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Complete plastid genome of two *Dalbergia* species (Fabaceae), and their significance in conservation and phylogeny

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ABSTRACT

Dalbergia (Fabaceae) is a pantropical genus. Due to high economic and ecological values, many *Dalbergia* species were assessed as threatened taxa. In this study, we reported the complete plastome of two *Dalbergia* species, *D. odorifera* and *D. oliveri*, that was 155,838 bp and 156,074 bp in size, respectively. Comparative analyses showed *Dalbergia* plastomes were conserved in genome size, structure, and gene contents. Four nucleotide diversity hotspots of *Dalbergia* genomes were *rpl32-ndhF*, *rpl32-trnL^{UAG}*, *trnL^{UAA}*, and *trnT^{ACA}-trnL^{UAA}*. Phylogenetic analysis revealed the sister relationship between *D. odorifera* and *D. hainanensis*, and between *D. oliveri* and *D. assamica*, respectively. The complete plastomes can provide the important information for investigations on conservation genetics and phylogenies of *Dalbergia* and Fabaceae.

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Dalbergia L. f. (Faboideae, Fabaceae), comprises approximately 250 species as trees, shrubs, and woody climbers, which are widely distributed in tropical and sub-tropical regions around the world (Vatanparast et al. 2013; Hassold et al. 2016). Due to high economic and ecological values, all *Dalbergia* species have been included in appendices list of Wild Fauna and Flora by international trade regulations under the Convention on International Trade (CITES 2019). Meanwhile, 108 *Dalbergia* species are included in the IUCN Red List (IUCN 2020). Unfortunately, some *Dalbergia* species become endangered as the consequence of illegal timber trades. To enforce protective legislation and ensure effective conservation on *Dalbergia* species, the wood identity of the traded timber must be effective and accurate. Therefore, it would be highly desirable to develop reliable identification techniques being rapidly applied without specialists (Song et al. 2019). To date, DNA barcoding approach has been demonstrated as an effective molecular technique for species identification and community phylogenies (CBOL Plant Working Group 2009; Li et al. 2011; Kress 2017; Zeng et al. 2018). Chloroplast genomes of angiosperms have a circular structure and are composed of four regions, including two inverted repeat regions that are separated by a large single-copy (LSC) and a small single-copy (SSC) region (Palmer 1983; Wicke et al. 2011; Ruhlman and Jansen 2014). Chloroplast genomes of *Dalbergia* spp. range from 155,726 to 156,698 bp in size (Wariss et al. 2018; Deng et al. 2019; Song et al. 2019). In this study, we *de novo* assembled the

complete chloroplast genome of two *Dalbergia* species, *D. odorifera* T.C.Chen and *D. oliveri* Gamble ex Prain, from China and Myanmar. The main goals of this study were to: (1) characterize the chloroplast genome of *Dalbergia* spp.; (2) assess high variable plastid regions for species identification and evolutionary investigations; and (3) reconstruct the plastid phylogenomics of *Dalbergia*, as well as Faboideae.

Fresh specimens of *D. odorifera* were collected from Xishuangbanna Tropical Botany Garden, Chinese Academy of Sciences, Yunnan, China (21°55'14"N and 101°16'34"E), and those of *D. oliveri* were collected from Ngaliak Reserve, Nay Pyi Taw Union Territory, Myanmar (19°92'68"N and 95°97'52"E). Voucher specimens of *D. oliveri* (P.P. Win et al. PPW-029) and *D. odorifera* (W-B Yu et al. Dai 166) were deposited at the Herbarium of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (HITBC).

Genomic DNAs were extracted from silica gel dried leaves using a modified CTAB method (Doyle and Doyle 1987). The purified DNA was fragmented to approximately 350 bp in size for library construction, and 150 bp pair-end reads were generated by Illumina NovaSeq 6000 (Annoroad, Beijing, China). The raw reads were *de novo* assembled using GetOrganelle toolkit (Jin et al. 2018). The plastomes were annotated using CPGAVAS2 (Shi et al. 2019), then manually adjusted in Geneious (Kearse et al. 2012). Fifty-two plastomes of Faboideae were achieved from GenBank, and *Cercis canadensis* L. was chosen as outgroup. The whole plastome sequences with on IR region were aligned using MAFFT

