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PLASTOME REPORT

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The complete chloroplast genome sequence of *Primula medogensis* (Primulaceae) and its phylogeny

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ABSTRACT

Primula medogensis W.B Ju, B. Xu & X.F. Gao 2023, a new species categorized under *P.* sect. *Cordifoliae*, was officially described in 2023. Given its recent classification, the genetic resources for this species are currently very limited. Here, we sequenced and assembled the first complete chloroplast genome of *P. medogensis* using Illumina sequencing technology. The complete chloroplast genome of *P. medogensis* is 151,486 bp in length, exhibiting a typical quadripartite structure. It consists of a large single-copy region (LSC; 83,407 bp) and a small single-copy region (SSC;17675 bp), separated by a pair of inverted repeat regions (IRs; 25202 bp). A total of 131 genes were annotated, including 86 protein-coding, 37 tRNA, and eight rRNA genes. The overall GC content was 37.1%. Phylogenetic analysis of 59 *Primula* species revealed a close relationship between *P. medogensis* and *P. calliantha* subsp. *bryophila*.

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Introduction

The genus *Primula* L. represents the largest genus within the family Primulaceae, comprising more than 500 species (Hu 1994). This genus mainly thrives in temperate regions and mountainous areas of the Northern Hemisphere, with only a few species found in the Southern Hemisphere. The distribution center of *Primula* spans the Himalayas to Yunnan and the western Sichuan Province (Flora of China 1990). Notably, more than 300 species of the genus are distributed in China (Richards 2003). *Primula medogensis*, a newly discovered perennial herbaceous plant, is native to Motuo County, Tibet Autonomous Region, China (Figure 1, Ju et al. 2023).

As described before, *Primula medogensis* thrives among mosses, typically found in the moist fissures of wet cliffs and the surrounding boulders (Ju et al. 2023). It is classified under *P.* sect. *Cordifoliae* and is morphologically closest to *P. baileyana* Kingdon-Ward. 1926 and *P. rotundifolia* Wall. 1916. However, it can be distinguished by its hairy roots and a petiole that is more than three times the length of the leaf blade, which serve as important diagnostic features (Ju et al. 2023). The limited known distribution of this species, along with its undefined conservation status, underscores the need for immediate further studies. As of April 24, 2024, only 63 chloroplast genome sequences for *Primula* species are available, representing approximately 8% of the total species diversity within the genus.

Therefore, in this study, we aim to delineate the complete chloroplast genome of *P. medogensis*, examining its phylogenetic relationships with other closely related species at the chloroplast genome scale. Such an investigation seeks to address the scarcity of chloroplast genome data within this genus, concurrently offering novel molecular insights into the phylogeny and conservation biology of *Primula*.

Materials and methods

The fresh leaves of *P. medogensis* (Figure 1) were collected from Motuo County, Xizang Province, China (31°04′N, 103°11′E, elevation ca. 3607 m) on 18 May 2021. The voucher specimen (voucher: *YLZB07293*; contact person: Bo Xu, xubo@cib.ac.cn) has been deposited at the Herbarium of the Chengdu Institute of Biology (CDBI), Chinese Academy of Sciences. Genomic DNA was extracted from silica-gel dried leaves through Plant DNA Isolation Kit (Cat. No. DE-06111) and sequenced *via* Illumina pair-end technology. The raw sequencing data were processed using fastp v0.23.2 (Chen et al. 2018) for quality control and data cleaning. Trimming of low-quality bases and adapter sequences removal were performed according to the default parameters of the fastp tool. The chloroplast genome was assembled using

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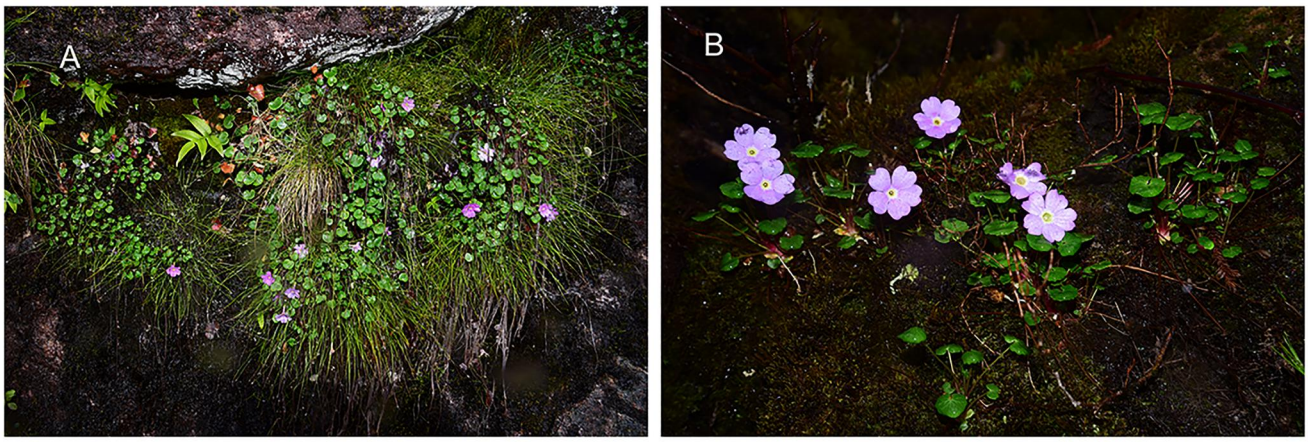

