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PLASTOME REPORT

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The complete chloroplast genome of *Meconopsis torquata* (Papaveraceae), a traditional Tibetan medicine

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ABSTRACT

Meconopsis torquata Prain 1906, a national second-class rare and endangered plant, is reported here for the first time for its complete chloroplast genome. The genome is 153,290 bp in length, comprising a large single-copy region (LSC, 83,918 bp), a small single-copy region (SSC, 17,740 bp), and two inverted repeat sequences (IRa and IRb, each 25,816 bp). The overall GC content is 38.7%, with the IR region having the highest content (43.1%). The genome is annotated with 112 unique genes, including 4 rRNA genes, 29 tRNA genes, and 79 protein-coding genes. Analysis of codon usage bias reveals that codons ending in A/T account for 96.7% of those with a Relative Synonymous Codon Usage (RSCU) value above 1. This predominance of A/T-ending codons might be indicative of *M. torquata* adaptation to high-altitude environments. Phylogenetic analysis reveals a close kinship between *M. torquata* and *M. pinnatifolia* and *M. paniculata*, indicating that the ancestral groups of these species might have a complex evolutionary history. This study uncovers the genetic characteristics and adaptive evolution of *M. torquata*, offering a new perspective in understanding the phylogenetic relationships within the genus. The findings not only provide a solid theoretical foundation for the conservation and sustainable use of this rare and endangered species but also offer significant scientific support for the conservation of biodiversity.

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Meconopsis torquata; Papaveraceae; chloroplast genome; phylogenetic analysis

Introduction

Meconopsis, belonging to the Papaveraceae family, comprises over 70 species, predominantly distributed in the Himalayas, the Tibetan Plateau, and the Hengduan Mountains (Xiao and Simpson 2017; Shi et al. 2022). These species have adapted to various ecological conditions ranging from 2000 to 5800 m in altitude, including temperate forests, alpine meadows, scree slopes, and snow zones (Xie et al. 2014). The Eastern Himalayan-Hengduan Mountains region, in particular, is a center of diversity for the *Meconopsis*, playing a pivotal role in maintaining local ecosystem balance and biodiversity (Duffy et al. 2017; Shi et al. 2022). Known for their diverse flower colors and elegant forms, *Meconopsis* species are not only valuable resources for horticultural cultivation but also widely used in traditional Tibetan medicine for their unique medicinal values. Classical texts, such as The Four Medical Tantras and Jing Zhu Materia Medica, document their utilization in treating inflammation and pain. Modern pharmacological studies have confirmed that the isoquinoline alkaloids and flavonoid compounds present in these species exhibit a range of biological activities, including anti-tumor, hepatoprotective, analgesic, antibacterial, antioxidant, antitussive, and anti-inflammatory effects (Guo et al. 2016). However, *Meconopsis* species are facing severe challenges due to habitat fragility and human disturbances, leading to several species, including *Meconopsis torquata* Prain, being listed as national second-class protected plants (Shi et al. 2022). This situation underscores the urgent need for the conservation and sustainable use of their genetic resources and habitats. Despite this, chloroplast genome studies on *M. torquata* and other *Meconopsis* species remain insufficient. This study reports the complete chloroplast genome sequence of *M. torquata*, aiming to provide crucial molecular data for the formulation of its conservation strategies and understanding of its evolutionary history.

Materials and methods

Plant material, DNA extraction and sequencing

In this study, fresh leaf samples of *M. torquata* were collected from Lhasa, Tibet, China (91°3′6.85″, 29°42′52.76″; altitude 4739 m) (Figure 1). The collection and identification were

