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PLASTOME REPORT

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The complete plastid genome of *Polygonatum gracile* P. Y. Li (Asparagaceae): characterization and phylogeny

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ABSTRACT

Polygonatum gracile P. Y. Li is a perennial rhizomatous medicinal herb endemic to China with recognized medicinal value. Here, the first complete plastid genome of *P. gracile* is reported and characterized, and the phylogenetic relationships of *P. gracile* with other *Polygonatum* species are clarified by phylogenetic inferences based on complete plastid genome sequences. The complete plastid genome of *P. gracile* is 155,734 bp in length and exhibits the typical quadripartite circular structure consisting of a large single-copy (LSC, 84,385 bp) region, a small single-copy (SSC, 18,519 bp) region, and a pair of inverted repeat regions (IRa/b, 26,415 bp). A total of 112 unique genes, including 78 protein-coding genes, 30 tRNA genes, and four rRNA genes were identified. Seven protein-coding genes, eight tRNA genes, and four rRNA genes were duplicated in the IR regions. Phylogenetic reconstructions indicated that *P. gracile* belongs to sect. *Verticillata* and is most closely related to *P. zanlanscianense* Pamp. This study provides a basis for further phylogenetic reconstructions, conservation and utilization of *Polygonatum* species.

GRAPHICAL ABSTRACT

Introduction

Polygonatum Mill. is the largest genus in tribe Polygonateae of Asparagaceae, which comprises approximately 78 accepted species (https://powo.science.kew.org/) widely distributed in the Northern Hemisphere. *Polygonatum* species are popular in China for their significant medicinal value (Zhang et al. 2014). Accordingly, taxonomy and phylogeny of *Polygonatum* have received considerable attention since the establishment

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Figure 1. Photographs of *Polygonatum gracile* P. Y. Li. taken by Ming-ying Zhang in Taibai County, Shaanxi province, China (34.054706 N, 107.338426 E). (A) Plant and habit, (B) flowers, and (C) rhizome. *Polygonatum gracile* is a perennial rhizomatous herb 10–30 cm in height; leaves are commonly verticillate, inflorescences usually exhibit two flowers; perianths are pale yellow; rhizome is terete with 2–3 mm in diameter.

of the genus. Numerous morphological and karyological characters, complemented by molecular evidence have been used to address phylogenetic relationships and classify *Polygonatum* species (Baker 1875; Tang 1978; Tamura 1993; Meng et al. 2014; Zhao et al. 2019; Xia et al. 2022). The most widely accepted infrageneric classification, confirmed by multiple subsequent phylogenetic studies, subdivided *Polygonatum* into three sections: section (sect.) *Polygonatum*, sect. *Sibirica*, and sect. *Verticillata* (Meng et al. 2014). However, the interspecific relationships of some species in sect. *Polygonatum* and sect. *Verticillata* are still to be clarified (Flodena and Schillingb 2018; Zhao et al. 2019; Xia et al. 2022).

Polygonatum gracile P. Y. Li (1966) (Figure 1) is a perennial rhizomatous medicinal herb endemic to China, which is mainly confined to southeast Gansu, Shaanxi (Qinling Mountains) and south Shanxi provinces (Chen and Tamura 2000). The rhizome of *P. gracile* has traditional and scientifically assessed medicinal value (Zhang et al. 2014). Nevertheless, this species has rarely been considered in previous phylogenetic research. Zhao et al. (2019)'s phylogenetic analyses utilizing four plastid DNA regions (*atp*B-*rbc*L, *mat*K, *rbc*L, and *rps*16) indicated *P. gracile* belongs to sect. *Verticillata*. However, the phylogenetic relationships of *P. gracile* with other *Polygonatum* species remain to be solved. Besides, the plastid genome of *P. gracile* has not been reported yet.

In this study, the complete plastid genome of *P. gracile* was investigated for the first time, and phylogenetic analyses using complete plastid genome sequences were performed to elucidate the phylogenetic relationships of this taxon with other *Polygonatum* species.