Figure 1. Habitat (A) and flowering plants (B) of *Primula medogensis* by W.B. Ju.

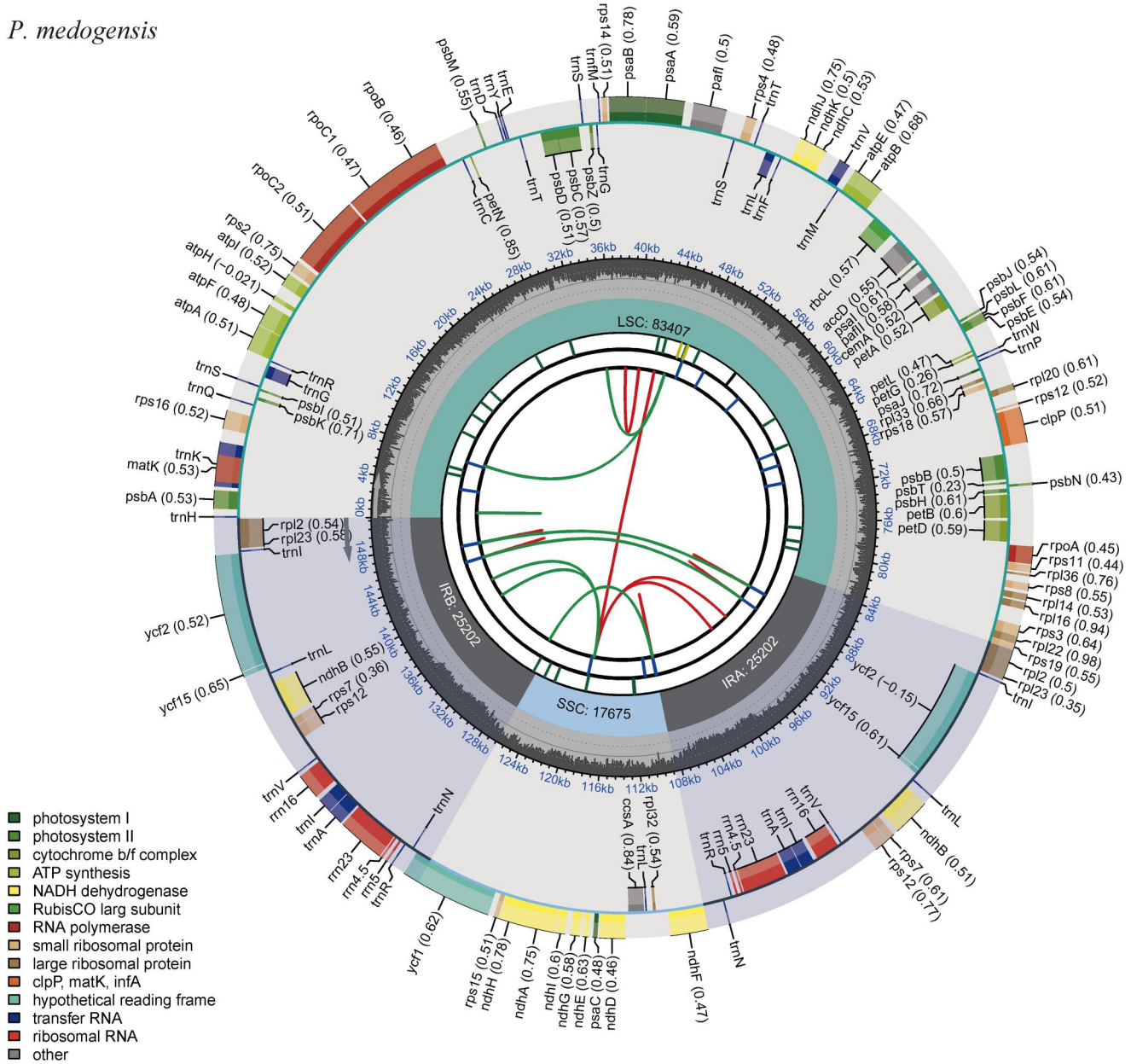

Figure 2. Chloroplast genome map of *Primula medogensis*. The map was generated by CPGView. Genes located on the inner and outer of circle are transcribed clockwise and anticlockwise, respectively. The dark grey inner circle indicates GC content. Large single-copy (LSC), small single-copy (SSC), and inverted repeats (IRA and IRB) are indicated in the inner layer. The functional classification of the genes is provided in the bottom left corner.