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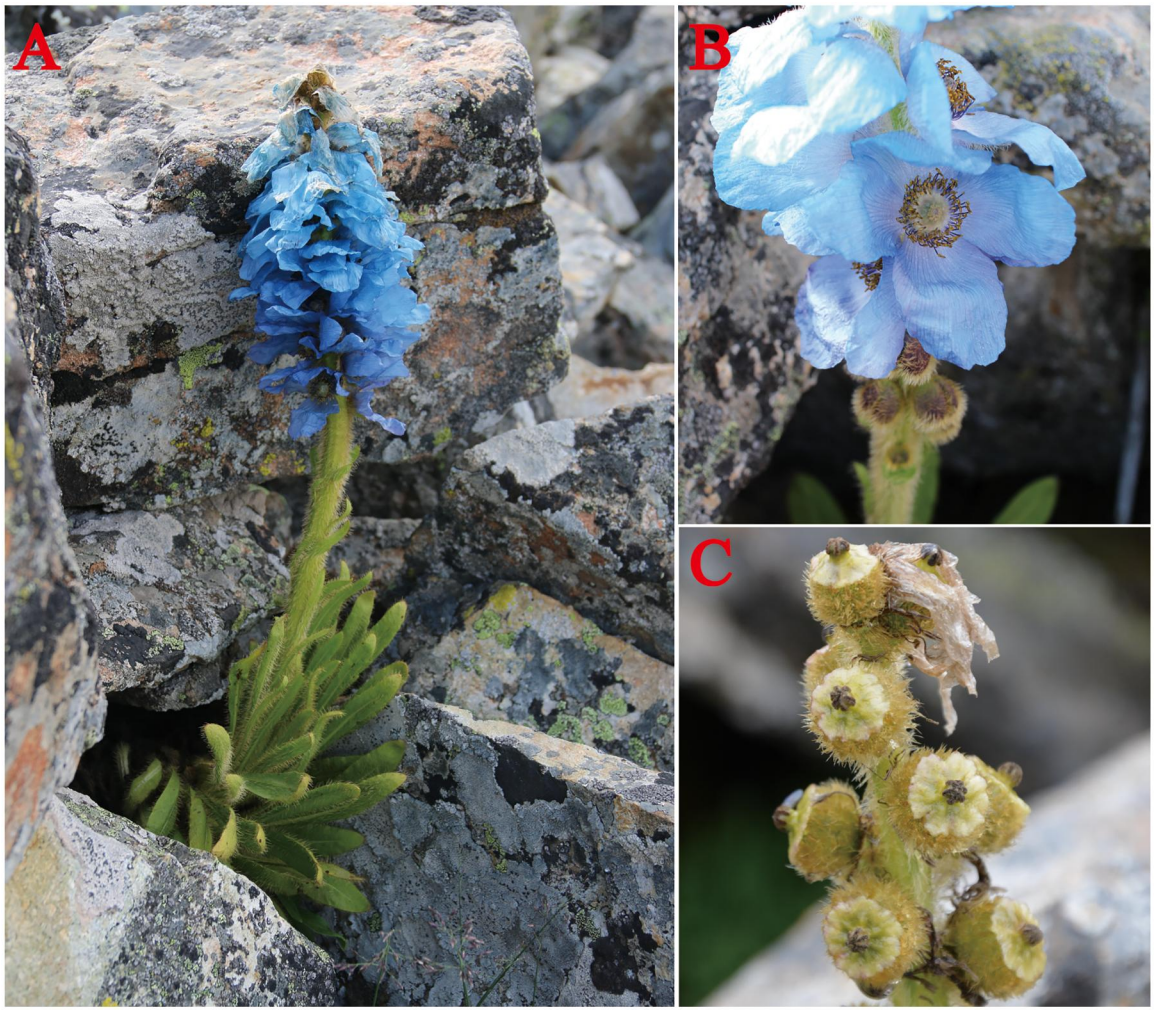

Figure 1. The morphological characteristics of *M. torquata*. (A) Habit, (B) Inflorescence, (C) capsule fruit. The photograph was taken by junwei Wang, La Qiong, and Min Xu in Lhasa, Tibet, China. The most characteristic features of the specimen include: an annual herbaceous plant with basal, rosette-forming leaves; large, blue flowers that are densely arranged at the stem's tip; the fruit is an inverted-ovate capsule.

conducted by Jun-Wei Wang (email: jwyx12240315@126.com). The leaf samples were dried with silica gel and then stored at −20 °C for further use. The voucher specimens are preserved in the Key Laboratory of Biodiversity and Environment on the Qinghai-Tibetan Plateau, Ministry of Education, School of Ecology and Environment, Tibet University, Lhasa 850000, China, with the voucher number wang20180019. Total DNA was extracted using a modified CTAB method (Li et al. 2013). The extracted DNA samples were assessed for integrity and concentration, then used to construct a 150 bp paired-end sequencing library, sequenced on the Illumina NovaSeq X platform at Novogene Co., Ltd., Beijing, China. This produced 45,332,282 raw sequencing reads, and after quality filtering, 44,681,980 high-quality reads were obtained with a Q20 ratio of 97.37%.

