The complete chloroplast genome of *Gaultheria fragrantissima* Wall. (Ericaceae) from Yunnan, China, an aromatic medicinal plant in the wintergreens

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**ABSTRACT**

*Gaultheria fragrantissima* (Ericaceae) is an aromatic medicinal plant with high concentrations of the secondary metabolite methyl salicylate (oil of wintergreen). In this study, the complete chloroplast genome of *G. fragrantissima* was sequenced. The complete plastome is 176,196 bp in length, and the GC content is 36.6%. The plastome comprises 110 unique genes (76 protein-coding, 30 tRNA and 4 rRNA). Phylogenetic analysis fully supported a sister relationship between *G. fragrantissima* and *G. hookeri* within the Leucothoides clade of Gaultheria. This chloroplast genome will serve as a valuable reference for future taxonomic and phylogenetic research.

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*Gaultheria* Kalm ex L., a genus classified in the family Ericaceae, contains about 288 species (Kron et al. 2020). In Asia, the genus is known for the medicinal species *Gaultheria fragrantissima* Wall., which contains methyl salicylate (oil of wintergreen). This phenolic compound has pharmacological activities is antimicrobial, an insecticide and antioxidant (Mukhopadhyay et al. 2016; Park et al. 2016; Lu et al. 2019). As a Chinese ethnomedicinal plant species, it has been used to treat rheumatism and arthritis due to containing lignan glycoside, flavonoid, organic acid, terpenoid, and steroid, etc. (Ma et al. 2002; Cheng et al. 2009). *Gaultheria fragrantissima* was phylogenetically placed within the Leucothoides clade, but its relationship among species has not been clearly resolved because of possible evolutionary reticulation (Lu et al. 2010, 2019). The chloroplast genome has been successfully utilized for reconstructing phylogenetic relationships in plants (Jung et al. 2014; Huang et al. 2020; Lee et al. 2021). In this study, we analyzed the complete chloroplast genome of *G. fragrantissima* to contribute to the molecular systematics and biology of the species.

The specimen was collected from Dawei Mountain in Pingbian, Yunnan, China (22°55′39″N, 103°41′25″E). A specimen was deposited at the herbarium of Kunming Institute of Botany (collection number: Lu001; contact person: Lu Lu, lulukmu@163.com; https://www.cvh.ac.cn/spms/detail.php?id=ea98cfc63) under the voucher number 1251725. Genomic DNA was extracted from fresh leaves using the modified CTAB method (Doyle and Doyle 1987). Illumina Solexa platform (Illumina, San Diego, CA; New England Biolabs, Cedex, France) was used to sequence 150 bp pair-end reads of the 500 bp insert-size libraries. *De novo* assembly was performed with GetOrganelle toolkit (Jin et al. 2020). Reference guided connection and annotation were subsequently conducted using Bandage 0.8.1 (Wick et al. 2015) and Geneious 9.1.4 (Biomatters Ltd., Auckland, New Zealand) with *Vaccinium macrocarpon* Aiton. (NC019616) used as the reference. The plastome structure was further verified by PCR and Sanger sequencing.

Raw reads were deposited in the NCBI Sequence Read Archive (SRA: SRX10204411) and the final annotated chloroplast genome sequence was deposited in NCBI GenBank (accession no. MW563322). The genome sequence is 176,196 bp in total length with a typical quadripartite structure, consisting of a large single-copy region with 107,747 bp (LSC), a small single-copy region with 3509 bp (SSC), and two inverted repeat regions with 32,470 bp (IRs), respectively. The GC content in the chloroplast genome is 36.6%. The plastome comprises 110 unique genes, including 76 protein-coding, 30 tRNA, and 4 rRNA genes.

To identify the phylogenetic position of *G. fragrantissima*, 15 published plastomes from both *Vaccinium* and *Gaultheria*, and the newly obtained plastome were aligned using MAFFT (Katoh et al. 2005). A maximum-likelihood phylogenetic tree was reconstructed by RAxML (Stamatakis, 2014) with 1000 rapid bootstrap replicates and GTR + GAMMA + I substitution model. A sister relationship between *G. fragrantissima* and *G. hookeri* C. B. Clarke in the Leucothoides clade of *Gaultheria* was supported by 100% bootstrap value (Figure 1). These two species were both supported within the Leucothoides clade based on separate and combined DNA sequence data.
from five genic regions (ITS, matK, rpl16, trnL-trnF, and trnS-trnG) in Lu et al. (2010) and our study further strongly supported their sister relationship. Our results provide fundamental information for further taxonomic and phylogenetic researches on the Leucothoides clade of *Gaultheria*.

**Disclosure statement**

The authors declare no conflicts of interest.

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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/](https://www.ncbi.nlm.nih.gov/) under the accession no. MW563322. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA705850, SRX10204411, and SAMN18104638, respectively.

**References**

Cheng Y, Miao J, Ma LY, Lai JY, Xu XD, Yang JS. 2009. Study advances on chemical constituents and bioactivities from plants of genus *Gaultheria*. Lishizhen Med Materia Med Res. 20(2):399–402.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 19:11–15.

Huang R, Liang Q, Wang Y, Yang TJ, Zhang Y. 2020. The complete plastome genome of *Epimedium pubescens* Maxim. (Berberidaceae), a traditional Chinese medicine herb. Mitochondrial DNA Part B, 5(3): 2042–2044.

Jin JJ, Yu WB, Yang JB, Song Y, DePamphilis CW, Yi TS, Li DZ. 2020. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. Genome Biol. 21(1):241.

Jung J, Kim KH, Yang K, Bang KH, Yang TJ. 2014. Practical application of DNA markers for high-throughput authentication of *Panax ginseng* and *Panax quinquefolius* from commercial ginseng products. J Ginseng Res. 38(2):123–129.

Katoh K, Kuma KI, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. 33(2): 511–518.

Kron KA, Fritsch PW, Lu L, Judd WS. 2020. New combinations and new and resurrected names in *Gaultheria* (Ericaceae). GBS. 72(2):299–317.

Lee YS, Kim J, Woo S, Park JY, Park HS, Shim H, Choi HI, Kang JH, Lee TJ, Sung SH, et al. 2021. Assessing the genetic and chemical diversity of *Taraxacum* species in the Korean Peninsula. Phytochemistry. 181: 112576.

Lu L, Fritsch PW, Bush CM, Wang H, Kron KA, Li DZ. 2019. Allopolyploidy in the Wintergreen Group of tribe Gaultherieae (Ericaceae) inferred from low-copy nuclear genes. Nord J Bot. 37(6):e02077.

Ma XJ, Zhao L, Han ZT, Zheng JH, Chen XZ. 2002. Comparison of the contents of lignans glycosides in 5 medicinal plants of *Gaultheria* [J]. Plant Resour Environ. 11(2):61–62.

Mukhopadhyay M, Bantawa P, Mondal TK, Nandi SK. 2016. Biological and phylogenetic advancements of *Gaultheria fragrantissima*: economically important oil bearing medicinal plant. Ind Crops Prod. 81:91–99.

Park CG, Shin E, Kim J. 2016. Insecticidal activities of essential oils, *Gaultheria fragrantissima* and *Illicium verum*, their components and analogs against Callosobruchus chinensis adults. J Asia-Pac Entomol. 19(2):269–273.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30(9):1312–1313.

Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualization of de novo genome assemblies. Bioinformatics. 31(20): 3350–3352.

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**Figure 1.** A maximum-likelihood tree based on 16 plastomes reconstructed using RAxML. Bootstrap support values are shown next to the nodes.