

The complete chloroplast genome sequence of *Ludisia discolor* from Hainan of China

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ABSTRACT

Ludisia discolor is one of the most important ornamental and medicinal orchids. To improve our understanding of the evolution of chloroplast, we resequenced complete chloroplast (cp) genome of *L. discolor* from Hainan, China. The cp genome sequence of *L. discolor* of Hainan was 153,324 bp in length, with a large single-copy (LSC) region of 82,922 bp, a small single-copy (SSC) region of 17,258 bp, and a pair of inverted repeats (IR) regions of 26,572 bp. Complete chloroplast genome contain 132 genes, there were 86 protein-coding genes, 38 tRNA genes and eight rRNA genes. The phylogenetic tree showed that *L. discolor* of Hainan is sister to *L. discolor* (unknown distributed region). Their cp genomes have same gene number but different in length of genome, indicating high conserved among them.

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Shican, *Ludisia discolor*, is a perennial orchid which belongs to Orchidoideae, Orchidaceae (Chen et al. 2009). It usually grows on the rocks of ravines or slopes in evergreen broad-leaved forests at altitude 900–1300 metres and is distributed in China (Guangdong, Guangxi, Hainan and Yunnan), Myanmar, Laos, Cambodia, Vietnam, Thailand, Malaysia, Philippines, Indonesia (Chen et al. 2009). *L. discolor* is a traditional Chinese herbal medicine and has the effect of tonifying kidney, moistening lung and clearing heat and so on (Lin 2012). Unfortunately, wild *L. discolor* is dying out due to over-exploitation in recent years. It is now under the second-grade protection state in China (Su et al. 2017). It is imperative to establish effective conservation strategies for this important plant. The complete chloroplast genome data will provide useful information for the study of population dynamics, phylogeny and species evolution (Shaw et al. 2014). Previously, a cp genome of *L. discolor* (unknown distributed region) has been reported. To compare chloroplast of *L. discolor* from different distributed regions, we sequenced complete chloroplast genome of *L. discolor* from Hainan, China.

All of the cp genome came from fresh leaves of *L. discolor* from Jianfenglin mountain (18°73'42.70"N, 108°85'81.07"E), Hainan Province of China. The voucher specimen was kept at the herbarium of College of Landscape Architecture, Fujian Agriculture and Forestry University (specimen code Ld190801). DNA was extracted from fresh leaf tissue, with 450 bp randomly interrupted by the Covaris ultrasonic

breaker for library construction. The constructed library was sequenced by the Illumina Hiseq Xten platform, with approximately 2GB data generated. Illumina data was filtered by script in the cluster (default parameter: -L 5, -p 0.5, -N 0.1). Complete chloroplast genome of *Goodyera velutina* (GeneBank accession: NC_029365) was used as reference, chloroplast genome of *L. discolor* was assembled by GetOrganelle pipe-line (<https://github.com/Kinggerm/GetOrganelle>) to obtain plastid-like reads, and the reads were viewed and edited by Bandage (Wick et al. 2015). Assembled chloroplast genome was annotated base on comparing with *G. velutina* by Geneious v 11.1.5 (Biomatters Ltd., Auckland, New Zealand) (Kearse et al. 2012). The annotation result was drawn with the online tool OGDRAW (<http://ogdraw.mpimgolm.mpg.de/>) (Lohse et al. 2013).

The complete chloroplast genome sequence of *L. discolor* (GenBank accession: MN317571) was 153,324 bp in length, with a large single-copy (LSC) region of 82,922 bp, a small single-copy (SSC) region of 17,258 bp, and a pair of inverted repeats (IR) regions of 26,572 bp. Complete chloroplast genome contained 132 genes, including 86 protein-coding genes, 38 tRNA genes, and eight rRNA genes. The complete genome GC content was 36.9%. The released cp genome sequence on NCBI of *L. discolor* (GenBank accession: NC_030540, not show the distributed region) was 153,054 bp in length, and the corresponding values in LSC, SSC and IR regions were 83,879 bp, 17,233 bp and 25,971 bp,