Genome assembly and annotation

Under the Linux operating system, the chloroplast genome of *M. torquata* was assembled using GetOrganelle v1.7.7 (Jin et al. 2020) with default parameters. Coverage of the assembly results was calculated using a script provided by Ni et al. (2023) (Figure S1). The chloroplast genome annotation was initially performed using the online tools CPGAVAS2 (Shi et al. 2019) and GeSeq (Tillich et al. 2017), followed by manual corrections in Geneious Prime (Kearse et al. 2012), including adjustments to intron and exon boundaries of protein-coding genes and the removal of annotations with low coverage. The genome map was drawn using CPGView (Liu et al. 2023). Additionally, codon usage bias analysis of the annotated protein-coding sequences was performed using CodonW1.4.4 (http://codonw.sourceforge.net/), with subsequent visualization conducted on the bioinformatics cloud platform (http://112.86.217.82:9919/#/home) (Figure S2). The complete annotated chloroplast genome sequence was converted to GenBank format using GB2sequin (Lehwark and Greiner 2019) and submitted to the GenBank database.

Phylogenetic analysis

To clarify the phylogenetic position of *M. torquata*, this study employed both Maximum Likelihood (ML) and Bayesian Inference (BI) methods to construct the phylogenetic relationship within the *Meconopsis*. All sequences, except for *M. torquata*, were downloaded from the NCBI database. The 14 sequences were aligned using MAFFT v7.453 (Katoh and

