The complete chloroplast genome sequence of *Intsia bijuga* (Colebr.) Kuntze (Fabaceae: Detarioideae: Afzelieae)

Sonicha U-thoomporna, Wasitthee Kongkachana, Nukoon Jomchai, Nattapol Narong, Pitchaporn Waiyamitra, Pasin Maprasop, Sithichoke Tangphatsornruang, and Wirulda Pootakhama

National Omics Center, National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand; Department of Marine and Coastal Resources, Bangkok, Thailand

**ABSTRACT**

*Intsia bijuga* (Colebr.) Kuntze. (1891) is a threatened mangrove species, belonging to the Fabaceae family and is native to the western Pacific coast and Southeast Asia. Here, we applied short-read Illumina technology to sequence and assemble its chloroplast genome. The complete chloroplast genome is 158,363 bp in length, composed of one large single-copy (LSC) region of 87,489 bp, one small single-copy (SSC) region of 19,438 bp, and a pair of inverted repeats (IRs) of 25,719 bp. A total of 129 unique genes were annotated, comprising 84 protein-coding genes, eight rRNA genes, and 37 tRNA genes. Our phylogenetic analysis showed the placement of *I. bijuga* (OL699920.1) with *Afzelia* species within Fabaceae family.

*Intsia bijuga* (Colebr.) Kuntze. (1891) is a threatened mangrove species, belonging to the Fabaceae family, commonly known as Ipil, Moluccan ironwood, Borneo teak or Vesi (Nugroho et al. 2010). They are prevalent in the western Pacific coast and Southeast Asia, especially in Indonesia, Malaysia, Thailand, and the Philippines (Hu et al. 2012; Hartiadi et al. 2020). Their typical habitats are lowland areas and riparian zones (Thaman et al. 2006). With careful preparation, *I. bijuga* seeds can be made edible and juice extracted from parts of bark has been used in traditional medicine to treat rheumatism, chills, diarrhea, muscular rigidity, and adult rheumatoid arthritis (Cambie and Ash 1994). To date, there have been very few genetics or genomic studies on *I. bijuga*. Here, we assembled and characterized the complete chloroplast genome sequence of *I. bijuga*. This information enables future comparative studies that can provide a better understanding of its evolution and genetic diversity.

In this study, fresh leaves of *I. bijuga* were collected from the Mangrove Forest Research Center, Ranong, Thailand (9°53’10.2”N 98°32’51.6”E). The sample collection permit was issued by Department of Marine and Coastal Resources (Ministry of Natural Resources and Environment, Thailand) under the project number P-1952261. To prepare high molecular weight DNA for 10x Genomics linked-read sequencing, the total genomic DNA was extracted using MagAttract HMW DNA Kit following the manufacturer’s protocol. The herbarium voucher specimen and DNA of *I. bijuga* were deposited at National Biobank of Thailand (NBT), National Science and Technology Development Agency (NSTDA) (contact: Anuttara Nathalang; Email: anuttara.nat@nstda.or.th and Panyavut Aumpuchin; Email: panyavut.aum@nstda.or.th) under the voucher numbers BBH004053 and NBTG000001, respectively. A total of 1.25 ng of high molecular weight DNA was used to prepare the link-read library, and the 10x library was sequenced on single lane of Illumina Hiseq X Ten (2 x 150 bp paired-end read, Illumina, San Diego, CA). Illumina short-read data were assembled by GetOrganelle v1.7.3.5 (Jin et al. 2020), and the assembly was annotated with the GeSeq (Tillich et al. 2017). The complete chloroplast genome sequence and annotation were deposited in the NCBI GenBank database under the accession number OL699920.1. The *I. bijuga* chloroplast genome was aligned with that of two other *I. bijuga* accessions and 133 related species. Maximum-likelihood phylogenetic analysis was constructed on this dataset using RAxML 68 v1.0.0 with 1000 bootstrap replications (Stamatakis 2014). *Quillaja saponaria* was used as an outgroup (Figure 1).

