


The complete chloroplast genome of *Hedychium flavum* Roxb., an ornamental, edible and medicinal plant

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ABSTRACT

Hedychium flavum Roxb. 1820 is a perennial herb mainly distributed in China, India, Myanmar and Thailand with ornamental, edible and medicinal value. It is extensively cultivated as a source of aromatic essential oils, ornamental plant, food flavorings and vegetables, and folk medicine. In this study, we sequence the complete chloroplast genome of *H. flavum* by *de novo* assembly. The assembled genome has a typical quadripartite circular structure with 163,909 bp in length, containing a large single-copy region (LSC, 88,589 bp), a small single-copy region (SSC, 15,762 bp), and two inverted repeat regions (IRs, 29,779 bp). The cp genome contains 133 genes, including 87 protein-coding genes, 38 tRNA genes and 8 rRNA genes. Phylogenetic analysis based on the complete cp genome shows a close affinity of *H. flavum* and *H. neocarneum* with 100% bootstrap support. This study will provide useful genetic resource for further phylogenetic analysis of the genus *Hedychium* and Zingiberaceae.

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Introduction



The genus *Hedychium* (Zingiberaceae), commonly known as ginger lily, comprises approximately 93 species, which are mainly distributed in China, India, and Southeast Asia (Tian et al. 2023; The plant list 2012). The *Hedychium* species are herbs with thick, fleshy and aromatic rhizomes and extensively cultivated for their various uses in fragrance, paper, ornamental, cosmetics, medicine, and food industries (Sakhanokho et al. 2013; Hartati et al. 2014; Tian et al. 2020). *Hedychium* plants are rich in essential oil extracted from leaves, flowers and rhizomes and widely used in high-quality perfumes and traditional medicine for the treatment of asthma, bronchitis, diarrhea, gastric diseases, flu, nausea, snake bites, and leishmaniasis (Basak et al. 2010; Hartati et al. 2014). Moreover, some species of *Hedychium* were grown for their edible flowers (He 2000).


Hedychium flavum Roxb. 1820 (Roxburgh 1820), a perennial tuberous plant, is distributed in China, Thailand, India and Myanmar (Wu and Larsen 2000). It is extensively cultivated for its aromatic essential oil or as an ornamental plant. (Wu and Larsen 2000; Tian et al. 2020). *H. flavum* has large aromatic flowers forming a terminal spike with an orange spot in the petals (Figure 1) and blooming from August to September (Wu and Larsen 2000; Huang et al. 2023). Its flowers can remain open for 5-6 days after being cut and placed in bottle, making it an aromatic cut flower with great breeding prospects (Gao et al. 2002; Pan and Zhang 2013). Furthermore, the rhizomes (commonly known as *yehansu*) and flowers are

widely used in Chinese folk medicine and has remarkable efficacy in treating various diseases, like coughs, stomach pain, bruises, rheumatism and headache (Ai 2013; Zhou et al. 2018). Meanwhile, their extracted essential oils have a variety of pharmacological effects such as antibacterial, anticancer, insecticidal, anti-inflammatory, and tyrosinase inhibitory properties, with the essential oils of the flowers also being used in cosmetics. (Sakhanokho et al. 2013; Tian et al. 2020, 2023). The



Figure 1. Species reference image of *H. flavum* with following distinguished characters: terminal spikes, imbricate bracts, yellow flowers, yellow obcordate labellum with orange at base, and long filament ca. 3 cm. (photo taken by Yong-Hong Zhang in Songming County, yunan province, China).

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et al. 2021) and then manually corrected. The annotated chloroplast genome sequence was deposited into GenBank under the accession numbers of OQ308833, and the original reads were also deposited in GenBank. The circular genome map was drawn using CPGView program (Liu et al. 2023).

