

PLASTOME ANNOUNCEMENT



Complete plastid genome of *Gentiana zollingeri* Fawcett (Gentianaceae) and phylogenetic analysis

Jing Yu^a, Pengcheng Fu^b and Shanshan Sun^b

^aAffiliated Hospital of Zhengzhou University, Zhengzhou, China; ^bSchool of Life Science, Luoyang Normal University, Luoyang, China

ABSTRACT

Gentiana zollingeri Fawcett (Gentianaceae) belongs to the most species-rich section, *Chondrophyllae*, in the Gentianaceae, but its phylogenetic relationship with other members of this section is unclear. To confirm its phylogenetic position, the complete plastid genome of *G. zollingeri* was determined and analyzed. The plastome was sequenced using the Illumina HiSeq platform, assembled with GetOrganelle, and annotated with GeSeq. The genome is circular with a length of 130,762 bp. It contains a large single-copy (LSC) region of 74,236 bp, a small single-copy (SSC) region of 10,598 bp, and two inverted repeat (IR) regions of 22,964 bp each. The plastome of *G. zollingeri* shows considerable structural differences from those of other *Gentiana* plastomes, such as the absence of the *ndh* gene. In phylogenetic analyses, section *Chondrophyllae*, including *G. zollingeri* and its sisters, formed a long branch sistering with section *Cruciata*. The plastome sequence described here represents an important contribution to phylogenetic and evolutionary studies on *Gentiana*.

ARTICLE HISTORY

Received 22 August 2022
Accepted 23 November 2022

KEYWORDS

Gentiana zollingeri;
plastome; phylogenetic
analysis

Gentiana is an alpine genus of about 360 species that are distributed worldwide, with the Qinghai-Tibet Plateau as the center of its distribution and diversity (Ho and Liu 2001; Favre et al. 2016). Out of the 13 sections in *Gentiana*, section *Chondrophyllae* Bunge is the most species-rich taxon, containing about 180 species (Ho and Liu 2001; Favre et al. 2020). Although many *Gentiana* plastome sequences are available, genome data are very limited for members of section *Chondrophyllae* (Fu et al. 2021). *Gentiana zollingeri* Fawcett, 1883 (Figure 1), having value in Chinese traditional medicine, belongs to section *Chondrophyllae* series *Fastigiatae* T.N. Ho, and is widely distributed in China, Russia, Korea, and Japan (Ho and Liu 2001).

Herein, we report the complete plastome of *G. zollingeri* (MZ934753) and its main characteristics. One *G. zollingeri* individual was collected from Liangjiatai Town, Hebei Province, China (40°46'N, 118°48'E). The voucher specimen was deposited at the Herbarium of Luoyang Normal University (Bin Cai, 987869364@qq.com) under the voucher number LP161491, and was identified by Dr. Pengcheng Fu. We extracted total DNA using a Dzip plant genomic DNA extraction kit (Sangon, Shanghai, China). The fragmented genomic DNA was sequenced using the Illumina HiSeq 2500 platform (Novogene, Tianjin, China), yielding 2 Gb clean data consisting of paired-end reads. The plastome was assembled using GetOrganelle version 1.7.1 (Jin et al. 2020) and annotated using GeSeq (Tillich et al. 2017) with the default parameters. The genome map was drawn using CPGview (<http://www.1kmpg.cn/cpgview>) with the GenBank accession number (MZ934753).



Figure 1. *Gentiana zollingeri*. *Gentiana zollingeri* is an annual, 3–6 cm tall. Stems purple; flowers few to many; corolla pale blue; stamens equal. Photographs by Pan Li.

The plastome was compared with those of species from the main clades of *Gentiana* (Zhou et al. 2018; Sun et al. 2018; Sun, Wang, et al. 2019; Fu et al. 2021), and structural changes were detected using mVISTA (Frazer et al. 2004). To confirm the phylogenetic position of *G. zollingeri*, the shared protein-

CONTACT Shanshan Sun ✉ sss823129@126.com School of Life Science, Luoyang Normal University, Luoyang, China

© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

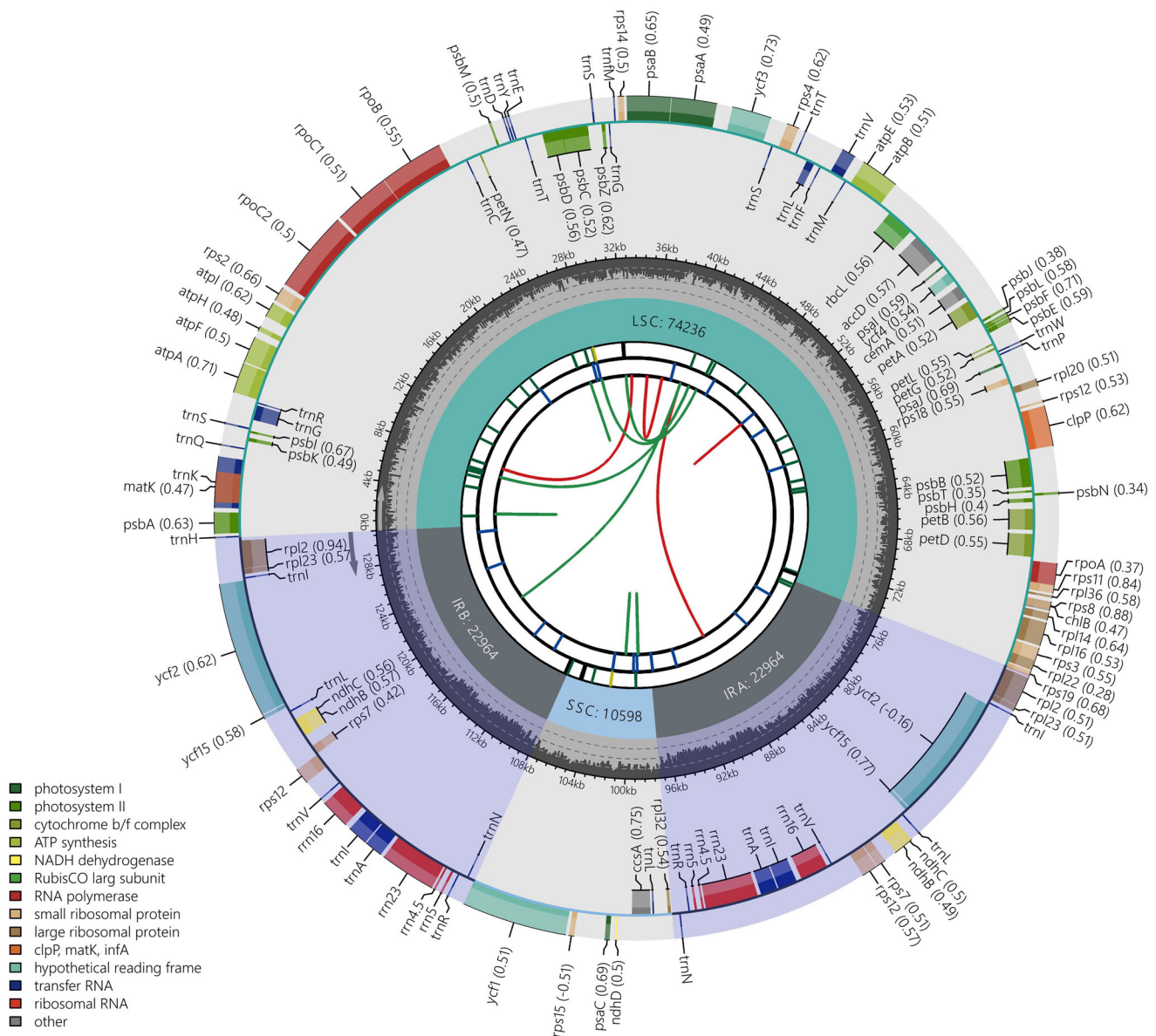


Figure 2. Schematic map of overall features of the chloroplast genome of *Gentiana zollingeri*. Graphic showing features of the *Gentiana zollingeri* plastome was generated using CPGview (<http://www.1kmppg.cn/cpgview>). The map contains seven circles. From center outwards, first circle shows distributed repeats connected with red (forward direction) and green (reverse direction) arcs. Second circle shows tandem repeats marked with short bars. Third circle shows microsatellite sequences as short bars. Fourth circle shows sizes of long single copy (LSC) and short single copy (SSC) regions. Fifth circle shows two inverted repeat (IR) regions: IRA and IRB. Sixth circle shows GC contents along plastome. Seventh circle shows genes marked with different colors according to their functional groups.

coding genes in plastomes of all main *Gentiana* clades were extracted and then aligned using MAFFT version 7 (Katoh and Standley 2013) in PhyloSuite version 1.1.15 (Zhang et al. 2020). Using concatenated data, maximum likelihood phylogenetic analyses were conducted using IQ-TREE version 1.6.12 (Nguyen et al. 2015) with 1000 replicates. The substitution model was chosen using ModelFinder 2 (Kalyaanamoorthy et al. 2017). *Metagentiana rhodantha* (GenBank accession no. MN199153, Fu et al. 2021) was used as the outgroup.