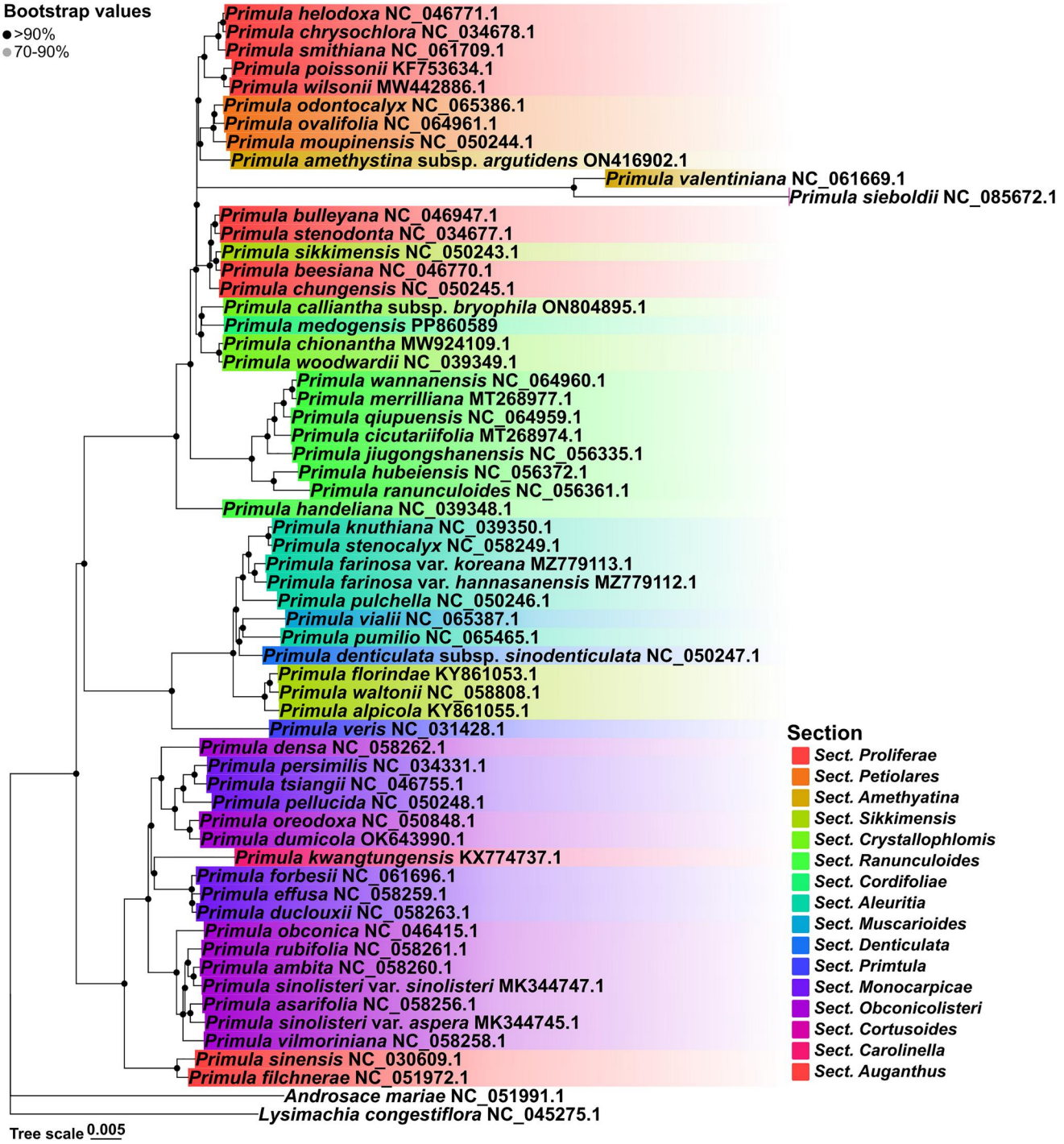

Figure 3. ML phylogenetic tree of *Primula* obtained from 61 complete chloroplast sequences. The 60 species were *Lysimachia congestiflora* (NC_045275.1, outgroup) (Li et al. 2019), *Androsace mariae* (NC_051991.1), *P. chrysochlora* (NC_034678.1) (Zhang et al. 2017), *P. helodoxa* (NC_046771.1) (Zhang et al. 2019). *P. smithiana* (NC_061709.1), *P. wilsonii* (MW442886.1) (Xie et al. 2023), *P. poissonii* (KF753634.1) (Yang et al. 2014), *P. odontocalyx* (NC_065386.1), *P. ovalifolia* (NC_064961.1), *P. moupinensis* (NC_050244.1) (Zhang et al. 2016), *P. amethystina* subsp. *argutidens* (ON416902.1), *P. sikkimensis* (NC_050243.1) (Zhang et al. 2016), *P. beesiana* (NC_046770.1) (Wu et al. 2019), *P. stenodonta* (NC_034677.1) (Zhang et al. 2016), *P. bulleyana* (NC_046947.1) (Chen et al. 2019). *P. chungensis* (NC_050245.1) (Zhang et al. 2016), *P. woodwardii* (NC_039349.1) (Ren et al. 2018), *P. chionantha* (MW924109.1), *P. calliantha* subsp. *bryophila* (ON804895.1), *P. merrilliana* (MT268977.1) (Xu et al. 2020), *P. wannanensis* (NC_064960.1) (Xu et al. 2020), *P. qiupuensis* (NC_064959.1) (Xu et al. 2020), *P. cicutariifolia* (MT268974.1) (Xu et al. 2020), *P. jiugongshanensis* (NC_056335.1) (Xu et al. 2020), *P. ranunculoides* (NC_056361.1) (Xu et al. 2020), *P. hubeiensis* (NC_056372.1) (Xu et al. 2020), *P. handeliana* (NC_039348.1) (Ren et al. 2018), *P. knuthiana* (NC_039350.1) (Ren et