

Complete chloroplast genome of *Zingiber mioga* by *de novo* sequencing

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ABSTRACT

Zingiber mioga (Thunb.) Rosc. (*Zingiber mioga*) is an important edible species, which also has important medical and natural pigment value. This article is firstly reported the *Zingiber mioga's* chloroplast genomes which detect by *de novo* sequencing. The results showed that the length sequence of *Zingiber mioga's* chloroplast genome was 163,541 bp, and the length of LSC, SSC, and two IR regions was 88,035, 15,886, and 29,810 bp, respectively. *Zingiber mioga's* chloroplast genome was encoded 135 genes involving 10 *rRNA*, 38 *tRNA*, and 87 protein-coding genes. After phylogenetic and cluster analysis, the *Zingiber* were closest approach to *Zingiber mioga*, followed by *Kaempferia*, *Curcuma*, *Hedychium*, and *Roscoea*.

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Zingiber mioga (Thunb.) Rosc. (*Zingiber mioga*) is a Zingiberaceae family from China, Japan, and Korea. Its young flower buds and fruit has been used as a traditional food (Jo et al. 2016), and it is used medicinally to treat the irregular menstruation, dysmenorrhea, cough, and asthma in China and consumed throughout Japan (Huang et al. 2016; Lee et al. 2016). Moreover, *Zingiber mioga* also has important medical and natural pigment value (Huang and Li 2013; Huang et al. 2016). However, due to human over-exploitation and consumption of wild resources, the *Zingiber mioga* resources have been faced seriously challenges in China. Compared with nuclear genome, the chloroplast genome has conserved structure and orthologous (Aldrich et al. 1988; Yi et al. 2016; Ali et al. 2018; Wang et al. 2020), and it plays an important role in *Zingiber mioga's* heredity and evolution. Nonetheless, there are no related studies about the *Zingiber mioga's* chloroplast genome, that blocked the process of molecular genetics research. Therefore, we submit a complete *Zingiber mioga's* chloroplast genomes by *de novo* sequencing.

We collected the fresh leaves of *Zingiber mioga* (stored in herbarium of Traditional Chinese Veterinary Medicine Laboratory of West Anhui University, Voucher numbers: TCVM202004150125) from Yuexi county, Anhui province, PR China (N:30.84939°, E:116.35999°), and used Plant DNA extraction kit (TIANGEN, Beijing, China) to extract total DNA of *Zingiber mioga's* fresh leaves, and then we use micro-volume spectrophotometer (OD_{260nm} and OD_{280nm}) and 1% agarose electrophoresis method to detect DNA quality. When the DNA quality meets the sequencing requirements, the DNA was sent to Beijing Zhongxing Bomai Technology Co., LTD for sequencing using Collibri PCR-free PS DNA Library Prep Kit for Illumina Systems by Illumina NovaSep platform

(template size: 500 bp). The Raw data were filtered to obtain Clean Data, then the Get Organelle pipeline (<https://github.com/Kinggerm/GetOrganelle>) was been ran to cut out the top 15 million reads from Cleandata, and then the SOAP *de novo* software (Luo et al. 2012) was used to assemble the Clean data to obtain the contig sequence. The BLAT (Kent 2002) was used to get the relative position of the genome in the contig sequences reference to the genome (NC 024157.1, NC 011942.1, NC 009618.1, NC 000932.1, and KX 352464.1). The Bandage tool (Wick et al. 2015) was run to obtain the full-length frame diagram of chloroplast genome. The Geseq program (<https://chlorobox.mpimp-golm.mpg.de/geseq.html>) was used to annotate chloroplast genome. The OGDRAW software (Lohse et al. 2013) was used to draw the physical map of chloroplast genome (GenBank accession number: MW285081).

The result of genome analysis showed that the *Zingiber mioga's* chloroplast genome has a typical four-segment structure, including a large single copy region (LSC), a small single copy region (SSC), and two inverted repeat region (IR) (Figure 1). And the full-length sequence of *Zingiber mioga's* chloroplast genome was 163,541 bp, with the length 88,035 bp, 15,886 bp, and 29,810 bp of LSC, SSC, and two IR regions, respectively. Its GC content was 36.04% and encoded 135 genes involving 10 *rRNA*, 38 *tRNA*, and 87 protein-coding genes.

To confirm the phylogenetic status of *Zingiber mioga* in *Scitamineae* plants, we used 87 chloroplast protein-coding genes from eight *Zingiberales* plants and 1 out group (*Ravenala*) in NCBI for phylogenetic analysis and performed raxmlGUI version 1.5 b (<https://sourceforge.net/projects/raxml-gui/>) by GTRCATX model with 1000 bootstrap replicates. All 10 plants were divided into seven genus. The first genus