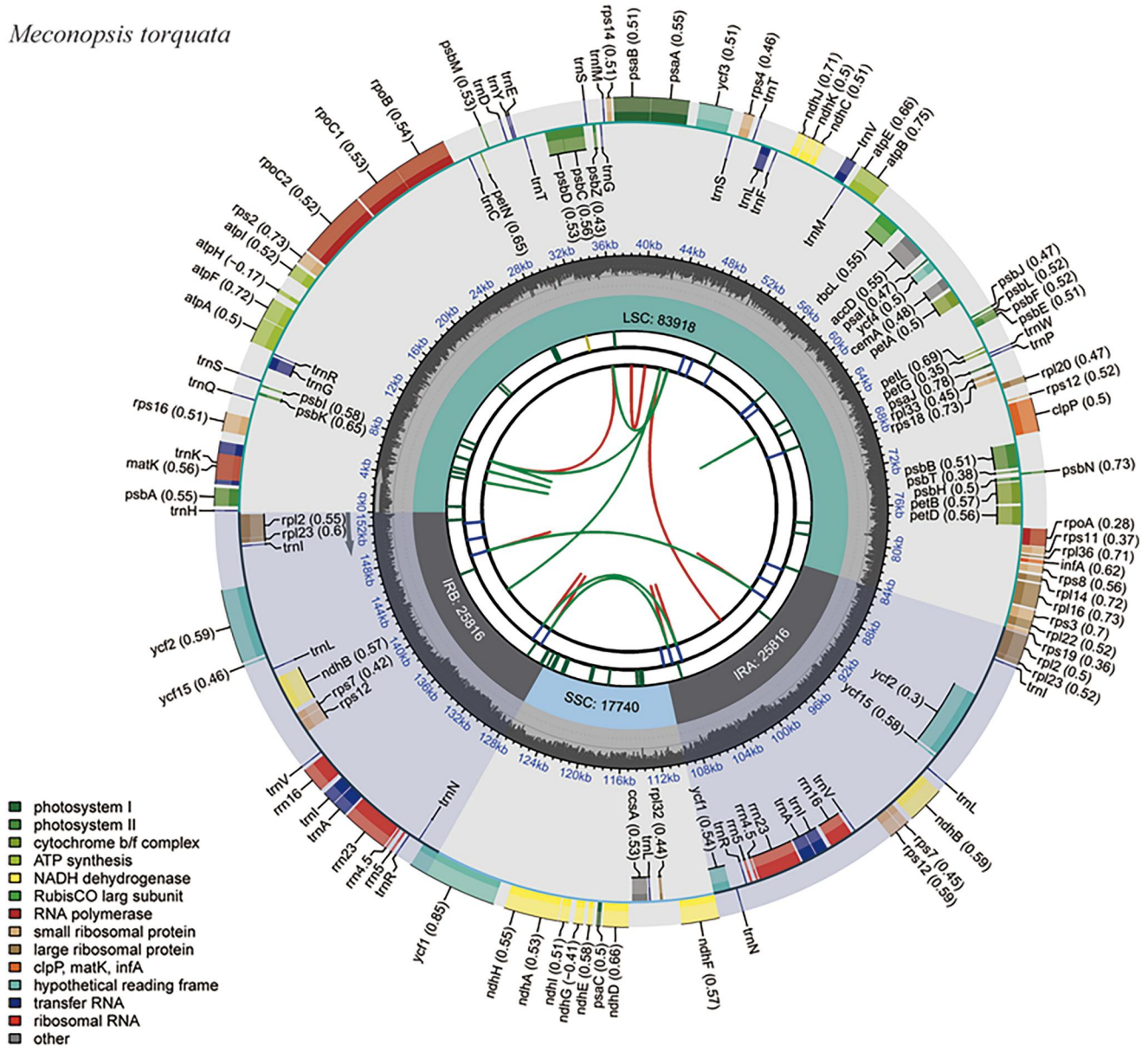

Figure 2. Chloroplast genome map of *M. torquata*. From the center outward, the map is composed of six concentric rings. The first circle represents the forward and reverse repeats, connected with red and green arcs, respectively. The second circle marks the tandem repeats. The third circle displays the microsatellite sequences. The fourth circle indicates the sizes of feature regions, including a large single-copy (LSC), a small single-copy (SSC), and two inverted repeats (IRa and IRb). The fifth circle exhibits the GC content distribution. The sixth circle displays genes organized by function, with their associated codon usage bias indicated in parentheses following each gene name.

Standley 2013), and the alignment was manually corrected in Geneious Prime. Phylogenetic analysis was conducted in Phylosuite v1.2.2 (Zhang et al. 2020), employing Gblocks 0.91b (Talavera and Castresana 2007) to select conserved regions, and the best nucleotide substitution model was calculated using ModelFinder v.1.6.8 (Kalyaanamoorthy et al. 2017) based on the Bayesian Information Criterion (BIC). Maximum Likelihood analysis was performed using IQTREE (Nguyen et al. 2015), with 1000 bootstrap replications for confidence assessment. Bayesian Inference analysis was conducted in Mrbayes v3.2 (Ronquist et al. 2012), running for 2,000,000 generations, sampling every 100 generations, with the first 25% of trees discarded as burn-in. The constructed tree file was viewed using FigTree 1.4.4 (http://tree.bio.ed.ac.uk/software/Figtree/), considering branches with Bootstrap Support (BS) >75 and Posterior Probability (PP) >95% as highly credible.

Results

The complete chloroplast genome of *M. torquata* is 153,290 bp in length with an average sequencing depth of 2405.66× (Figure S1). The chloroplast genome exhibited a typical quadripartite structure, consisting of a large single-copy region (LSC, 83,918 bp), a small single-copy region (SSC, 17,740 bp), and two inverted repeat regions (IRa and IRb, each 25,816 bp) (Figure 2). The overall GC content of the genome is 38.7%, with significant heterogeneity across different