al. 2018), *P. stenocalyx* (NC_058249.1), *P. farinosa* var. *hannasanensis* (MZ779112.1) (Kim et al. 2022), *P. farinosa* var. *koreana* (MZ779113.1) (Kim et al. 2022), *P. pulchella* (NC_050246.1) (Zhang et al. 2016), *P. pumilio* (NC_065465.1), *P. vialii* (NC_065387.1), *P. denticulata* subsp. *sinodenticulata* (NC_050247.1), *P. waltonii* (NC_058808.1), *P. florindae* (KY861053.1), *P. alpicola* (KY861055.1), *P. veris* (NC_031428.1) (Zhou et al. 2016), *P. persimilis* (NC_034331.1) (Zhang et al. 2016), *P. tsiangii* (NC_046755.1) (Chen et al. 2019), *P. pellucida* (NC_050248.1) (Zhang et al. 2016), *P. oreodoxa* (NC_050848.1), *P. dumicola* (OK643990.1), *P. sieboldii* (NC_085672.1) (Saneyoshi et al. 2005), *P. valentiniana* (NC_061669.1), *P. densa* (NC_058262.1) (Zhong et al. 2019), *P. duclouxii* (NC_058263.1) (Zhong et al. 2019), *P. effusa* (NC_058259.1) (Zhong et al. 2019), *P. forbesii* (NC_061696.1), *P. kwangtungensis* (KX774737.1) (Zhang et al. 2016), *P. ambita* (NC_058260.1) (Zhong et al. 2019), *P. sinolisteri* var. *aspera* (MK344745.1) (Zhong et al. 2019), *P. sinolisteri* var. *sinolisteri* (MK344747.1) (Zhong et al. 2019), *P. rubifolia* (NC_058261.1) (Zhong et al. 2019), *P. asarifolia* (NC_058256.1) (Zhong et al. 2019), *P. vilmoriniana* (NC_058258.1) (Zhong et al. 2019), *P. obconica* (NC_046415.1) (Zhang et al. 2019), *P. filchnerae* (NC_051972.1) (Liu et al. 2020), *P. sinensis* (NC_030609.1) (Liu et al. 2016).