Materials and methods

Plant material, DNA extraction, and sequencing

Fresh leaves of *P. gracile* were obtained from Taibai County, Shaanxi province, China (34.054706 N, 107.338426 E), and preserved in silica. The voucher specimen was deposited in the herbarium of traditional Chinese Medicine, Shaanxi University of Chinese Medicine (Ming-ying Zhang, 2051075@sntcm.edu.cn) under the voucher number XGJ001CP28. Total genomic DNA was extracted from silica-dried leaves utilizing the Hi-DNAsecure Plant kit (DP350, TIANGEN, Beijing, China). The qualified total genomic DNA was fragmented for library construction using the NEBNext Ultra™ II DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA). Paired-end (2 × 150 bp) sequencing was conducted on the HiSeq X Ten platform at the BGI Company (Wuhan, China).

Genome assembly and annotation

Approximately, 6 GB raw data were generated with total 12,266,742 paired-end reads. Raw data were trimmed by removing adapters and low-quality reads using the NGS QC Toolkit v.2.3.3 (Patel and Jain 2012). The cutoff value for percentage of read length was 80, and that for PHRED quality score was 30, with 1.24% of low-quality reads being discarded. Trimmed high-quality reads were assembled into contigs using GetOrganelle v.1.7.5 with the recommended script (Jin et al. 2020) using the complete plastid genome of *P. verticillatum* (GenBank accession: MZ150866) as a reference (seed sequence). The assembled contigs were then connected to generate a complete circular plastid genome with the redundant sequences removed using Bandage v.0.8 (Wick et al. 2015). The directions of the two inverted repeat regions were determined in Geneious v.8.0.2 (Kearse et al. 2012) with the same reference genome of *P. verticillatum* (MZ150866). The assembled complete plastid genome was then annotated by CPGAVAS2 (Shi et al. 2019). The plastid genome diagram was drawn by OrganellarGenomeDRAW (OGDRAW) 1.3.1 (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html) (Greiner et al. 2019). The complete plastid genome sequence was deposited in GenBank (accession no.: OM219009).

Phylogenetic analysis

There are 36 accepted *Polygonatum* species (excluding the unidentified, hybrid and unverified ones) with plastid genome sequences available in GenBank (https://www.ncbi.nlm.nih.gov, accessed 25 July 2024). To figure out the phylogenetic relationships of *P. gracile* with other *Polygonatum* species, all those 36 species, each represented by one sequence, were included for phylogenetic reconstruction. *Heteropolygonatum*

alternicirrhosum (Hand.-Mazz.) Floden and *Disporopsis aspersa* (Hua) Engl. ex K. Krause were selected as outgroups. Their plastid genome sequences were retrieved from GenBank. Sequences those were produced in published studies were preferred selected (Supplementary Table S1). Together with that of the newly sequenced *P. gracile* in the present study, all 39 plastid genome sequences were aligned in MAFFT (Katoh and Standley 2013) under default settings with manual adjustment in Geneious v.8.0.2.

Maximum-likelihood (ML) and Bayesian's methods were employed for phylogenetic inferences. ML tree was inferred by the RAxML-HPC BlackBox under the GTR + G model with 1000 bootstrap replicates (Stamatakis 2014). Bayesian's inference was conducted with MrBayes v.3.2.7a (Ronquist et al. 2012), the nucleotide substitution model (GTR + I + G) and parameter settings were determined based on the Akaike information criterion (AIC) using JModelTest (Darriba et al. 2012). Two independent Markov chain Monte Carlo (MCMC) runs each with four chains were computed for 10 million generations and sampling trees every 1000 generations. The first 25% of the calculated trees were discarded as burn-in and the remaining trees were used to construct a consensus tree and estimate the posterior probabilities. Both ML and Bayesian inferences were performed on the CIPRES Science Gateway (Miller et al. 2010; https://www.phylo.org).