The complete plastome of *G. zollingeri* is 130,762 bp in length. The LSC and SSC regions consist of 74,236 and 10,598 bp, respectively, and there are two IRs of 22,964 bp each. A total of 122 genes are annotated, consisting of 80 protein-coding genes, 34 tRNA genes, and eight rRNA genes (Figure 2). Comparative analyses indicate that the *ndh* complex in the plastome of *G. zollingeri* has undergone numerous gene

loss (*ndhA*, *ndhC*, *ndhE*, *ndhF*, *ndhG*, *ndhI*, *ndhJ* and *ndhK*) and pseudogenization events (*ndhB*, *ndhD* and *ndhH*). The absence of the *ndh* complex is also detected in the *Gentiana* sections *Chondrophyllae* (Fu et al. 2021) and *Kudoa* (Sun et al. 2018).

The phylogenetic relationships among all main *Gentiana* sections are fully supported. The phylogenetic analysis shows that *G. zollingeri* is more closely related to *G. aristata* and *G. producta* than to *G. leucomelaena*, *G. haynaldii*, and *G. cuneibarba* (Figure 3), indicating that the series *Humiles* (containing *G. aristata* and *G. leucomelaena*) and *Dolichocarpa* (containing *G. producta* and *G. haynaldii*) in section *Chondrophyllae* are not monophyletic. Notably, *G. filistyla* forms a sister group with section *Kudoa* rather than with section *Isomeria* to which it belongs (Ho and Liu 2001). This finding is also supported by nuclear data (Favre et al. 2020). The plastome sequence of *G. zollingeri* reported here provides