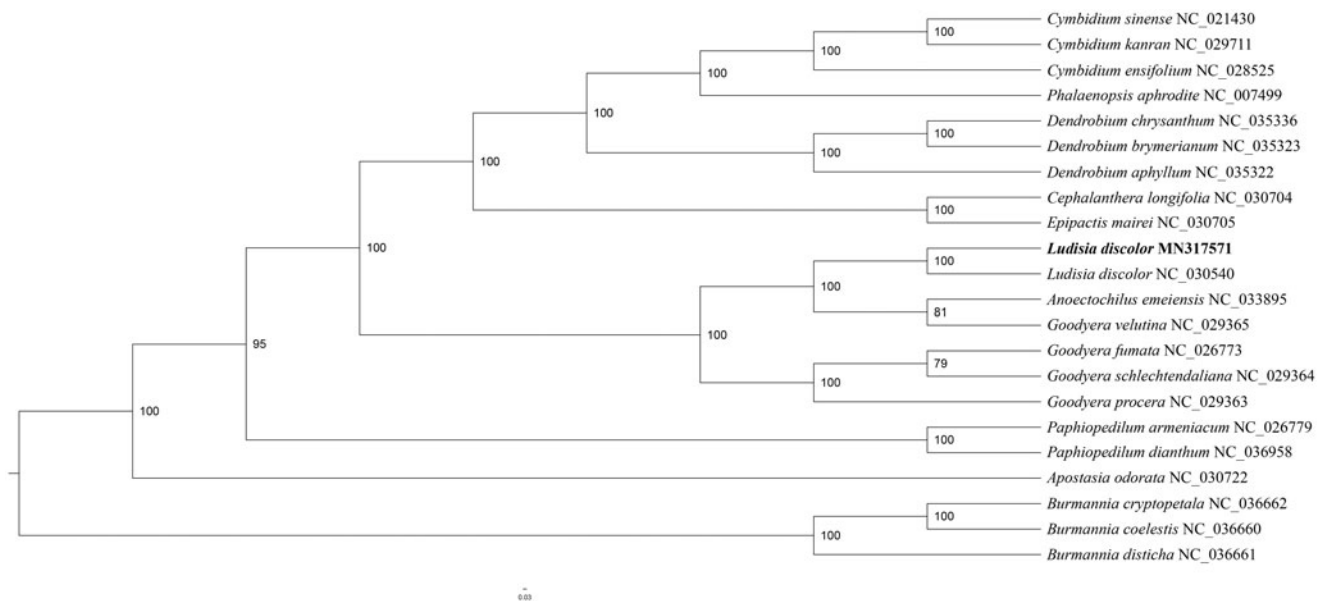


Figure 1. Phylogenetic analysis of 19 species of Orchidaceae, including two *L. discolor*, and three taxa (*Burmannia coelestis*, *B. cryptopetala*, *B. disticha*) as outgroup based on chloroplast genome sequences by RAxML, bootstrap support value near the branch.

respectively. The genome contained 132 genes, 86 protein-coding genes, 38 tRNA genes, eight rRNA genes and 37.0% GC content. The result shows that cp genomes of the two *L. discolor* have same gene number but different in length of genome, indicating their cp genomes were highly conserved. The difference between these two genomes is the result of the different species used for reference annotation and the variations between plant populations.

In order to reveal the phylogenetic position of *L. discolor* with other members of Orchidaceae, a phylogenetic analysis was performed based on 19 complete chloroplast genomes of Orchidaceae, including two *L. discolor*, and three species (*Burmannia coelestis*, *B. cryptopetala*, *B. disticha*) as outgroup, they were all downloaded from NCBI GenBank (Jiang et al. 2019). The sequences were aligned by MAFFT v7.307 (Katoh and Standley 2013), and phylogenetic tree was constructed by RAxML (Stamatakis 2014). The phylogenetic tree showed that *L. discolor* nest into *Goodyera* clade and is sister to the released cp genome on NCBI of *L. discolor* (GenBank accession: NC_030540) with strong support (Figure 1).

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

- Chen X, Gale SW, Cribb PJ. 2009. *Ludisia* A. Richard in Wu Z, Ravan PH, Hong D. Flora of China. 25:55.
- Jiang YT, Lin RQ, Liu B, Zeng QM, Liu ZJ, Chen SP. 2019. Complete chloroplast genome of *Cymbidium ensifolium* (Orchidaceae). Mitochondrial DNA B. 4:2236–2237.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 30:772–780.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28: 1647–1649.
- Lin ZX. 2012. Research progress on *Ludisia discolor* of ornamental south China medicinal plants resource. Fujian Sci Technol Tropical Crops. 37: 4–5.
- Lohse M, Drechsel O, Kahlau S, Bock R. 2013. Organellar Genome DRAW—a suite of tools for generating physical maps of chloroplast and mitochondrial genomes and visualizing expression data sets. Nucleic Acids Res. 41:W575–W581.
- Shaw J, Shafer HL, Leonard OR, Kovach MJ, Schorr M, Morris AB. 2014. Chloroplast DNA sequence utility for the lowest phylogenetic and phylogeographic inferences in angiosperms: the tortoise and the hare IV. Am J Bot. 101:1987–2004.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30:1312–1313.
- Su JM, Zhu H, Huang ZY, Mo ZZ, Chen RX, Dong YP, Zheng CX. 2017. Study on the proliferation of axillary buds and rooting of *Ludisia discolor*. J Yulin Normal University. 38:90–92.
- Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualization of de novo genome assemblies. Bioinformatics. 31:3350–3352.