Results

Plastid genome characterization

The sequencing depth ranged from 35× to 1458× (average 1086.3×, Supplementary Figure S1). The complete plastid
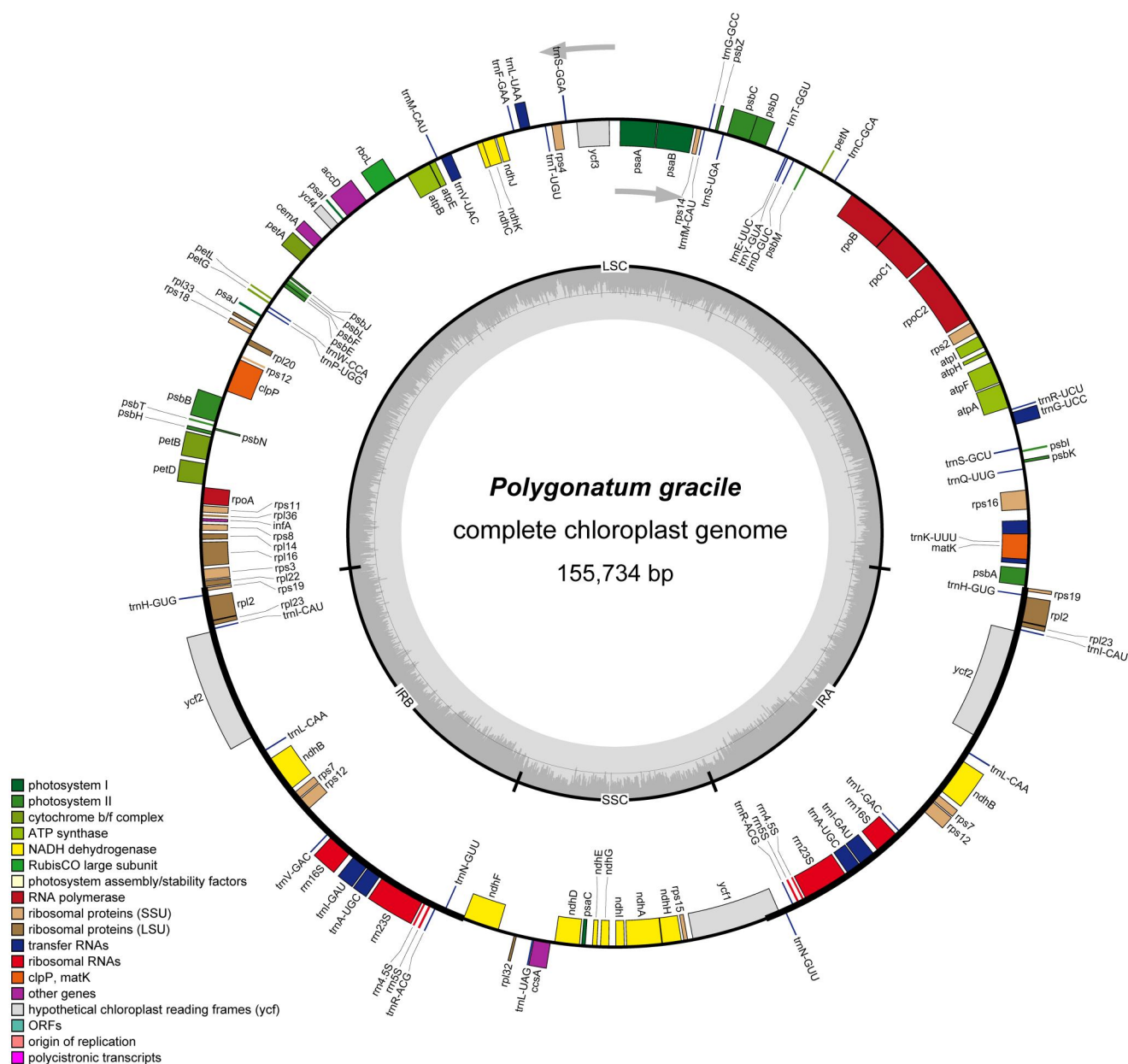
Figure 2. The plastid genome map of *P. gracile* drawn by OrganellarGenomeDRAW. Genes inside and outside the circle are transcribed in clockwise and counter-clockwise directions, respectively. Genes are color-coded based on their functions. The grey area in the inner circle indicates the GC content of the chloroplast genome, and the middle gray line is the 50% threshold line.
