

The first complete chloroplast genome of *Cyclamen persicum* and its phylogenetic position within Primulaceae

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ABSTRACT

Cyclamen persicum Mill., 1768, is a perennial herb of Primulaceae and is native to the Mediterranean region. In this study, we sequenced the chloroplast genome of *Cyclamen persicum*. Its complete chloroplast contained 151,911 nucleotides, including a large single-copy (LSC) region with a length of 83,191 bp, a small single-copy (SSC) region with a length of 17,922 bp, and a pair of reverse repeat IR regions (25,399 bp). The *C. persicum* chloroplast genome encoded 112 unique genes, including 78 protein-coding genes, 30 tRNA genes, and four rRNA genes. The GC content of the entire genome was 37.25%, lower than that of many angiosperm plastome. The phylogenetic result indicated that *C. persicum* exhibited the closest relationship with *Cyclamen rohlfsianum*, and provided new information for the phylogeny relationship of genus *Cyclamen*.

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Introduction

Cyclamen persicum Mill., 1786, is a perennial herb of Primulaceae. *Cyclamen* is native to the Mediterranean region, from Spain to Iran in the east, and from northeast Africa to Somalia in the south. It has green leaves with white or gray halo spots on the leaf surface, and green or dark red on the


back. The flowers are unique in shape, beautiful and dazzling, brilliant and colorful, some varieties have fragrance. *Cyclamen* is suitable for potted plants and has high ornamental value. *Cyclamen* was once the next genus of Ardisidae. In APG III classification, because Ardisidae was canceled, *Cyclamen* was merged into Primulaceae. In 1938, Schwarz established a



Figure 1. Plant image of *C. persicum*. This photo was taken by Xiaohua Liu with the author's approval for use. The most characteristic feature of the *C. persicum* is its green leaves with white or gray halo spots on the leaf surface and green or dark red on the back.

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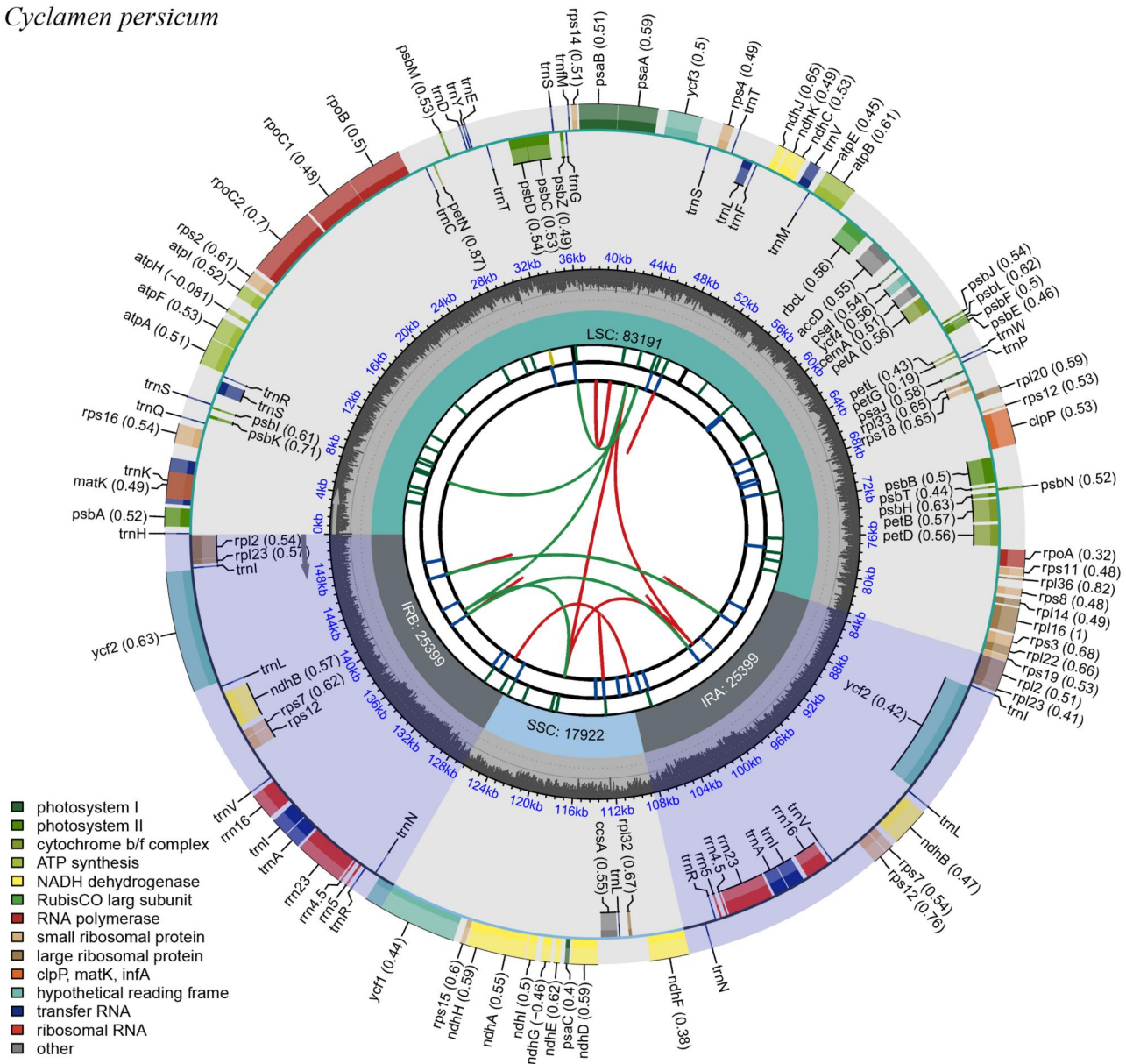
Cyclamen persicum