GetOrganelle v1.7.5 with parameter '-R 10 -t 20 -w 0.6 -k 21,45,65,85,105 -F embplant_pt' (Jin et al. 2020) and annotated using Plastid Genome Annotator (Qu et al. 2019). The average coverage for the assembled cp genome was 3594.97× (Figure S1). Manual adjustments were performed by referencing homologous genes (genbank: NC_039350.1) of *P. medogensis* and corrections for start and stop codons in Geneious v11 (Matthew et al. 2012). Finally, the annotated chloroplast genome was visualized by CPGview (http://www.1kmpg.cn/cpgview).

The chloroplast genome sequences of 58 *Primula* species were downloaded from the NCBI database. Two species from closely related genera, *Lysimachia congestiflora* Hemsl. 1889 (accession number: NC_045275.1) and *Androsace mariae* Kanitz 1891 (accession number: NC_051991.1) were included as outgroup. Then, the sequences of all genomes were aligned using MAFFT v7.475 (Katoh and Standley 2013). The best-fit nucleotide substitution model (TVM+F + R3) for the matric was estimated using the software jModelTest v2.1.6 (Posada 2008). The phylogenetic tree was reconstructed using the maximum likelihood method (ML) implemented in IQ-TREE v2.0.3 (Bui et al. 2020) and visualized using the Chiplot online site (Xie et al. 2023).

Results

The complete chloroplast genome of *P. medogensis* comes in a typical quadripartite structure and spans a length of 151,486 bp (accession number: PP860589; Figure 2). The chloroplast genome comprises a large single-copy (LSC) region measuring 83,407 bp and a small single-copy (SSC) region spanning 17,675 bp, which are separated by a pair of inverted-repeat (IR) regions, each 25,202 bp in length. In total, 131 genes were annotated, including 86 protein-coding (CDS), 37 transfer RNA (tRNA), and 8 ribosomal RNA (rRNA) genes. Among them, 11 genes (*rps16, atpF, petB, petD, rpl16, rpl2*2, ndhA, ndhB*2, rpoC1, trnA-UGC*2, trnG-UCC*2, trnI-GAU*2, trnK-UUU*2, trnL-UAA, trnV-UAC*) contain one intron, and four genes (*pafI*, *clpP, rps12*2*) contain two introns (Figure S2). The trans-splicing gene *rps12* had three unique exons (Figure S3). The overall GC content of the chloroplast genome was 37.1%.

The phylogeny reconstructed based on chloroplast genomes of 59 *Primula* species indicated that *Primula* can be roughly divided into three major clades (Figure 3). Clade I consists of the *P.* sects. *Prolifera*, *Petiolares*, *Amethyatina*, *Crystallophlomis* and *Ranunculoides*. Clade II consists of *P.* sects. *Aleuritia*, *Denticulata*, *Muscarioides*, *Sikkimensis* and *Primtula*. Clade III consists of *P.* sects. *Obconicolisteri*, *Cortusoides*, *Carolinella* and *Auganthus*. *Primula medogensis* was closely related to *P. calliantha* subsp. *bryophila*, which belongs to *P.* sect. *Crystallophlomis*.

Discussion and conclusion

In this study, the chloroplast genome sequence of *P. medogensis* was reported for the first time. The genome size, gene content, and gene order are similar to previously published chloroplast genomes in *Primula* (Xu et al. 2020). Results show that *P. medogensis* was nested within *P.* sect. *Crystallophlomis*. However, due to the lack of sequence information for other species from *P.* sect. *Cordifoliae*, the phylogenetic tree in this study cannot reflect which section *P. medogensis* belongs to. Moreover, this study used a relatively small number of species for phylogenetic analysis (less than 10% of the entire genus), and only utilized the chloroplast genome as a molecular marker for phylogenetic analysis. Therefore, further in-depth research on the phylogenetic relationships within the genus *Primula* is recommended. The results and findings will provide a vital phylogenetic framework for future studies on population genetics and conservation efforts in *Primula*.

Ethical approval

The collection of leaf samples conformed to the requirement of international ethics, which did not cause damage to the local environment. No endangered or protected species were involved in the study, and the collecting of the samples did not require specific permission from authorities.

Authors' contributions

YF and YJW designed the study. JTL, WBJ, XL, YZ, and TYC performed data analysis and drafted the manuscript. YF and YJW revised the manuscript. All authors reviewed and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The chloroplast genome of *P. medogensis* was publicly available in NCBI GenBank database with accession number PP860589 (https://www.ncbi.nlm.nih.gov/nuccore/PP860589). Raw sequencing data were deposited to the Genome Sequence Archive (GSA, CRA017623) of China National Center for Bioinformation (CNCB) under the BioProject number PRJCA027723 (https://ngdc.cncb.ac.cn/bioproject/browse/PRJCA027723).

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