To explore the phylogenetic relationship of *H. flavum* with its closely related species, a phylogenetic tree was constructed using complete chloroplast genomes of 35 species from Zingiberaceae and three species from Musaceae (as outgroups) downloaded from NCBI GenBank. Total of 39 chloroplast genomes were aligned by the MAFFT v7.490 software (Kato and Standley 2013). Subsequently, nucleotide substitution models were estimated using MEGA 11 (Tamura et al. 2021) and the model of GTR+G+I was chosen. The maximum likelihood (ML) tree was reconstructed by using RAxML v8.2.11 (Stamatakis 2014). Bootstrap values were calculated from 1,000 replicates to evaluate the reliability of each node.

Results

General feature of the chloroplast genome

The newly assembled plastid genome of *H. flavum* is 163,909 bp in length. The clean reads (24,029,978) were

subjected to assembly to produce a circular molecule of complete chloroplast with about an average 731.4 coverage, which indicated that the genome assembly is reliable (Figure S1). The chloroplast genome of *H. flavum* had a typical quadripartite structure (Figure 2). It is divided into four distinct regions: a large single-copy (LSC) region of 88,589 bp, a small single-copy (SSC) region of 15,762 bp, and a pair of inverted repeats (IR) region of 29,779 bp. The GC content in whole cp genome, LSC region, SSC region, and IR regions were 36.1%, 33.9%, 29.6% and 41.1%, respectively.

A total of 133 genes were annotated in the assembled chloroplast genome, including 87 protein-coding genes, 38 transfer RNA genes and eight ribosomal RNA genes. There are 11 cis-splicing genes including *rps16*, *atpF*, *rpoC1*, *petB*, *ycf3*, *clpP*, *petD*, *rpl6*, *rpl2*, *ndhB*, and *ndhA* (Figure S2A) and one trans-splicing genes *rps12* (Figure S2B). Most of the genes occurred in a single copy, while 20 genes occurred in double copies (*rps19*, *trnH-GUG*, *rpl2*, *rpl23*, *trnL-CAU*, *ycf2*, *trnL-CAA*, *ndhB*, *rps7*, *rps12*, *trnV-GAC*, *rrn16*, *trnI-GAU*, *trnA-UGC*, *rrn23*, *rrn4.5*, *rrn5*, *trnR-ACG*, *trnN-GUU*, and *ycf1*). There were 9 genes with one intron (*rps16*, *atpF*, *rpoC1*, *petB*, *petD*, *rpl6*, *rpl2*, *ndhB*, and *ndhA*), and 3 genes with two introns (*rps12*, *ycf3*, and *clpP*).