Figure 2. Chloroplast genome map of *C. persicum*. Genes drawn outside the outer circle are transcribed clockwise, and those inside are transcribed counter-clockwise. Genes belonging to different functional groups are color-coded. The dark gray in the inner circle indicates GC content of the chloroplast genomes.

formal subgenus for *Cyclamen*, and identified 13 species in two parts (Schwarz 1938). After careful research by many scholars, *Cyclamen* has developed into a genus of no less than 30 species over the last 80 years (Schwarz 1938, 1955, 1964; Grey-Wilson 1988, 1997, 2003; Anderberg 1994; Anderberg et al. 2000; Clennett 2002). *Cyclamen* is traditionally classified into Primulaceae, but the evidence from chloroplast DNA sequence data shows that *Cyclamen* belongs to the *Mahogany* family (Källersjö et al. 2000). In this paper, we sequenced the chloroplast genome of *Cyclamen persicum*, providing a method guidance for the study of the evolution and phylogeny of *Cyclamen* plants.

Materials and methods

The fresh and healthy original plants of *C. persicum* were collected and identified by Jinlei Ba (henrybajin@163.com), from

Shihuiyao village, Shihuiyao Town, Chengde City in Hebei Province (N40°89', E118°25', picture see Figure 1) (this sample was neither collected from a protected area nor listed on any endangered species list, such as CITES; and, the collection of plant material was in accordance with local regulations). The voucher specimen were deposited at the Chengde Academy of Agricultural and Forestry Science (Jinlei Ba, henrybajin@163.com) with the voucher number as CDXKL01. The genomic DNA was extracted using the plant genomic DNA extraction kit from the fresh leaves (Tiangen Biochemical Technology (Beijing) Co., Ltd., Beijing, China) and make some modifications according to the agreement provided by the manufacturer (Zhang et al. 2021). The DNA quantity and quality were examined using a Qubit 4.0 Fluorometer (Thermo Fisher Scientific Inc., Waltham, MA), followed by shearing to prepare a PCR-free library according to the preparation guide. The Illumina NovaSeq system was used for