The length of the *I. bijuga* chloroplast genome was 158,363 bp, and the GC content was 36.23%, which was consistent with the GC content of the previously reported genomes (36.18%; MN709818.1, Zhang et al. 2020, 35.98%; KX673214.1, Donkpegan et al. 2017). The chloroplast genome consisted of a large single-copy (LSC) region of 87,489 bp with 33.89% GC content and a small single-copy (SSC) region of 19,438 bp with 29.69% GC content separated by two inverted repeat regions (IRs) of 25,719 bp with 42.67% GC content. The genome annotation contained a total of 129 genes, including 84 protein-coding genes, 37 tRNA genes, and eight rRNA genes.
The phylogenetic tree revealed a monophyletic group of our *I. bijuga* (OL699920.1) with the previously reported *I. bijuga* (KX673214.1 and MN709818.1) and five *Afzelia* species with a 100% bootstrap support value. The phylogenetic tree was consistent with the trees from other studies (Donkpeigan et al. 2017; Zhang et al. 2020). Our reported chloroplast genome of *I. bijuga* will be useful for future conservation genetic research of mangroves.

**Author contributions**

SU, WP, and ST designed research study and obtained the funding. NJ, NN, PW, and PM performed laboratory work (sample collection, DNA extraction, library construction, and sequencing). WK performed bioinformatics analyses. SU wrote and revised the manuscript, and all authors reviewed it.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This study was supported by the National Science and Technology Development Agency (NSTDA), Thailand under Grant Number [P1952261].
ORCID

Sithichoke Tangphatsornruang http://orcid.org/0000-0003-2673-0012

Data availability statement

The data that support the findings of this study are openly available in the GenBank database https://www.ncbi.nlm.nih.gov/genbank/ under the accession number OL699920.1. The associated BioProject, SRA, and BioSample numbers are: PRJNA783380, SRR17035385, and SAMN23429392, respectively.

References

Cambie RC, Ash J. 1994. Fijian medicinal plants. Collingwood, Australia: CSIRO Publishing.
Donkpegan ASL, Doucet J-L, Migliore J, Duminil J, Pinheiro R, Wieringa JJ, Champluvier D, Hardy OJ. 2017. Evolution in African tropical trees displaying ploidy-habitat association: the genus Afzelia (Leguminosae). Mol Phylogenet Evol. 107:270–281.
Hartiadi LY, Sahamastuti AAT, Chandra CV, Febriani E, Adiyanto SA, Daeli GBC, Clarissa GC. 2020. Protective effect of merbau (Intsia bijuga) extract on hydrogen peroxide-treated HaCaT human keratinocytes and its formulation as antioxidant cream. Pharm Sci. 27(1):131–138.
Hu C, Jiang G, Xiao M, Zhou J, Yi Z. 2012. Effects of heat treatment on water-soluble extractives and color changes of merbau heartwood. J Wood Sci. 58(5):465–469.
Jin J-J, Yu W-B, Yang J-B, Song Y, dePamphilis CW, Yi T-S, Li D-Z. 2020. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. Genome Biol. 21(1):1–31.
Nugroho JD, Mansur I, Purwito A, Suhendang E. 2010. Morphological characteristics of Ectomycorrhizas on merbau [Intsia bijuga (Colebr.) O. Kuntze]. HAYATI J Biosci. 17(2):68–72.
Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30(9):1312–1313.
Thaman RR, Thomson LA, DeMeo R, Areki F, Elevitch CR. 2006. Intsia bijuga (vesi). Species profiles for Pacific Island Agroforestry. (www.traditionaltree.org).
Tillich M, Lehwark P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R, Greiner S. 2017. GeSeq: versatile and accurate annotation of organelle genomes. Nucleic Acids Res. 45(W1):W6–W11.
Zhang R, Wang Y-H, Jin J-J, Stull GW, Bruneau A, Cardoso D, De Queiroz LP, Moore MJ, Zhang S-D, Chen S-Y, et al. 2020. Exploration of plastid phylogenomic conflict yields new insights into the deep relationships of Leguminosae. Syst Biol. 69(4):613–622.