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PLASTOME REPORT

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The complete chloroplast genome sequence of *Calystegia hederacea* Wall. in Roxb. 1824 (Convolvulaceae) in Enshi, Hubei

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ABSTRACT

Calystegia hederacea Wall. in Roxb. 1824 is a perennial herbaceous vine in the family Convolvulaceae and has several biological effects. Herein, we reported the first complete chloroplast genome of *C. hederacea*. The chloroplast genome sequence was 152,057 bp in length, comparing a large single-copy (LSC) region of 87,891 bp, a small single-copy (SSC) region of 19,866 bp, and a pair of inverted repeat (IR) regions of 22,150 bp. This sequenced chloroplast genome contained 126 predicted genes, including 81 protein-coding genes, 37 tRNA genes, and eight rRNA genes, and the total GC content of the chloroplast genome was 37.79%. Phylogenetic analysis revealed that *C. hederacea* was closely related to *C. soldanella*. The chloroplast genome presented in this study will enrich the genome information of the genus *Calystegia* and provide deeper insights into the evolution study of the family Convolvulaceae.

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1. Introduction

The genus *Calystegia* R. Br. comprises approximately 26 species with wide distribution in temperate zones (Brummitt 1963; Brummitt and Staples 2007). *Calystegia hederacea* Wall. in Roxb. 1824, belonging to the family Convolvulaceae, is a perennial herbaceous vine with strong twining stems and large white trumpet flowers and is widely spread in India and East Asia (Hotta et al. 1989). Numerous studies have revealed that *C. hederacea* contains a series of glycosidic acids, including calyhedic acids A–F (Ono et al. 2020, 2022), calyhedins I–VI (Ono et al. 2021), and calysolins V–IX (Ono et al. 2014), and further has several biological effects, such as cytotoxicity to cancer cells, anti-bacterial, ionophoric (Pereda-Miranda et al. 2010), anti-viral (Ono et al. 2014), multidrug resistance modulatory (Figueroa-González et al. 2012), and anti-inflammatory (Yoshikawa et al. 2010). In recent years, the chloroplast genomes of several *Calystegia* species have been published, including *C. soldanella* (Wu et al. 2022) and *C. arvensis* (Wang et al. 2021). Nevertheless, the complete chloroplast genome of *C. hederacea* is still unknown. Therefore, we reported the first complete chloroplast genome of *C. hederacea* and further confirmed its phylogenetic position. This presented chloroplast genome will provide insight into the genomic resource for comparative genomic and species identification of the genus *Calystegia*.

2. Materials

The experimental samples of *C. hederacea* were taken from the germplasm resource nursery of Enshi Tujia and Miao Autonomous Prefecture Academy of Agricultural Sciences, Hubei Province, China (N 30°31′53″, E 109°48′11″) (Figure 1). A specimen was deposited at the Herbarium of Enshi Tujia and Miao Autonomous Prefecture Academy of Agricultural Sciences (Contact: Wei Fu, fuwei5@mail2.sysu.edu.cn) under the voucher number *XH-202309*.

3. Methods

Total genomic DNA was extracted from the fresh leaves using the modified CTAB method (Doyle and Doyle 1987) and then randomly fragmented by sonication. Subsequently, the DNA library with an insertion size of 350 bp was constructed, and library quality was assessed on the Agilent 5400 system (Santa Clara, CA). Paired-end sequencing was performed on the DNBSEQ-T7 platform (Beijing Biomics Tech Co., Ltd., Beijing, China), yielding approximately 3.13 GB of raw data. After removing the low-quality sequences and adapter sequences by fastp (Chen et al. 2018), a total of 41,728,832 clean reads were obtained to *de novo* assemble the chloroplast genome using GetOrganelle (version 1.7.1) (Jin et al. 2020) with the k-mer length: 21, 35, 45, 65, 85, and 105. We further annotated the assembled chloroplast genome using CPGAVAS2 (http://47.96.249.172:16019/analyzer/annotate) (Shi