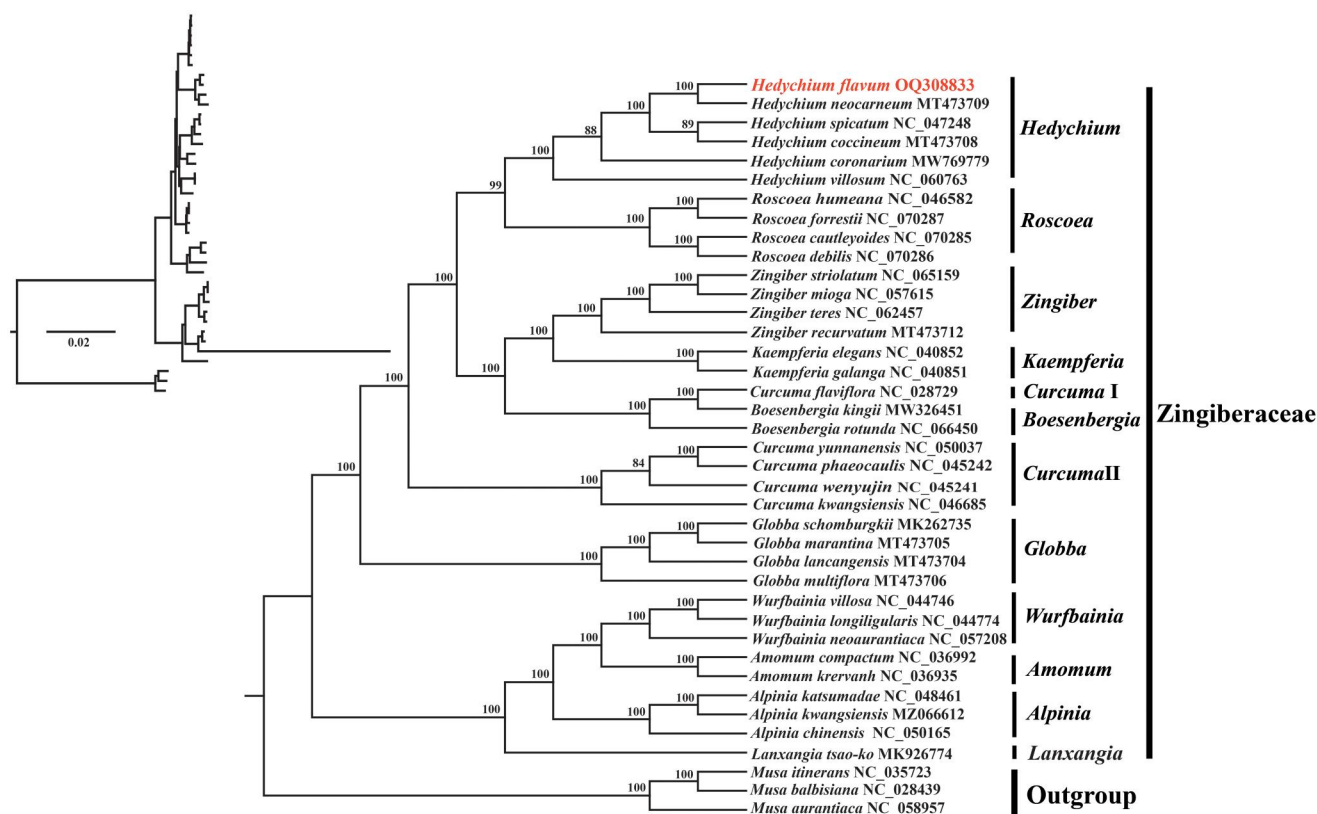


Figure 3. The maximum likelihood tree of *H. flavum* and its related relatives based on the complete chloroplast genomes. Bootstrap values were shown next to the nodes. The following sequences were used: *Alpinia chinensis* NC_050165 (Mei et al. 2020), *A. katsumadae* NC_048461 (Li et al. 2020), *A. kwangsiensis* MZ066612 (Zhang et al. 2021), *Amomum compactum* NC_036992 (Wu et al. 2018), *A. krervanh* NC_036935 (Wu et al. 2017), *Boesenbergia kingii* MW326451 (Liang and Chen 2021), *B. rotunda* NC_066450 (Liew et al. 2022), *Curcuma flaviflora* NC_028729 (Zhang et al. 2016), *C. kwangsiensis* NC_046685 (Gui et al. 2020), *C. phaeocaulis* NC_045242, *C. wenyujin* NC_045241 (Kim et al. 2021), *C. yunnanensis* NC_050037 (Liang et al. 2020), *Globba lancangensis* MT473704, *G. marantina* MT473705, *G. multiflora* MT473706, *G. schomburgkii* MK262735 (Li et al. 2021), *H. coccineum* MT473708 (Li et al. 2021), *H. coronarium* MW769779 (Yang et al. 2021), *H. neocarneum* MT473709 (Li et al. 2021), *H. spicatum* NC_047248 (Unpublished), *H. villosum* NC_060763 (Yang et al. 2021), *Kaempferia elegans* NC_040852, *K. galanga* NC_040851 (Li et al. 2019), *Lanxangia tsao-ko* MK926774 (Ma and Lu 2020), *Musa aurantiaca* NC_058957 (Feng et al. 2020), *M. balbisiana* NC_028439 (Niu et al. 2018), *M. itinerans* NC_035723 (Zhang et al. 2019), *Roscoea cautleyoides* NC_070285 (Unpublished), *R. debilis* NC_070286 (Unpublished), *R. forrestii* NC_070287 (Unpublished), *R. humeana* NC_046582 (Zhu et al. 2019), *Wurfbainia longiligularis* NC_044774 (Cui et al. 2019), *W. neaurantiaca* NC_057208 (Li et al. 2019), *W. villosa* NC_044746 (Cui et al. 2019), *Zingiber mioga* NC_057615 (Unpublished), *Z. recurvatum* MT473712 (Li et al. 2021), *Z. striolatum* NC_065159 (Tian et al. 2023), *Z. teres* NC_062457 (Unpublished).

