

## Complete chloroplast genome sequence and phylogenetic analysis of *Ilex viridis* Champ. ex Benth

Zhenyu Shan<sup>a\*</sup>, Biyao Zhou<sup>a\*</sup>, Yao Li<sup>a,b</sup>, Daosen Liu<sup>c</sup>, Wei Li<sup>a</sup>, Julin Ma<sup>a</sup> and Tao Su<sup>a,b</sup>

<sup>a</sup>Co-Innovation Center for Sustainable Forestry in Southern China, College of Biology and the Environment, Nanjing Forestry University, Nanjing, China; <sup>b</sup>Key Laboratory of State Forestry Administration on Subtropical Forest Biodiversity Conservation, Nanjing Forestry University, Nanjing, China; <sup>c</sup>Institute of Communication and Electronic Engineering, Qiqihar University, Qiqihar, China

### ABSTRACT

*Ilex viridis* Champ. ex Benth. is domestic to southern China. In the present work, the complete chloroplast (cp) genome sequence of *Ilex viridis* was assembled and characterized by high-throughput sequencing analyses. The chloroplast genome was 157,701 bp in length, consisting of large single-copy (LSC) and small single-copy (SSC) regions of 87,177 bp and 18,394 bp, respectively, which were separated by a pair of 26,065 bp inverted repeat (IR) regions. The genome was predicted to contain 134 genes, including 89 protein-coding genes, 37 tRNA genes, and 8 rRNA genes. The overall GC content of the genome is 37.7%. The phylogenetic tree reconstructed using 13 chloroplast genomes reveals that *I. viridis* is mostly related to *Ilex szechwanensis*.

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*Ilex viridis*; complete chloroplast genome; phylogenetic analysis



*Ilex* is the only living genus in the monogeneric family Aquifoliaceae which contains 600 species varying in leaf morphologies (Yao et al., 2016). The geographic distribution of *Ilex* is associated with climatic patterns. *Ilex viridis* Champ. ex Benth. is native to China and grows in dense or miscellaneous forests on the altitude of 960–1250 m (Peng et al., 2013). Plants in the holly family have long been cultivated as ornamentals and herbal medicine. *I. viridis* showed extremely high medicinal values owing to the abundance of the secondary metabolites and the potential healthy activities (Li et al., 2013). Its vegetative tissues are used for curing hemolysis, detoxification, removal of phlegm, and metabolic disorder (Hao et al., 2013). *I. viridis* leaves were applied externally to staunch bleeding caused by empyrosis and trauma, and the root extracts were taken orally to alleviate arthralgia (Yi et al., 2016). Besides, *I. viridis* is a kind of evergreen tree with a tall trunk and strong branchlets, which makes itself the fine ornamental species for roadside trees, woodlots and park greening. As yet, there remain no reports on the plastid genome information of *I. viridis*. Here, using next-generation sequencing, the complete cp genome determined through a combination of *de novo* and reference-guided assembly, will provide theoretical basis for the phylogeny of *Ilex* genus.

The fresh leaves of *I. viridis* were sampled from Yuhuan, Zhejiang, China (28°13'N, 121°10'E). The voucher specimen (accession number YL20190417015) was preserved at the

Herbarium of Nanjing Forestry University (HNFU). The cp DNA extraction was conducted according to a previous study (Su et al., 2020). The whole-genome sequencing was served by Hefei Biodata Biotechnologies Inc. (Hefei, China) on the Illumina Hiseq platform. The filtered sequences were assembled using the program SPAdes assembler 3.10.0 (Bankevich et al., 2012). The annotation was performed using the DOGMA (Wyman et al., 2004) and BLAST searches.

The complete cp genome of *I. viridis* comprises 157,701 bp double-stranded, circular DNA (GenBank no. MN830250). It contains two IR regions of 26,065 bp, separated by large LSC and small SSC regions of 87,177 bp and 18,394 bp, respectively. The overall GC content of *I. viridis* cp genome is 37.7% and the corresponding values in LSC, SSC and IR regions are 35.7%, 31.9% and 43.0%, respectively. The cp genome was predicted to contain 134 genes, including 89 protein-coding genes, 37 tRNA genes, and 8 rRNA genes. Eight protein-coding genes, eight tRNA genes, and four rRNA genes were duplicated in IR regions. Nineteen genes contained two exons and four genes (*clpP*, *ycf3* and two *rps12*) contained three exons.