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Figure 1. Species reference image of *C. hederacea*. The photo was taken by Lin Li at the germplasm resource nursery of Enshi Tujia and Miao Autonomous Prefecture Academy of Agricultural Sciences, Hubei Province, China, in September 2023.

et al. 2019) with the published chloroplast genome of *C. soldanella* (GenBank accession number: LC729542) (Wu et al. 2022) annotation as the reference. Finally, CPGView (http://47.96.249.172:16085/cpgview/view) was used to improve annotation and further identify the accuracy of *cis*- and *trans*-splicing genes (Liu et al. 2023).

To investigate the phylogenetic position of *C. hederacea*, the chloroplast genome sequence of 14 representative species was downloaded from the GenBank database to reconstruct the phylogenetic tree, with *Panax ginseng* (GenBank accession number: MH049735) (Wang et al. 2018) as an outgroup. First, PhyloSuite (version 1.2.2) (Zhang et al. 2020) was used to extract 69 protein-coding genes (PCGs) from the genome annotation files, then multiple sequence alignment of each gene was performed using the MAFFT (version 7.407) (Katoh and Standley 2013), and the aligned genes were further concatenated using PhyloSuite (version 1.2.2) (Zhang et al. 2020). A maximum-likelihood (ML) phylogenetic tree was constructed by IQtree (version 1.7) (Nguyen et al. 2015) under the best-fit model (TVM + F + I + G4) with 1000 bootstrap replicates.

4. Results

The complete chloroplast genome sequence of *C. hederacea* was a typical quadripartite structure with 152,057 bp in length, containing a large single-copy (LSC) region of 87,891 bp, a small single-copy (SSC) region of 19,866 bp, and a pair of inverted repeat (IR) regions of 22,150 bp (Figure 2). The average coverage depth for the *C. hederacea* chloroplast genome is shown in Supplementary Figure 1. The overall GC content of the genome was 37.79%, whereas the corresponding value in the IR regions was 43.30%, which was higher than that in the LSC region (36.17%) and the SSC region (32.66%). This chloroplast genome contained 126 predicted genes, including 81 PCGs, 37 tRNA genes (tRNAs), and eight ribosomal RNA genes (rRNAs). Meanwhile, 10 PCGs and eight tRNAs had a single intron, and two PCGs (*ycf3* and *clpP*) contained two introns. We also identified 12 cis-splicing genes and one trans-splicing gene in this chloroplast genome, and their structures are shown in Supplementary Figure 2.

To clarify the phylogenetic position of *C. hederacea*, we performed a phylogenetic analysis based on 16 chloroplast genome sequences. The phylogenetic tree (Figure 3) showed that all the species in our study were divided into three clades, using *Panax ginseng* as the outgroup. *Erycibe obtusifolia* and *Erycibe henryi* were clustered in clade I. *Evolvulus alsinoides* var. *oblongus*, *Evolvulus alsinoides*, *Jacquemontia paniculata*, and *Dichondra micrantha* were clustered in clade II. Other species were clustered in clade III. Moreover, *C. hederacea* has a close relationship with *C. soldanella*, which was generally consistent with previous studies (Wu et al. 2022). These results would facilitate the phylogenetic and population genetic diversity of *C. hederacea*.

5. Discussion and conclusions

In this study, the complete chloroplast genome of *C. hederacea* was first sequenced and found to exhibit a typical quadripartite structure with 152,057 bp in length. Moreover, a total of 126 genes were predicted, including 81 protein-encoding genes, 37 tRNAs, and eight rRNA genes. These features are similar to those of the homologous species *C. soldanella* (Wu et al. 2022) and *C. arvensis* (Wang et al. 2021). The phylogenetic tree analysis revealed that *C. hederacea* was closely related to *C. soldanella*, which provides new data on the phylogeny, species identification, species resource conservation, and genetic investigations of *C. hederacea*. In the future, increasing the number of chloroplast genomes in the species of *Calystegia* will provide deeper insights into the evolution of this important family.