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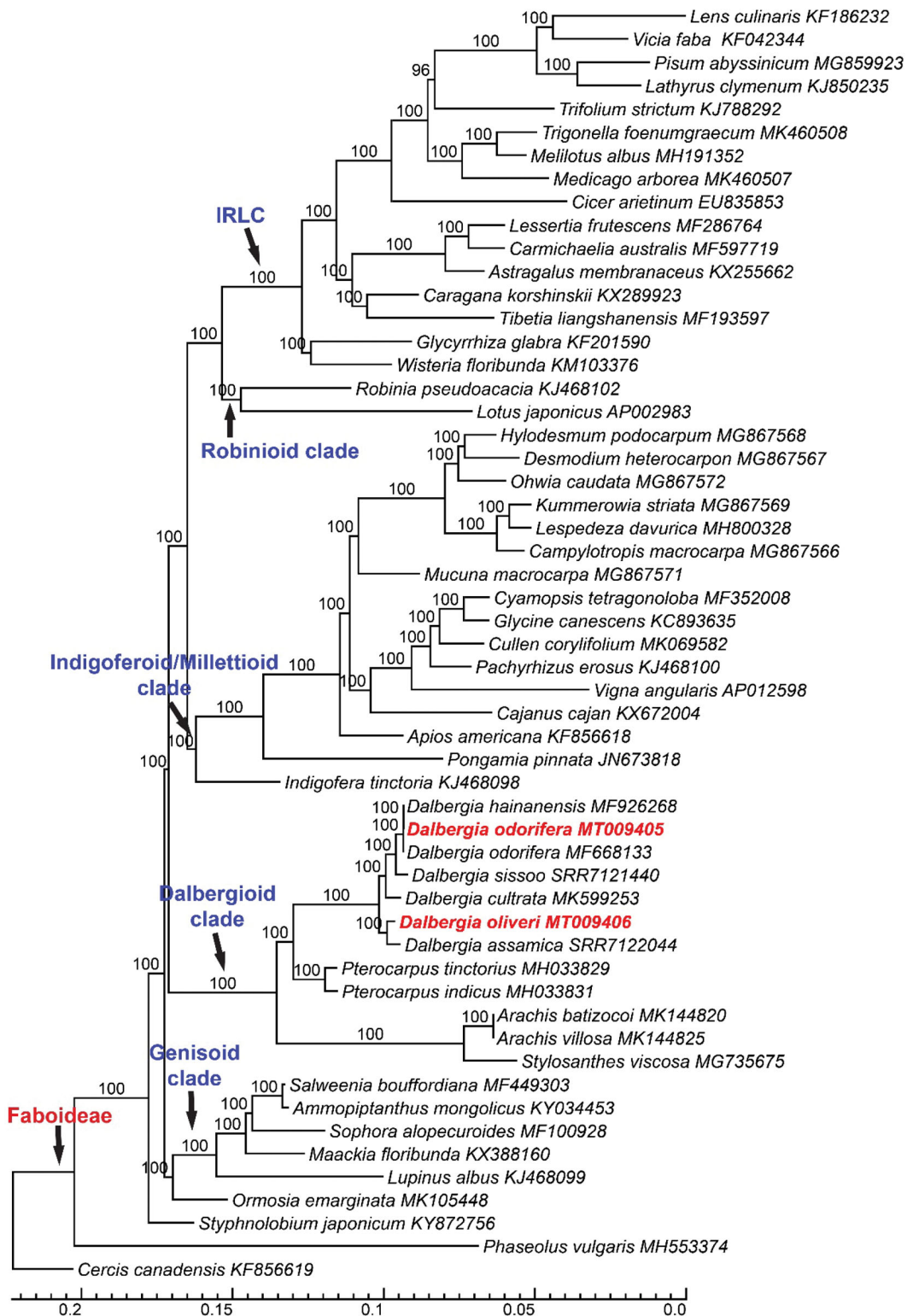


Figure 1. Phylogeny of *Dalbergia* species based on complete plastomes using maximum likelihood methods with bootstrap values on the branch. The bottom scale bar represents the number of substitutions per site. Two new plastomes (MT009405 and MT009406) were annotated using bold font.

(Kato and Standley 2013), gaps were trimmed by trimAl (Capella-Gutiérrez et al. 2009) using the command '-gt 0.5 cons 50'. The maximum likelihood tree was reconstructed by RAxML (Stamatakis 2014) using GTRGAMMAI model with 1000 bootstrap replicates on CIPRES (<https://www.phylo.org/>). The tree was visualized with the TreeGraph (Stover and Muller 2010). The dynamic nucleotide diversity (π) of seven

Dalbergia plastomes was estimated using DnaSP (Rozas et al. 2017) by a window size 500 bp and step size 250 bp. The region was considered as the divergence hotspot, when values of π were higher than 0.04%.

Two plastomes of *Dalbergia* were a typical quadripartite structure and 156,074 bp (*D. odorifera*, MT009405) and 155,838 bp (*D. oliveri*, MT009406) in length. The LSC region

for *D. odorifera* and *D. oliveri* was 85,766 bp and 85,829 bp, respectively, the SSC region was 18,841 bp and 18,686 bp, respectively, and the IR regions were 25,702 and 25,693 bp, respectively. GC contents were 36.1%. The genomes had 111 unique genes, including 77 protein coding genes (missing *infA*, *rpl22*, and *ycf15*), 30 transfer RNA (tRNA) genes, and four ribosomal RNA (rRNA). Four nucleotide diversity hotspots, *rpl32-ndhF*, *rpl32-trnL^{UAG}*, *trnL^{UAA}*, and *trnT^{ACA}-trnL^{UAA}*, were identified across the plastomes of *Dalbergia*, which are informative regions as DNA barcodes for molecular authentication of *Dalbergia* species.

Phylogenetic analysis showed that the backbone of Faboideae was well resolved, and five major clades were fully supported (Figure 1). Dalbergioid clade included four genera, showing a sister relationship between *Stylosanthes*+*Arachis* and *Dalbergia*+*Pterocarpus*. *Dalbergia* species formed a clade with 100% bootstrap values, and *D. assamica* Pittier and *D. oliveri* formed a clade, which was sister to the remaining *Dalbergia* species (Figure 1). *D. sissoo* was sister to *D. oliveri*+*D. hainanensis* Merr. & Chun. Therefore, this study demonstrated that the whole chloroplast genome sequences can reconstruct a robust phylogeny of *Dalbergia*, as well as Faboideae.

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Disclosure statement

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

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Data availability

The sequencing data using in this manuscript are deposited in the GenBank sequence database. The data were collected without violation of the protection of human subjects, or other valid ethical, privacy, or security concerns.

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