

## THE COMPLETE CHLOROPLAST GENOME SEQUENCE OF *ZINGIBER STRIOLATUM* (ZINGIBERACEAE) AND A PHYLOGENETIC ANALYSIS

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*Zingiber striolatum* Diels (also known as Yang-He in Chinese), is a unique and valuable Chinese medicinal herb that belongs to the tribe Zingibereae of the family Zingiberaceae. This study characterized the complete chloroplast genome of *Z. striolatum* sample from Yunnan, China, and compared it to other representative species from the Zingiberaceae family to reveal the phylogenetic relationships. The complete circular plastid genome of this species was 163,947 bp long and consisted of two inverted repeat regions (IRA and IRB) of 29,912 bp each, separated by two single-copy regions: a large single-copy region (LSC) of 88,267 bp and a small single-copy region (SSC) of 15,856 bp. The genome has 133 unique gene including 87 protein-coding genes, 38 tRNA genes, and 8 rRNA genes, respectively. The maximum likelihood (ML) method was used to generate a phylogenetic tree, and the results revealed that *Z. striolatum* belongs to the Sect. *Crytanthium* and it forms a clade with *Z. mioga* Roscoe and *Z. leptorrhizum* D.Fang.

*Zingiber striolatum* is widely distributed throughout China, including Guangxi, Guizhou, Guangdong, Hubei, Hunan, Jiangxi, and Sichuan, at elevations ranging from 300 to 1900 m above sea level (Wu and Larsen, 2000). Its edible part is its flower, which has an aromatic odor and can be made into dried fruit and steamed, stir-fried, or eaten raw. It is extensively distributed, primarily in wild form, but less so in cultivated form. The species has a high nutritional value since it contains a wide range of amino acids, cellulose, and proteins (Zhang *et al.*, 2014). *Z. striolatum* has gained popularity due to its numerous functional qualities and therapeutic significance. More than 100 *Z. striolatum*-related patents are now registered with the China Intellectual Property Office, and the market potential is enormous.

According to the “Compendium of Materia Medica, a famous Chinese medicine classic compiled by Shizhen Li in the 16th century”, *Z. striolatum* was used to promote blood circulation, eliminate phlegm, alleviate coughing, and relieve swelling and pain (Huang *et al.*, 2021). *Zingiber* species have identical leaf and other vegetative organ traits, making non-flowering stage species identification extremely challenging (Wu *et al.*, 2016). In recent years, efforts have been made to investigate the phylogenetic relationships of some *Zingiber* species using molecular sequence data (Kress *et al.*, 2002; Theerakulpisut *et al.*, 2012; Li *et al.*, 2020, 2021; Jiang *et al.*, 2023), but only a small number of samples were used, and the relationships among many species within the *Zingiber* genus remain unclear. In this study, we thoroughly sequenced and reconstructed the chloroplast genome sequence of *Z. striolatum*, including its gene content, and comparisons to closely related species. To clarify the taxonomic position of *Z. striolatum*, a phylogenetic analysis was conducted using previously published cp genomes from the Zingiberaceae family. These findings will provide useful genetic resources for future research on the phylogenetic position of *Z. striolatum* as well as evolutionary relationships with the Zingiberaceae family.

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The plant material was collected from Eshan County, Yunnan Province, China (102°6'9.07" E, 24°17'30.09" N, elevation 1911 m). The specimen was kept at Neijiang Normal University (<https://www.njtc.edu.cn/>; voucher no: KM001; Collector: Wang Hui). Silica-gel dried leaves were sent to Novogene (Tianjin, China) to extract whole genomic DNA for library preparation, and pair-end 250 bp read-length sequencing was performed on the Illumina HiSeq 2500 platform. The raw reads were filtered using Fastp v0.21.0 to remove sequencing adaptors and low-quality sequences. The high-quality reads were assembled with GetOrganelle v1.7.5 (Jin *et al.*, 2020). CPGAVAS2 (Shi *et al.*, 2019) was used to annotate the plastome with default parameters, and BLAST searches against the Swiss-Prot database were used to identify predicted protein-coding genes, which were then manually checked in Apollo software for a more precise annotation. The annotations of tRNA genes were confirmed by using tRNAscan-SE v.2.02. A fully annotated plastome circle diagram was created by a website IRscope (<https://irscope.shinyapps.io/Chloroplast/>) (Amiryousefi *et al.*, 2018). The cp genome of *Z. officinale* (NCBI accession number: NC\_044775) served as a reference for comparative analysis.