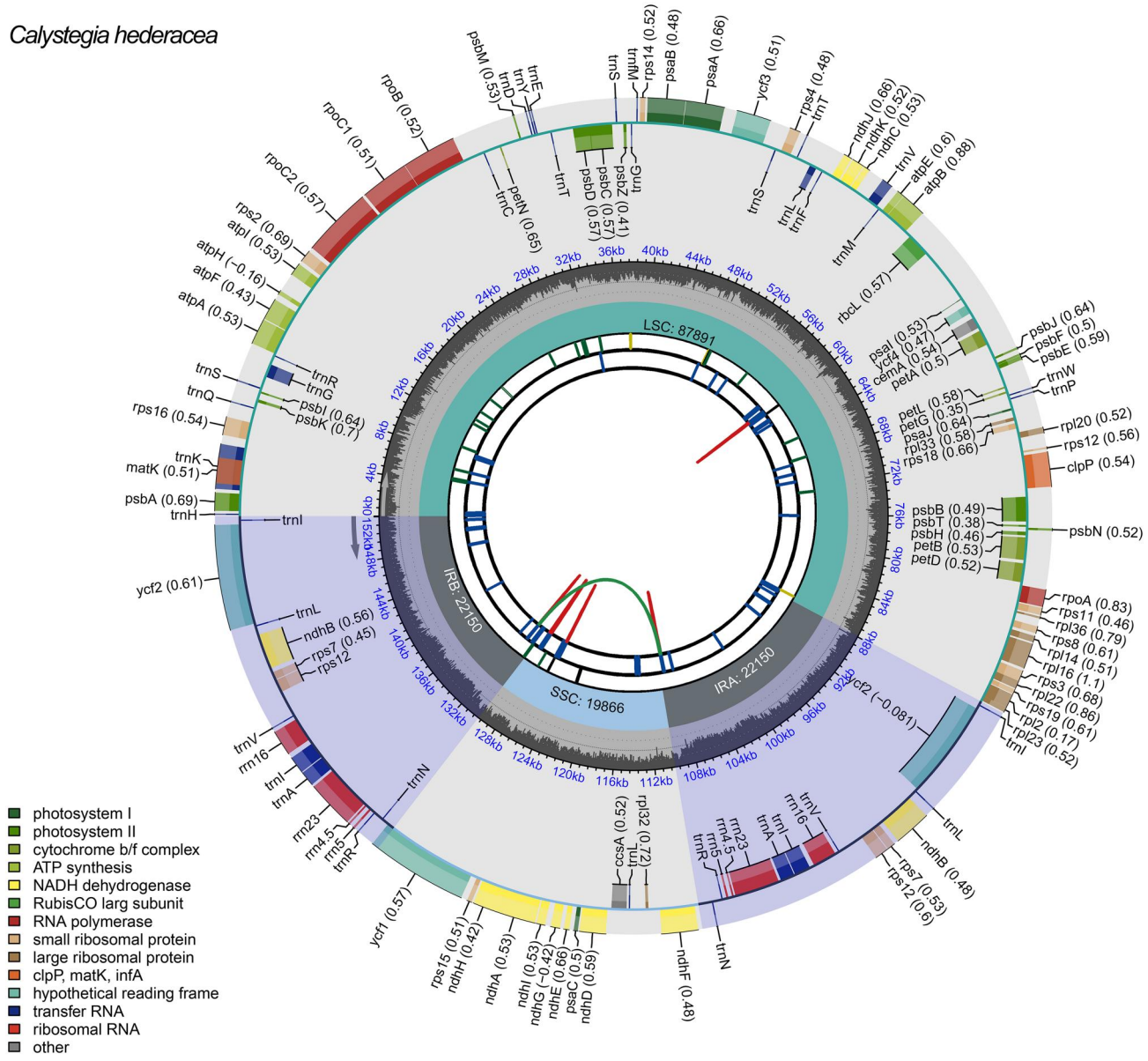

Figure 2. Genetic map of the chloroplast genome of *C. hederacea*. The large single-copy (LSC), small single-copy (SSC) region, and two inverted repeat regions (IRA and IRB) are shown in the inside track. Gene models, including protein-coding, tRNA, and rRNA genes, are shown with various colored boxes in the outer track.