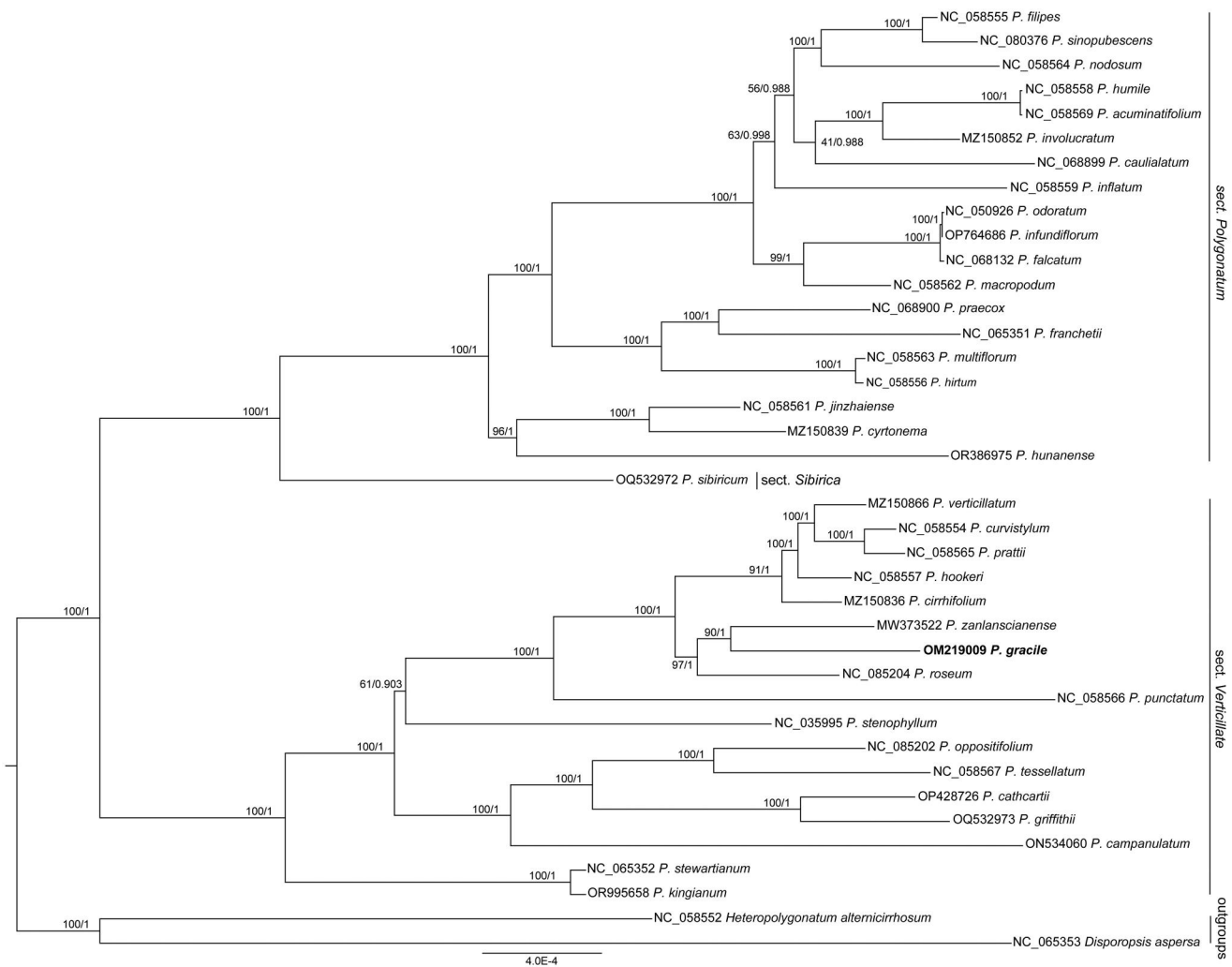
Figure 3. Phylogenetic tree of 37 *Polygonatum* species based on the complete plastid genome sequences, with *Heteropolygonatum alternicirrhosum* and *Disporopsis aspersa* as outgroups. Numbers along branches are bootstrap values (BS) from ML and posterior probabilities (PP) from Bayesian inference (BI). GenBank accession number for each plastid genome utilized for phylogenetic reconstruction is provided before the name of each taxon (see Table S1 for more details).