The complete circular plastid genome of *Z. striolatum* (SRA: PRJNA1099382) was 163,947 bp in length, consisting of a pair of inverted repeat regions (IRs with 29,912 bp each) divided by two single-copy regions (LSC with 88,267 bp; SSC with 15,856 bp). The plastid genome has an overall GC content of 36%, with LSC (51.09%), SSC (50.23%), and IR (RA 48.69% and IRB 51.31%), respectively. The genome consisted of 133 gene including 87 protein-coding genes, 38 tRNA genes, and 8 rRNA genes (Fig. 1).

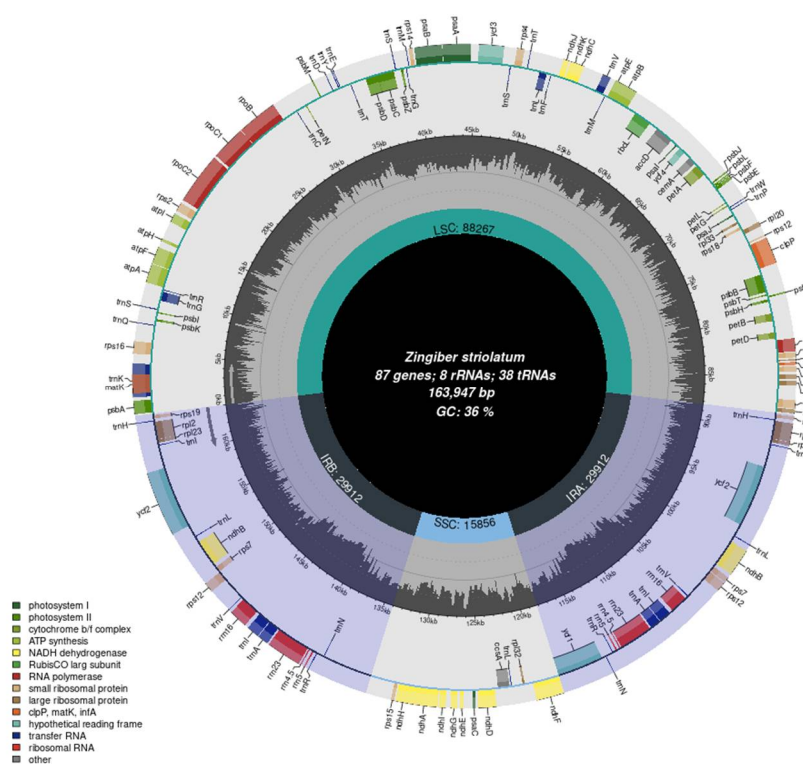


Fig. 1. The chloroplast genome map of *Zingiber striolatum* from Yunnan province, China. Genes within the circle are transcribed clockwise, while those beyond the circle are transcribed anti-clockwise. The tick lines showed the extent of the Inverted Repeats (IRA and IRB) that separate the Large Single-Copy (LSC) and the Small Single-Copy (SSC) regions. Genes with varying functions are shown by distinct colors.