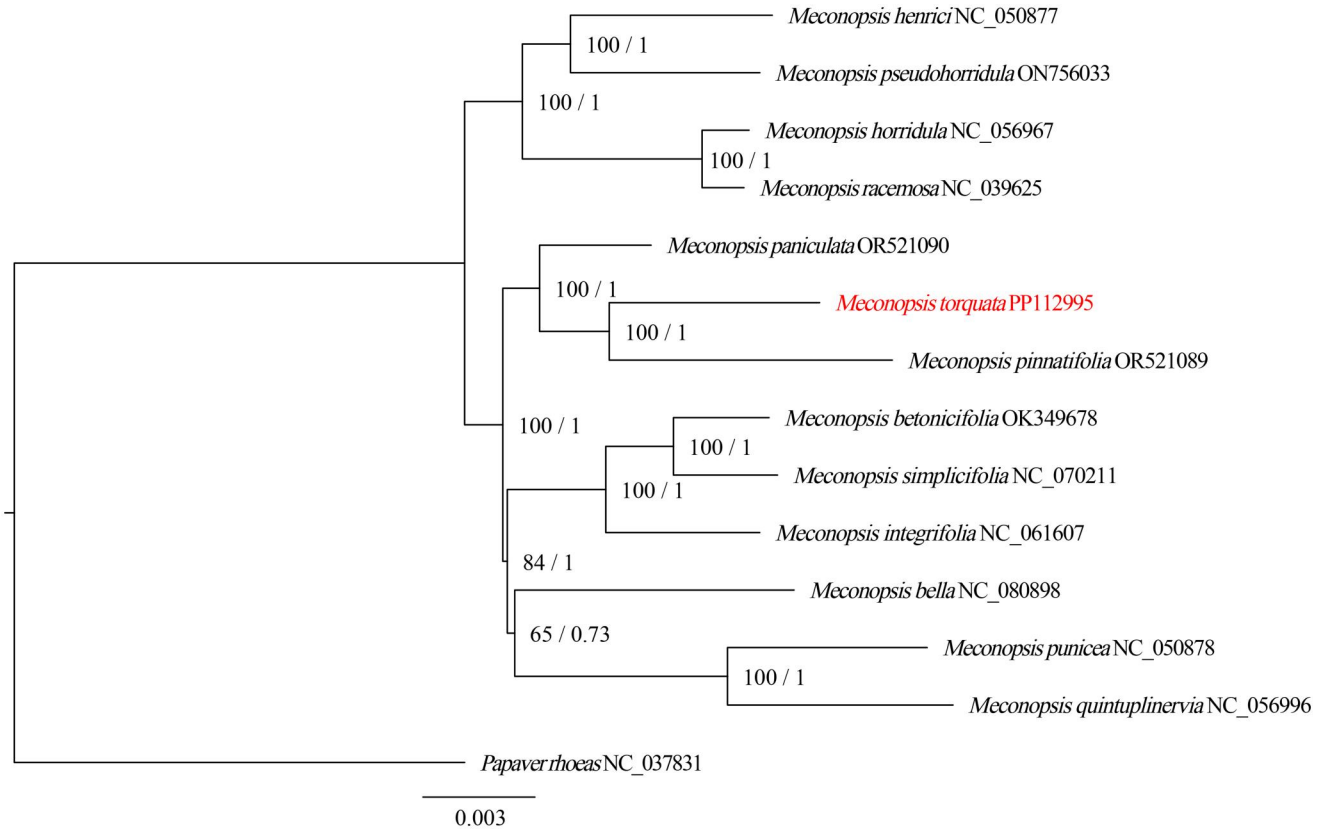

Figure 3. Phylogenetic tree of the complete chloroplast genomes in the genus *meconopsis*. The ML bootstrap (BS) values and BI posterior probabilities (PP) supporting each node are indicated beneath the branches. The sequences used were: *M. bella* NC_080898 (unpublished), *M. betonicifolia* OK349678 (unpublished), *M. henrici* NC_050877 (Zhu and Zhang 2020), *M. horridula* NC_056967 (Dan et al. 2021), *M. integrifolia* NC_061607 (Li et al. 2020), *M. paniculata* OR521090 (unpublished), *M. pinnatifolia* OR521089 (unpublished), *M. pseudohorridula* ON756033 (unpublished), *M. punicea* NC_050878 (Zhu and Zhang 2020), *M. quintuplinervia* NC_056996 (Xu et al. 2019), *M. racemosa* NC_039625 (Zeng et al. 2018), *M. simplicifolia* NC_070211 (Yang et al. 2023), and *papaver rhoeas* NC_037831 (Zhou et al. 2018) as the outgroup.

regions; the GC contents of the IR, LSC, and SSC regions are 43.1%, 37.2%, and 33.1%, respectively.