genome of *P. gracile* was 155,734 bp in length and exhibited the typical quadripartite circular structure including a large single-copy (LSC, 84,385 bp) region, a small single-copy (SSC, 18,519 bp) region, and a pair of inverted repeat regions (IRa/b, 26,415 bp). The GC content was 37.7%.

A total of 112 unique genes, including 78 protein-coding genes, 30 tRNA genes, and four rRNA genes were identified (Figure 2). Within which, seven protein-coding genes (*rps*7, *rps*12, *rps*19, *rpl*2, *rpl*23, *ycf*2, and *ndh*B), eight tRNA genes (*trn*H-GUG, *trn*I-CAU, *trn*I-GAU, *trn*L-CAA, *trn*V-GAC, *trn*A-UGC, *trn*R-ACG, and *trn*N-GUU), and four rRNA genes (*rrn*4.5, *rrn*5, *rrn*16, and *rrn*23) were duplicated in the inverted repeat regions. Besides, *rpl*2, *rpl*16, *rps*16, *rpo*C1, *atp*F, *pet*B, *pet*D, *ndh*A, and *ndh*B contained one intron, *ycf*3 and *clp*P harbored two introns, and *rps*12 gene was trans-spliced. The cis-splicing and trans-splicing genes are shown in Supplementary Figures S2 and S3.

Phylogenetic resolution

Maximum-likelihood and Bayesian inferences obtained the same phylogenetic topologies (Figure 3). All *Polygonatum* species were inferred forming a strongly supported monophyletic lineage

($BS_{ML} = 100/PP_{BI} = 1.00$) which could be further divided into three clades, corresponding to the previously recognized sect. *Verticillata* ($BS_{ML} = 100/PP_{BI} = 1.00$), sect. *Polygonatum* ($BS_{ML} = 100/PP_{BI} = 1.00$), and sect. *Sibirica* (contained only one species) (Meng et al. 2014; Xia et al. 2022). Among them, sect. *Verticillata* diverged first, sect. *Polygonatum*, and sect. *Sibirica* formed a sister relationship ($BS_{ML} = 100/PP_{BI} = 1.00$). *Polygonatum gracile* was inferred located in sect. *Verticillate* and most closely related to *P. zanlanscianense* with $BS_{ML} = 90/PP_{BI} = 1.00$.

Discussion and conclusions

Polygonatum gracile is a Chinese endemic medicinal herb, whose rhizome has acknowledged medicinal value. Yet, this species has received little attention in genomic research. In this study, the complete plastid genome of *P. gracile* was sequenced and described for the first time, which was demonstrated highly conserved in genome length, gene content and order, and GC content compared with other *Polygonatum* species (Wang et al. 2022; Cheng et al. 2023; Zhang et al. 2023).

Based on four plastid DNA regions, Zhao et al. (2019) grouped *P. gracile* in sect. *Verticillata*, but the phylogenetic

relationships of *P. gracile* with other *Polygonatum* species were unclear. Plastid genome sequences have been promoting species delimitation and phylogenetic resolution of plenty of angiosperm taxa including *Polygonatum* (Huang et al. 2021; Liu et al. 2022; Zhang et al. 2023; Chen et al. 2024). Utilizing the complete plastid genome sequences, our phylogenetic reconstructions confirmed the positioning of *P. gracile* in sect. *Verticillata*, as well as its closest phylogenetic affinity to *P. zanlanscianense*. These results will enhance further phylogenetic resolution, conservation and utilization of *Polygonatum* species.

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Author contributions

W.P.C. and J.H.C. conducted experiments, analyzed data, and wrote the manuscript, M.Y.Z. collected sample and specimen, identified species, and revised the manuscript. All authors contributed to this study and have read and agreed to the published version of the manuscript.

Ethics statement

According to the Wild Plants Protection Regulations of the People's Republic of China, *Polygonatum gracile* P. Y. Li is not included on the list of national protected wild plants, and does not belong to the IUCN Red List either. No specific permits are required for plant collection. The study does not require ethical approval or consent, as no endangered or protected plant species are involved.

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 (NCBI, https://www.ncbi.nlm.nih.gov/) under the accession number OM219009. The associated BioProject, SRA, and BioSample numbers are PRJNA1148805, SRR30274059, and SAMN43222264, respectively.

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