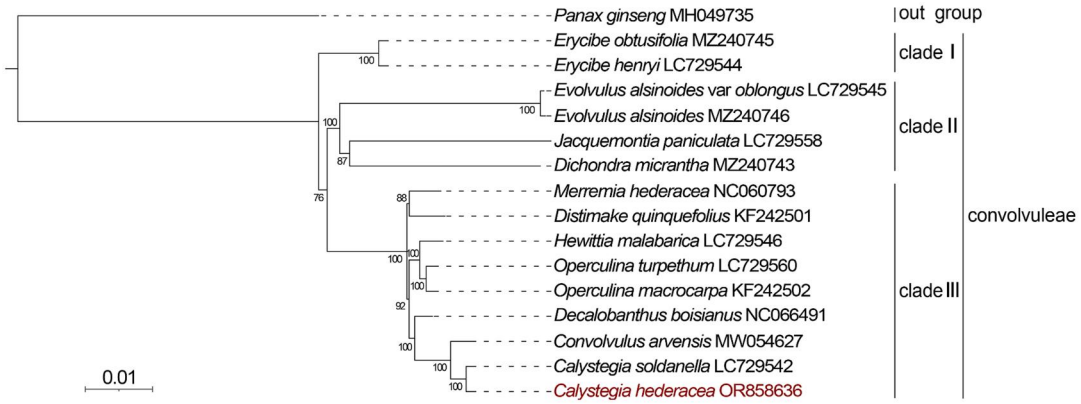

Figure 3. Phylogenetic relationships of *C. hederacea* based on the maximum-likelihood (ML) analysis of 69 protein-coding genes in chloroplast genomes. Bootstrap values next to the nodes are based on 1000 replications, and *Panax ginseng* was the outgroup. GenBank accession numbers: *Panax ginseng* MH049735 (Wang et al. 2018), *Erycibe obtusifolia* MZ240745 (Lin et al. 2022), *Erycibe henryi* LC729544 (Wu et al. 2022), *Evolvulus alsinoides* var. *oblongus* (Wu et al. 2022), *Evolvulus alsinoides* MZ240746 (Lin et al. 2022), *Jacquemontia paniculata* LC729558 (Wu et al. 2022), *Dichondra micrantha* MZ240743 (Lin et al. 2022), *Merremia hederacea* NC060793, *Distimake quinquefolius* KF242501 (Eserman et al. 2014), *Hewittia malabarica* LC729546 (Wu et al. 2022), *Operculina turpethum* LC729560 (Wu et al. 2022), *Operculina macrocarpa* KF242502 (Eserman et al. 2014), *Decalobanthus boisianus* NC 06649, *Calystegia soldanella* LC729542 (Wu et al. 2022), and *Convolvulus arvensis* MW054627 (Wang et al. 2021).

Author contributions

Yingchun Zou (Y.Z.) and Lan Long (L.L.) conceived and supervised the research. Wei Fu (W.F.), Lin Li (L.L.), and Xiaolong Wen (X.W.) conducted the bioinformatics analysis. Feifei Chen (F.C.) and Shuang Li (S.L.) contributed reagents/materials and performed the experiments. Wei Fu (W.F.) drafted the paper. Lin Li (L.L.) revised the paper critically for intellectual content. All of the authors have read and approved the final manuscript and have agreed to be accountable for all aspects of the work.

Ethical approval

No ethical approval/permission is required in this study. This study includes no human, animal, or endangered plant samples, and the sample was legally collected in accordance with guidelines provided by the authors' institution and national or international regulations.

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 no. OR858636. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1044543, SRR26944584, and SAMN38395455, respectively.

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