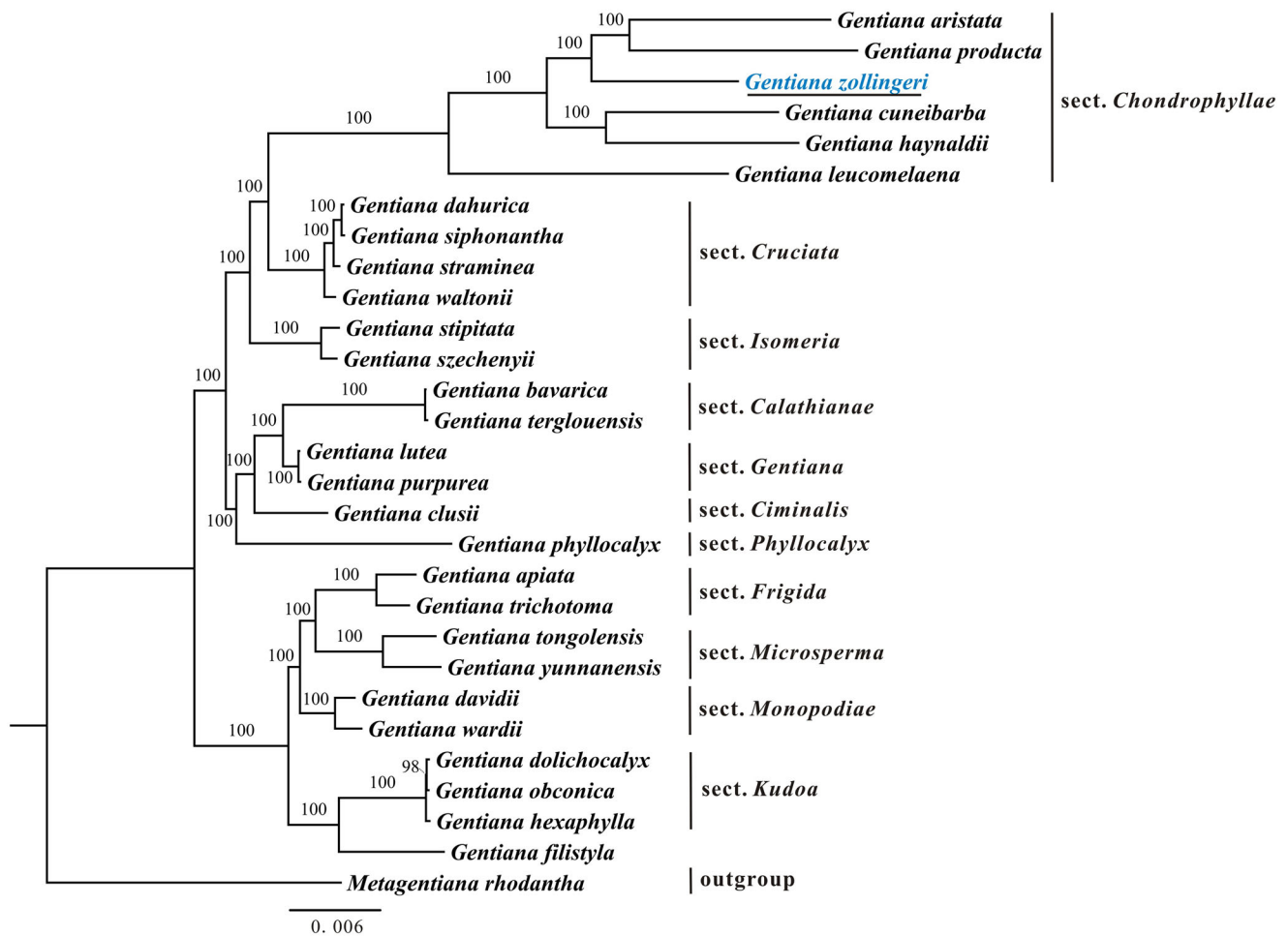


Figure 3. Maximal likelihood tree of *Gentiana* based on protein-coding genes in the plastome. Numbers above branches present bootstrap support. Species shown in blue is the newly sequenced in this study. The following sequences were used: *G. aristata* MN234139 (Fu et al. 2021), *G. producta* MN199163 (Fu et al. 2021), *G. cuneibarba* MN199137 (Fu et al. 2021), *G. haynaldii* MN234137 (Fu et al. 2021), *G. leucomelaena* MT905404 (Ya et al. 2021), *G. dahurica* MH261259 (Zhou et al. 2018), *G. siphonantha* MH261260 (Fu et al. 2021), *G. straminea* KJ657732 (Ni et al. 2016), *G. waltonii* MK780032 (unpublished), *G. stipitata* MG192309 (Sun et al. 2018), *G. szechenyii* MN199158 (Fu et al. 2021), *G. bavarica* MN199162 (Fu et al. 2021), *G. terglouensis* MN199132 (Fu et al. 2021), *G. lutea* MN199129 (Fu et al. 2021), *G. purpurea* MN199146 (Fu et al. 2021), *G. clusii* MN199142 (Fu et al. 2021), *G. phyllocalyx* MN199163 (Fu et al. 2021), *G. apiata* MK317975 (She et al. 2019), *G. trichotoma* MN089577 (Sun, Wang, et al. 2019), *G. tongolensis* MG251985 (Sun, Zhou, et al. 2019), *G. yunnanensis* MN199140 (Fu et al. 2021), *G. davidii* MN199156 (Fu et al. 2021), *G. wardii* MN234136 (Fu et al. 2021), *G. dolichocalyx* MN199161 (Fu et al. 2021), *G. obconica* MG192306 (Sun et al. 2018), *G. hexaphylla* MG192305 (Sun et al. 2018), *G. filistyla* MN199134 (Ya and Miao 2020), *Metagentiana rhodantha* MN199153 (Fu et al. 2021).

new molecular data that illuminate aspects of the phylogenetic and molecular evolution of *Gentiana*, particularly the most species-rich section, *Chondrophyllae*.

Acknowledgments

We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript. We also thank Rui Wang and Yizhuo Huang of Luoyang Normal University for their work in data analysis.

Ethical approval

The materials used in this study are not included in the IUCN Red List of Threatened Species, and the sampling site is not located in any protected area. The field study and laboratory research were conducted in accordance with the guidelines provided by Luoyang Normal University and Zhengzhou University.

Author contributions

J. Y. analyzed molecular data, prepare the illustrations and revised the manuscript. P. F. sampled individuals, prepared the illustration and wrote

the draft; S. S. designed the study and revised the manuscript. All the authors read and approved the manuscript.

Disclosure statement

All the authors declare no conflicts in this study. The collection of the plant material in this study was carried out in accordance with the national and local regulations.

Funding

This work was financially supported by National Natural Science Foundation of China [No. 82004142], and the China Postdoctoral Science Foundation [2020M672299].

