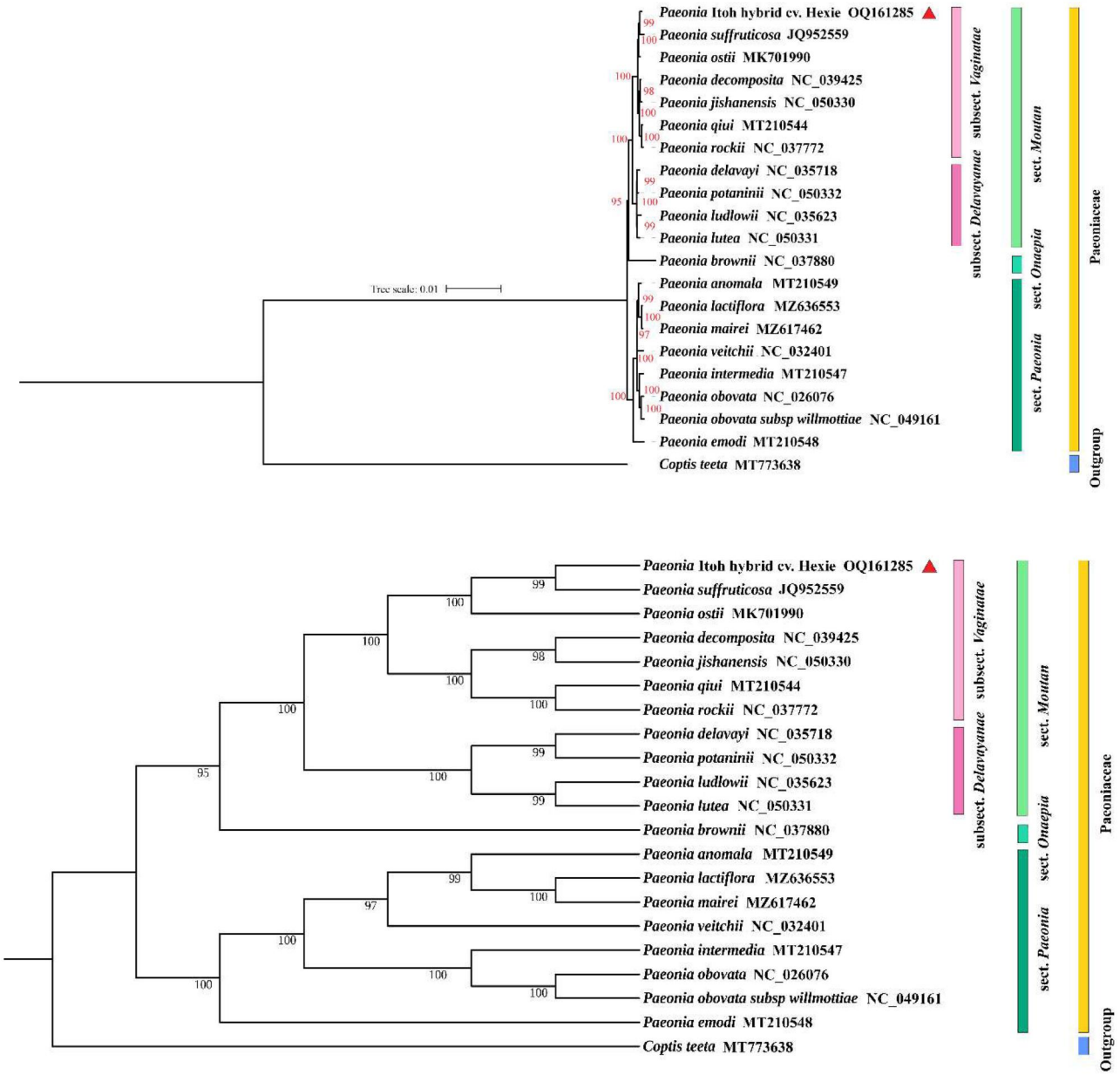

Figure 3. ML phylogenetic trees, shown as phylogram (up) and cladogram (down), were constructed based on 68 shared protein-coding genes (Table S1). *Coptis teeta* as an outer group. The figure represents the bootstrap values of ML. The following sequences were used: *P. suffruticosa* JQ952559 (Chen et al. 2023), *P. ostii* MK701990 (Guo et al. 2020), *P. decomposita* NC_039425 (Chen et al. 2019), *P. jishanensis* NC_050330 (Guo et al. 2020), *P. qiui* MT210544 (Wu et al. 2020), *P. rockii* NC_037772 (Bai et al. 2018), *P. delavayi* NC_035718 (Li et al. 2018), *P. potaninii* NC_050332 (Li et al. 2018), *P. ludlowii* NC_035623 (Guo et al. 2020), *P. lutea* NC_050331 (Guo et al. 2020), *P. brownii* NC_037880 (Dong et al. 2018), *P. anomala* MT210549 (Chen et al. 2023), *P. lactiflora* MZ636553 (unpublished), *P. mairei* MZ617462 (unpublished), *P. veitchii* NC_032401 (Samigullin et al. 2018), *P. intermedia* MT210547 (Chen et al. 2023), *P. ovovata* NC_026076 (Samigullin et al. 2018), *P. obovate* subsp. *willmottiae* NC_049161 (Zhang et al. 2020), *P. emodi* MT210548 (Chen et al. 2023), *Coptis teeta* MT773638 (Wang et al. 2022).

Ethical approval

This study includes no endangered plant species, and the sample was collected legally following guidelines provided by the authors' institution and national or international regulations.

Author contributions

B. F. and J. W. conceived the study and drafted the manuscript. S. D., R. D. and M. H. assembled, and annotated the chloroplast genome. D. K. S. and P. P. D. participated in the data analysis and revision of the manuscript. K. G. collected the plant sample. All authors contributed to the article and approved the submitted version.

Disclosure statement

No potential competing interest was reported by the 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 the accession no. OQ161285. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1069233, SAMN39515533 and SRR27676648 respectively.

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