A total of 112 unique genes were identified, including 4 rRNA genes, 29 tRNA genes, and 79 protein-coding genes (Figure 2). Most genes exist in a single-copy form, while 20 genes are duplicated in the IR regions, including 8 protein-coding genes, 8 tRNA genes, and 4 rRNA genes. Furthermore, 18 genes containing introns were discovered, with *clp*P, *rps*12, and *ycf*3 each containing two introns, and other genes like *atp*F, *ndh*A, *ndh*B each containing one intron. Additionally, the chloroplast genome contains 11 cis-spliced genes and one trans-spliced gene (*rps*12) (Figure S3). As shown in Figure S2, 30 codons with RSCU $>$ 1 exhibit a higher usage frequency compared to other synonymous codons. Notably, 29 of these codons terminate in an A/T base, representing 96.7% of the total.

The phylogenetic tree constructed in this study shows high consistency between the results of the Maximum Likelihood (ML) and Bayesian Inference (BI) methods, with the bootstrap support values (BS) of the ML tree and posterior probabilities (PP) of the BI tree marked on the respective branches (Figure 3). The phylogenetic analysis indicates that the relationships among most species within the *Meconopsis* are clearly resolved, with *M. torquata* being most closely related to *M. pinnatifolia* and *M. paniculata*. These species are predominantly distributed in the southeastern region of Tibet.

Discussion and conclusion

The chloroplast genome structure of *M. torquata* is similar to that of most angiosperms, exhibiting a typical quadripartite structure (Jansen et al. 2005). This finding aligns with the general range of terrestrial plant chloroplast genomes in angiosperms (120–160 kb, encoding about 110–130 unique genes) (Dobrogojski et al. 2020), indicating a distinct conservatism in genome size and gene content in *M. torquata*. Codon usage bias is a universal phenomenon in nature, formed over the course of evolution, and influenced by factors such as mutation, natural selection, gene length, and function (Parvathy et al. 2022). In *M. torquata*, an exceptionally high 96.7% of codons with an RSCU value greater than 1 end in A/T, far exceeding other species. This characteristic may be closely related to its evolutionary adaptation to high-altitude environments, reflecting the impact of specific environments on genomic adaptive changes (Chen et al. 2022).

The use of chloroplast genome data in plant phylogenetic analyses has become increasingly widespread (Parks et al. 2009). Based on the existing chloroplast genome sequences of the *Meconopsis*, this study analyzed their phylogenetic

relationships. The results reveal a close kinship between *M. torquata*, *M. pinnatifolia*, and *M. paniculata*. These species are distributed in the southeastern region of Tibet, suggesting that their ancestral groups may have had a broad distribution in this area and could have evolved diversely due to geographical isolation.

The in-depth analysis of the *M. torquata* chloroplast genome in this study not only reveals its unique genetic characteristics and adaptive evolution but also provides a new perspective in understanding the phylogenetic relationships within the *Meconopsis*. These findings are significant for understanding the impact of high-altitude environments on plant genome evolution and provide foundational data for biodiversity and ecological research of *Meconopsis* species in the southeastern region of Tibet. Furthermore, the comprehensive analysis of chloroplast genome structure, codon usage bias, and phylogenetic relationships lays an important foundation for future research in the fields of botany, genetics, and ecology.

Ethical approval

This study does not involve any research with human participants or animals conducted by any of the authors. We have strictly adhered to the "Regulations of the People's Republic of China on the Protection of Wild Plants", the International Union for Conservation of Nature (IUCN 2015) policies on research involving endangered species, the "Convention on Biological Diversity", and the "Convention on International Trade in Endangered Species of Wild Fauna and Flora". Throughout the research process, we have rigorously followed the relevant laws and regulations to ensure the legality and ethical integrity of the study.

Author contributions

Jun-Wei Wang and Zhe-Fei Zeng conceived and designed the study. Jun-Wei Wang, La Qiong, and Min Xu collected the plant samples. Junwei Wang and Zhe-Fei Zeng carried out the experiments and analyzed the data. Zhe-Fei Zeng wrote the initial draft, which was revised by La Qiong and Jun-Wei Wang. All authors contributed to the article and approved the submitted manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The complete chloroplast genome sequence of M. torquata is available in the NCBI GenBank database at https://www.ncbi.nlm.nih.gov/, under the accession number PP112995. The related project information can be found under the BioProject number PRJNA1063898. The corresponding BioSample and Sequence Read Archive (SRA) numbers are SAMN39412272 and SRR27499558, respectively.

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