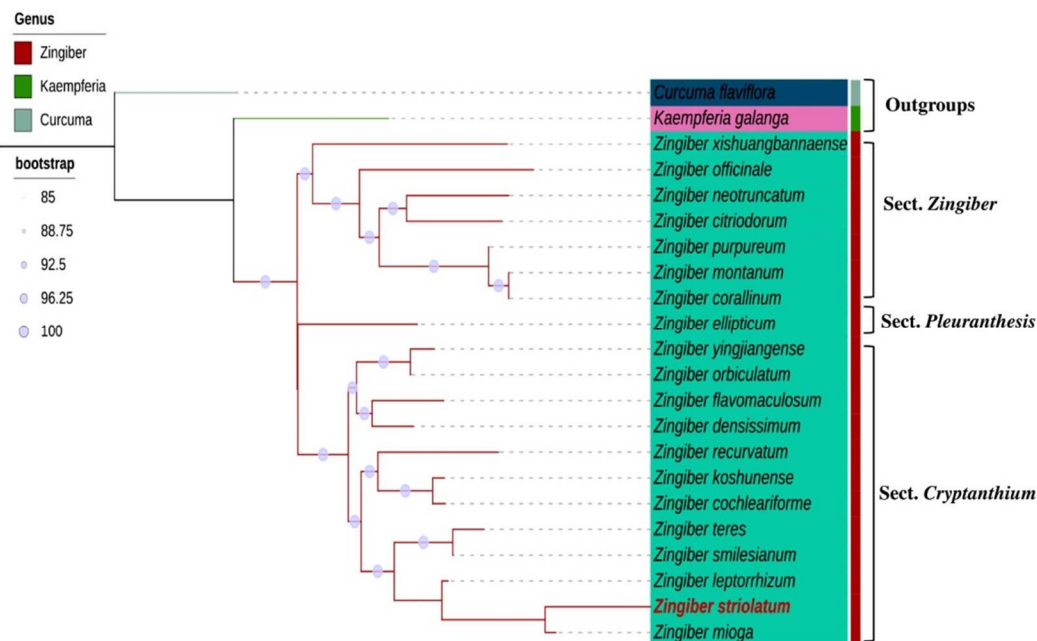


Fig. 2. Phylogenetic relationship of *Z. striolatum* based on the maximum-likelihood (ML) tree inferred from 22 chloroplast genomes of *Zingiber* (including *Kaempferia galanga* and *Curcuma flaviflora* as an outgroups). The chloroplast genome accession number used in this study: *Z. citriodorum* PP542025; *Z. cochleariforme* NC\_072310; *Z. corallinum* NC\_063565; *Z. densissimum* NC\_072300; *Z. ellipticum* NC\_072301; *Z. flavomaculosum* NC\_072311; *Z. koshunense* NC\_072302; *Z. leptorrhizum* NC\_072309; *Z. mioga* NC\_057615; *Z. montanum* MW801386; *Z. neotruncatum* NC\_072303; *Z. officinale* NC\_044775; *Z. orbiculatum* NC\_072304; *Z. purpureum* NC\_072305; *Z. recurvatum* MT473712; *Z. smilesianum* NC\_072306; *Z. teres* NC\_062457; *Z. xishuangbannaense* NC\_072307; *Z. yingjiangense* NC\_072308; *C. flaviflora* KR967361, and *K. galanga* NC\_040851.

In addition to the newly sequenced plastome, 22 publicly available plastomes retrieved from the NCBI (including twenty *Zingiber* species), with *Curcuma flaviflora* S.Q.Tong, and *Kaempferia galanga* L., were used as outgroups. PhyloSuite (v1.2.3) was used to extract protein-coding genes and RNAs from cp genomes; then, the sequences were aligned with MAFFT v7.313, and concatenated with Phylosuite (Zhang *et al.*, 2020). A phylogenetic analysis was carried out using the Maximum likelihood (ML) method in IQ-TREE version 2.2.0, with the *Edge-linked* partition model and 50,000 Ultrafast bootstrap replicates (Minh *et al.*, 2020). The tree was visualised with iTOL v6 (<https://itol.embl.de/upload.cgi>) (Letunic and Bork, 2024). The ML tree (Fig. 2) agreed with the most recent phylogenetic analysis of *Zingiber* (Jiang *et al.*, 2023; Theerakulpisut *et al.*, 2012). Our results revealed that the *Zingiber* species were monophyletic and split it into three sections: *Zingiber*, *Pleuranthesis* Benth., and *Cryptanthium* Horan. The ML tree showed that *Z. striolatum* clade is a sister group to *Z. mioga* Roscoe and *Z. leptorrhizum* D.Fang that belongs to the *Sect. Cryptanthium*. In conclusion, this study will provide valuable genomic information for phylogenetic and evolutionary investigations in the Zingiberaceae.

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