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 153,324bp in length, with a large single-copy (LSC) region of 82,922bp, a small single-copy (SSC) region of 17,258bp, and a pair of inverted repeats (IR) regions of 26,572bp. 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.

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.mpimp-golm.mpg.de/) (Lohse et al. 2013).

The complete chloroplast genome sequence of *L. discolor* (GenBank accession: MN317571) was 153,324bp in length, with a large single-copy (LSC) region of 82,922bp, a small single-copy (SSC) region of 17,258bp, and a pair of inverted repeats (IR) regions of 26,572bp. 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 153,054bp in length, and the corresponding values in LSC, SSC and IR regions were 83,879bp, 17,233bp and 25,971bp.
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).

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No potential conflict of interest was reported by the authors.

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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.