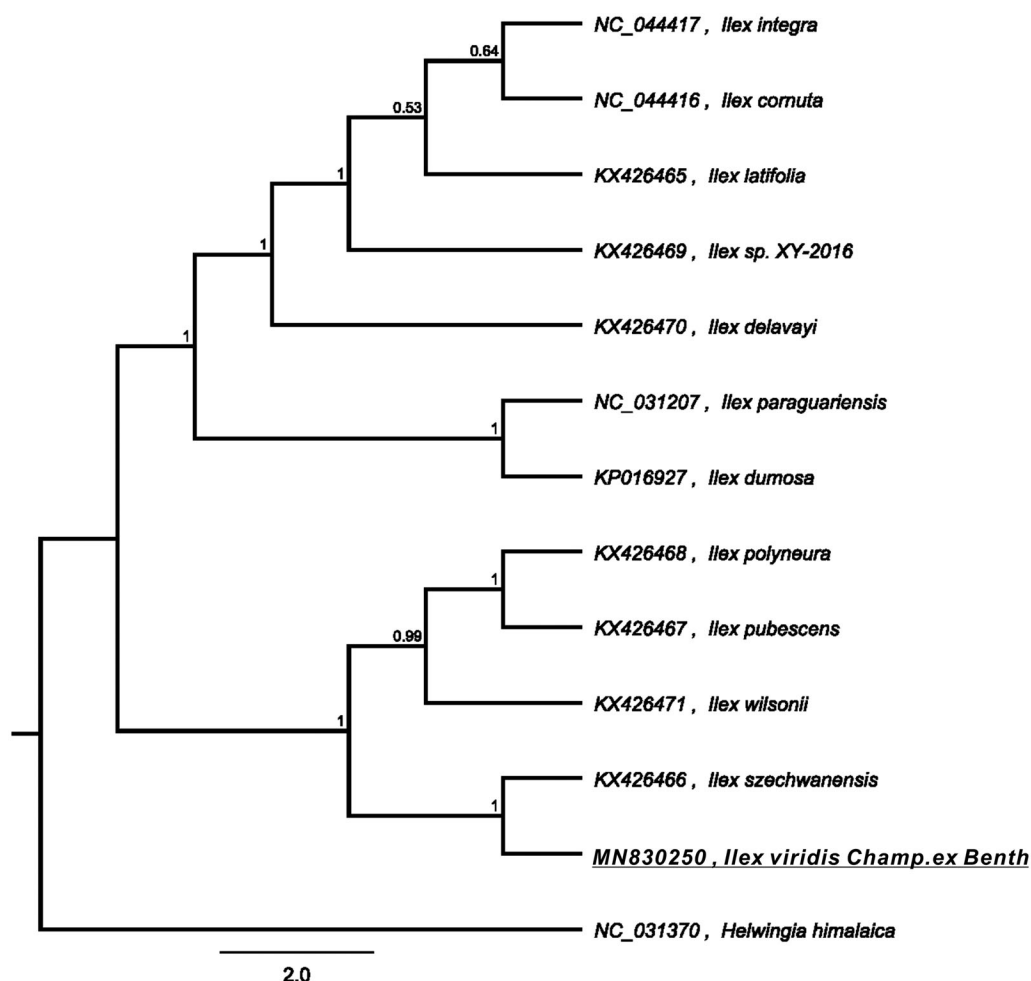
Thirteen cp genome sequences were aligned using MAFFT v7.307, and a maximum likelihood tree was constructed by FastTree version 2.1.10 (Kato and Standley, 2013; Price et al., 2010), showing that *I. viridis* is mostly related to *I. szechwanensis* (Figure 1). The cp sequences display superiority for the species discrimination. Thus, the complete cp genome

**CONTACT** Tao Su  [sutao@njfu.edu.cn](mailto:sutao@njfu.edu.cn)  Key Laboratory of State Forestry Administration on Subtropical Forest Biodiversity Conservation, Nanjing Forestry University, Nanjing 210037, China

\*These authors contributed equally to this work

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**Figure 1.** Phylogenetic tree inferred by Maximum Likelihood (ML) method based on 13 representative species. *Helwingia himalaica* was used as an outgroup control. A total of 1000 bootstrap replicates were computed and the support values were shown at the branches. The GenBank accession numbers were shown next to the name of the species.

sequence of *I. viridis* will provide an indispensable resource for the conservation genetics and the phylogenetic studies of Aquifoliales.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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