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/>] (<https://www.ncbi.nlm.nih.gov/>) under the accession no. MZ934753. The

associated BioProject, SRA, and Bio-Sample numbers are PRJNA758493, SRR15661475, and SAMN21028202, respectively.

References

- Favre A, Michalak I, Chen C, Wang J, Pringle JS, Matuszak S, Sun H, Yuan Y, Struwe L, Muellner-Riehl AN, et al. **2016**. Out-of-Tibet: the spatio-temporal evolution of *Gentiana* (Gentianaceae). *J Biogeogr.* 43(10): 1967–1978.
- Favre A, Pringle JS, Heckenhauer J, Kozuharova E, Gao Q, Lemmon EM, Lemmon AR, Sun H, Tkach N, Gebauer S, et al. **2020**. Phylogenetic relationships and sectional delineation within *Gentiana* (Gentianaceae). *Taxon.* 69(6):1221–1238.
- Frazer KA, Pachter L, Poliakov A, Rubin EM, Dubchak I. **2004**. VISTA: computational tools for comparative genomics. *Nucleic Acids Res.* 32(Web Server issue):W273–W279.
- Fu P-C, Sun S-S, Twyford AD, Li B-B, Zhou R-Q, Chen S-L, Gao Q-B, Favre A. **2021**. Lineage-specific plastid degradation in subtribe Gentianinae (Gentianaceae). *Ecol Evol.* 11(7):3286–3299.
- Ho TN, Liu SW. **2001**. A worldwide monograph of *Gentiana*. Beijing: science Press.
- Jin J-J, Yu W-B, Yang J-B, Song Y, dePamphilis CW, Yi T-S, Li D-Z. **2020**. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biol.* 21(1):31.
- Kalyaanamoorthy S, Minh BQ, Wong TK, von Haeseler A, Jermini LS. **2017**. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods.* 14(6):587–589.
- Katoh K, Standley DM. **2013**. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol.* 30(4):772–780.
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. **2015**. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol.* 32(1):268–274.
- Ni L, Zhao Z, Xu H, Chen S, Dorje G. **2016**. The complete chloroplast genome of *Gentiana straminea* (Gentianaceae), an endemic species to the Sino-Himalayan subregion. *Gene.* 577(2):281–288.
- She R-X, Li W, Xie X, Liu M, Wang L, Khan H, Ullah I, Zhao P. **2019**. The complete chloroplast genome of *Gentiana apiata* (Gentianaceae), an endemic species to Qinba Mountain in China. *Mitochondrial DNA B.* 4(1):1609–1610.
- Sun SS, Fu PC, Zhou XJ, Cheng YW, Zhang FQ, Chen SL, Gao QB. **2018**. The complete plastome sequences of seven species in *Gentiana* sect. *Kudoa* (Gentianaceae): insights into plastid gene loss and molecular evolution. *Front Plant Sci.* 9:493.
- Sun SS, Wang H, Fu PC. **2019**. Complete plastid genome of *Gentiana trichotoma* (Gentianaceae) and phylogenetic analysis. *Mitochondrial DNA B Resour.* 4(2):2775–2776.
- Sun SS, Zhou XJ, Li ZZ, Song HY, Long ZC, Fu PC. **2019**. Intra-individual heteroplasmy in the *Gentiana tongolensis* plastid genome (Gentianaceae). *PeerJ.* 7:e8025.
- Tillich M, Lehwark P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R, Greiner S. **2017**. GeSeq—versatile and accurate annotation of organelle genomes. *Nucleic Acids Res.* 45(W1):W6–W11.
- Ya HY, Miao YW. **2021**. Complete plastid genome of *Gentiana leucomelaena* Maxim. (Gentianaceae) and phylogenetic analysis. *Mitochondrial DNA B Resour.* 6(10):2953–2954.
- Ya HY, Zhou RQ, Li BB, Fu PC. **2020**. Complete plastid genome of an alpine plant *Gentiana filistyla* (Gentianaceae) and phylogenetic analysis. *Mitochondrial DNA B.* 5(2):1404–1405.
- Zhang D, Gao F, Li WX, Jakovlić I, Zou H, Zhang J, Wang GT. **2020**. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Mol Ecol Resour.* 20(1):348–355.
- Zhou T, Wang J, Jia Y, Li W, Xu F, Wang X. **2018**. Comparative chloroplast genome analyses of species in *Gentiana* section *Cruciata* (Gentianaceae) and the development of authentication markers. *IJMS.* 19(7):1962.