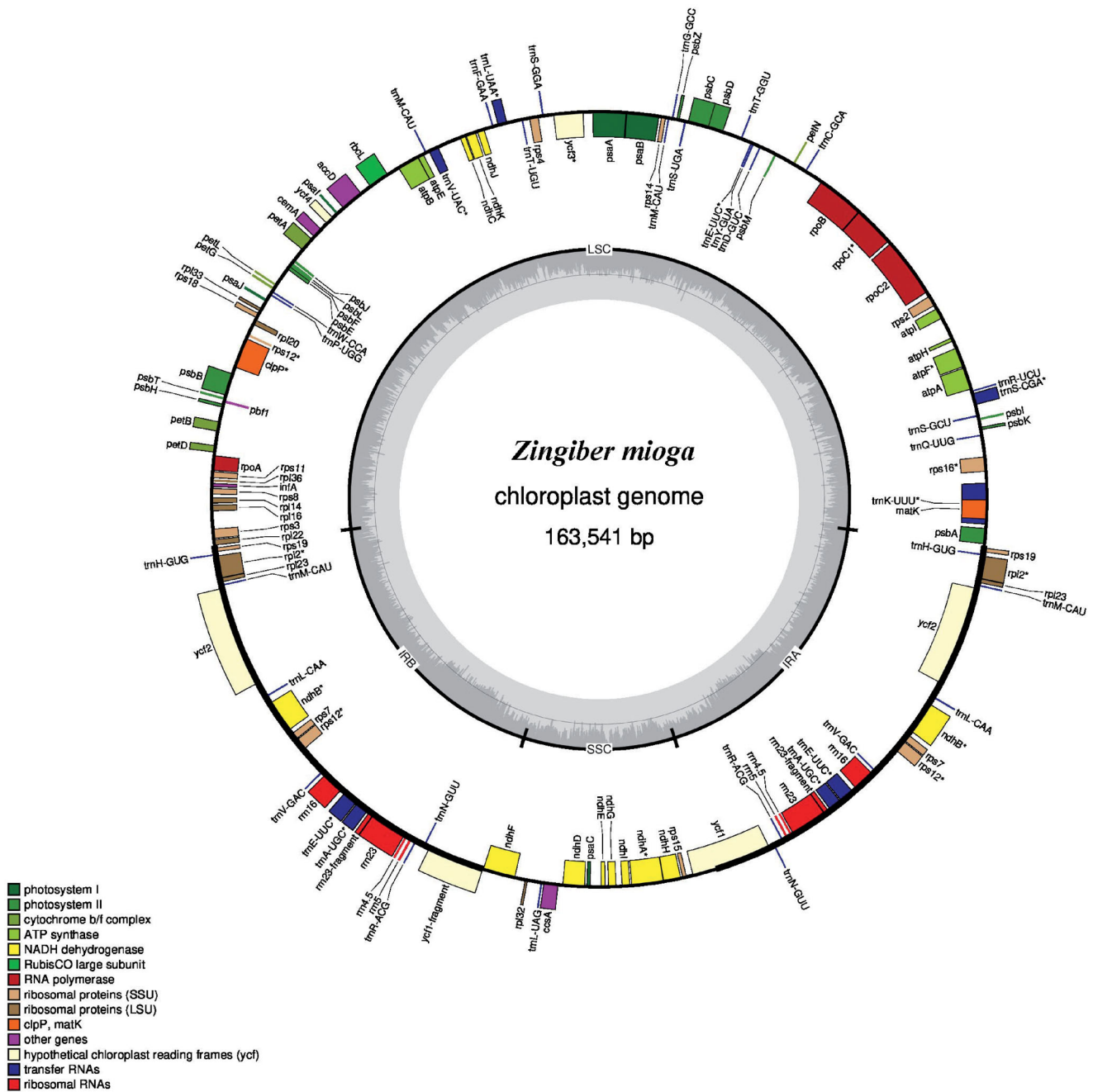


Figure 1. Gene map of the *Zingiber mioga*'s chloroplast genome.

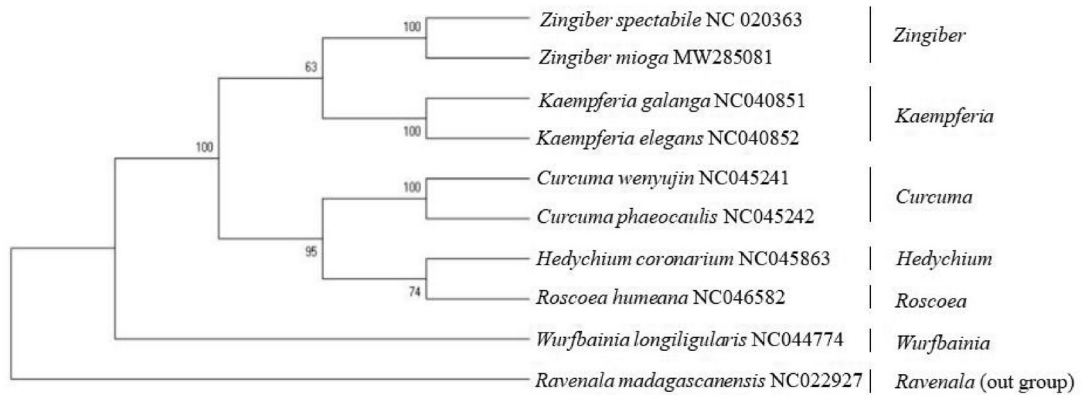


Figure 2. The ML phylogenetic tree of the *Zingiber mioga*.

consisted of two *Zingiber*, which showed that *Zingiber spectabile* and *Zingiber mioga* have a close relationship. The second genus was consisted by *Kaempferia galanga* and *Kaempferia elegans*, and the third and fourth genus were consisted by *Curcuma*, *Hedychium* and *Roscoea*, that four genera has a close relationship. However, *Wurfbainia longiligularis* was far apart from other *Zingiberales* plants. And *Ravenala madagascanensis* (*Musaceae*) as outgroup was away from each other plants. The cluster analysis results showed that *Zingiber* were closest to *Zingiber mioga*, followed by *Kaempferia*, *Curcuma*, *Hedychium*, and *Roscoea* (Figure 2). This research provides a basis for molecular genetics research for *Zingiber mioga*'s classification.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study have sent it up to BankIt (2403323) of National Center for Biotechnology Information, and provided GenBank accession number (MW285081). The associated BioProject and Bio-Sample numbers are PRJNA683709 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA683709>) and SRX9654415 (<https://www.ncbi.nlm.nih.gov/sra/SRX9654415/>), respectively.

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