Phylogenetic analysis of *H. flavum* in the family Zingiberaceae

Based on the chloroplast genome dataset, we generated a well-supported phylogenetic tree (Figure 3). The inferred phylogenetic tree indicated that the selected species from the Zingiberaceae were clustered within a lineage distinct from the outgroups. Within Zingiberaceae, the genus *Hedychium* forms a monophyly, and is closely related to the genus *Roscoea*. The phylogenetic tree also shows a close affinity of *H. flavum* and *H. neocarneum* with 100% bootstrap support.

Discussion and conclusion

In the present study, the chloroplast genome of *H. flavum* was sequenced, assembled, and annotated for the first time. The results showed that the genome size, overall GC content, genome quadripartite structure, and gene composition in the *H. flavum* chloroplast genome were highly similar to chloroplast genome of other species of *Hedychium* (Li et al. 2019; Yang et al. 2021). These results suggested that the chloroplast genomes of *Hedychium* species were highly conserved at the genus level. The IR regions have a higher GC content (41.1%) than LSC (33.9%) and SSC (29.6%). The higher GC content in the IR region may be due to the presence of rRNA genes, while the SSC region contains most of the NADH genes (Cai et al. 2006).

Phylogenetic analysis demonstrated that the genus *Hedychium* is monophyletic, which is consistent with the results of phylogenetic analysis based on nuclear *ITS*, chloroplast *matK/trnL-trnF* sequences, and single nucleotide polymorphism (SNP) matrix of chloroplast genome (Wood et al. 2000; Ngamriabsakul et al. 2004; Li et al. 2021). Based on the phylogenetic study of chloroplast genome SNP matrix, there is a close relationship between *Hedychium* and *Globba* (Li et al. 2021). However, our study showed that *Hedychium* is closely related to *Roscoea*, which is inconsistent with the previous findings. Phylogenetic analysis also showed a close affinity of *H. flavum* and *H. neocarneum*. However, due to the limited sequence of *Hedychium* cp genomes, the phylogenetic relationship in the genus requires further study. This study provides the cp genome information of *H. flavum*, which would contribute to the species identification and phylogenetic analysis within *Hedychium* and Zingiberaceae.

Author's contribution statement

Yong-Hong Zhang designed the research and revised the manuscript. Mei-Fei Li analyzed data and prepared a preliminary manuscript. Tao Xiao analyzed data and revised the manuscript. All authors read and approved the final manuscript, and agreed to be accountable for all aspects of the work.

Disclosure statement

No potential conflict of interest was reported by the authors.

Ethic statement

This research does not involve ethical issues. *H. flavum* does not belong to the China Species Red List. The collection of plant material carried out in accordance with guidelines provided by the authors' institution (Yunnan Normal University) and national regulations.

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Data availability statement

The data that support the findings of this study are openly available in GenBank of NCBI (<https://www.ncbi.nlm.nih.gov/>) with accession number OQ308833. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA941112, SRR23703126, SAMN33590324, respectively. The voucher specimens were deposited in the Herbarium of Yunnan Normal University (YNUB, Website: <https://life.ynnu.edu.cn/>, Contact: Jian-Lin Hang, Email: hjlynub@163.com) with the voucher number: LMF06, which is identified by Dr. Yong-Hong Zhang.

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