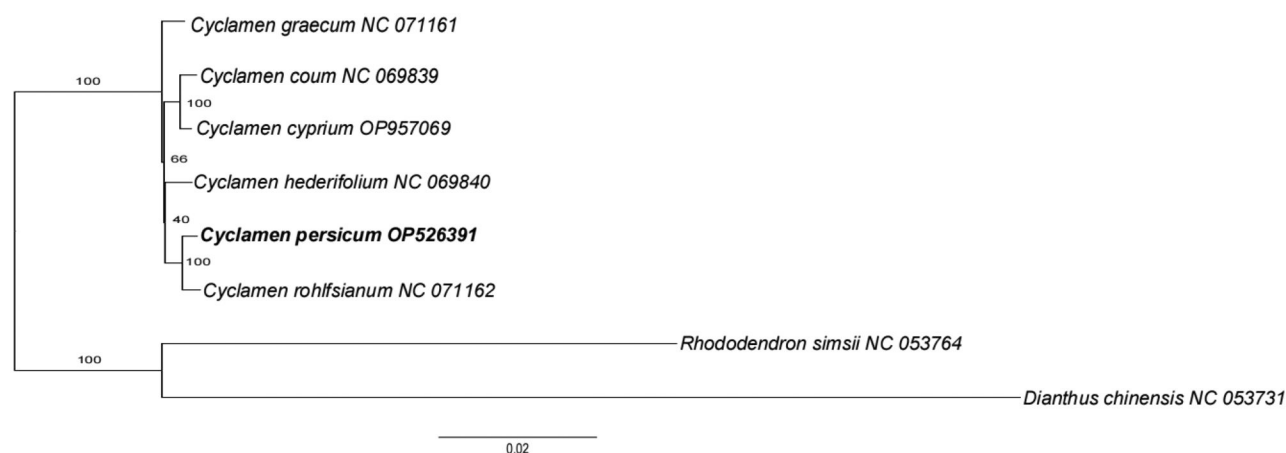


Figure 3. The phylogenetic tree recovered from eight complete chloroplast genome sequences by RAxML. Their accession numbers can be found after the species names. The following sequences were used: *Cyclamen coum* NC_069839, *Cyclamen hederifolium* NC_069840 (Mao 2022), *Cyclamen cypricum* OP957069, *Cyclamen graecum* NC_071161, *Cyclamen rolfsianum* NC_071162 (Mao et al. 2023), *Cyclamen persicum* OP526391 (Zhang 2022), *Dianthus chinensis* NC_053731 (Yang 2020), and *Rhododendron simsii* NC_053764 (Zhang 2020). *Dianthus chinensis* and *Rhododendron simsii* were used as the outgroups. The species with bold font was the chloroplast genome of our *C. persicum*.

high-throughput sequencing, which generated about 1.8 Gb of clean data with a paired end reading length of 150 bp. Trimmomatic v0.38 was used to clean the sequencing adapters and low-quality reads (Bolger et al. 2014). Then used GetOrganelle v1.7.3.5 to assemble the remaining readings into the complete chloroplast genome (Jin et al. 2020), and validated by reads mapping using bowtie2 (Langmead and Salzberg 2012). We annotated the chloroplast genome assembled by *C. persicum* by using CPGAVAS2 online web server (www.herbalgenomics.org/cpgavas2) (Shi et al. 2019). The complete chloroplast genome sequence of *C. persicum* was submitted to GenBank (accession number: OP526391).

In order to confirm the phylogenetic position of *C. persicum* in Primulaceae, eight chloroplast genomes were retrieved from NCBI. First, 72 protein coding genes shared by eight species were compared using MUSCLE v5 (Edgar 2022). Connect it into a super alignment sequence with a length of 53,850 bp. Subsequently, based on the maximum-likelihood (ML) method, a total of eight complete chloroplast genomes were phylogenetically analyzed using RAxML v8.2.12, with 1000 bootstrap replications (Stamatakis 2014). *Dianthus chinensis* and *Rhododendron simsii* were used as the outgroups.

Results

The assembled genome exhibited average, maximum, and minimum coverage depths were 2334 \times , 3279 \times , and 616 \times (Figure S1). The complete chloroplast genome of *C. persicum* contained 151,911 nucleotides, including a large single-copy (LSC) region with a length of 83,191 bp, a small single-copy (SSC) region with a length of 17,922 bp, and a pair of reverse repeat IR regions (25,399 bp). The schematic representation of comprehensive circular map of the chloroplast genome, cis-splicing genes, and trans-splicing genes (Figure 2 and Figure S2), was visualized through CPGView software (Liu et al. 2023). The GC content of the entire genome was 37.25% (Figure 2). There were 112 unique encoding genes in the chloroplast genome of *C. persicum*, including 78 protein-coding genes, 30 tRNA genes, and four rRNA genes (*rrn16s*,

rrn23S, *rrn4.5S*, and *rrn5S*). Eleven genes contained one intron, and two protein-coding genes (*ycf3*, *clpP*) contained two introns. In addition, small exons were annotated in three genes (*petB*, *petD*, and *rpl16*), and the lengths were 6 bp, 8 bp, and 9 bp. Moreover, a trans-splicing gene (*rps12*) was identified from the complete chloroplast genome. The phylogenetic result indicated that *C. persicum* exhibited the closest relationship with *Cyclamen rolfsianum* (Figure 3).

Discussion and conclusions

This study is the first to sequence the complete chloroplast genome of *C. persicum*, with a length of 151,911 bp. The genome size and gene content of *C. persicum* are closely consistent with the existing chloroplast genome in the genus *Cyclamen* (Mao et al. 2023). Our research findings constitute a valuable resource for *C. persicum*, enriching the existing database on the phylogenetic and molecular identification of *C. persicum*. It has substantial practicality in identifying *C. persicum*, and helps to analyze the genetic diversity and phylogenetic relationships of the genus *Cyclamen*. The availability of sequenced chloroplast genomes of the genus *Cyclamen* in the NCBI database is still limited. Consequently, more extensive chloroplast sequences need to be obtained from various species of *C. persicum* in order to gain a deeper understanding of the evolutionary history of *C. persicum*.

Author contributions

W.Z. and J.B. conceived and designed the experiments; W.Z., J.B., and J.G. performed the experiments; J.B. and Z.Z. analyzed the data; Z.Z., Z.J., and Z.C. contributed reagents/materials/analysis tools; W.Z. and J.B. wrote the paper.

Ethical approval

The material involved in the article does not involve ethical conflicts. This species is neither endangered in the cites catalogue nor collected from a natural reserve, so it does not need specific permissions or licenses. All collection and sequencing work was strictly executed under

local legislation and related laboratory regulations to protect wild resources.

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. OP526391. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA903544, SRR22708873, and SAMN32111963, respectively.

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