

Complete chloroplast genome of *Firmiana major* (Malvaceae), a critically endangered species endemic to southwest China

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Received: 11 October 2017 / Accepted: 13 October 2017
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Abstract *Firmiana major* is an endangered species in southwest China, which had been considered as extinct in the wild on the IUCN Red List of Threatened Species in 1998. Fortunately, around 2000 wild individuals were rediscovered along the valley of Jinshajiang river in south Sichuan and north Yunnan. In this study, we reported a complete chloroplast genome of *F. major*, which was de novo assembled using the next-generation sequencing data. The plastome was 161,302 bp in length, consisting of a pair of inverted repeat (25,543 bp for each), one large single copy (90,178 bp) and one small single copy (20,038 bp) regions. The whole genome contained 132 genes, including 87 protein-coding, 37 tRNA and 8 rRNA genes. The overall GC content of the whole genome was 36.9%. Maximum likelihood analysis showed that *F. major* was sister to *Tilia* spp.

Keywords *Firmiana major* · Plastid genome · Malvaceae · IUCN · Endangered species

Firmiana major (W. W. Smith) Handel-Mazzetti (Malvaceae) is a rare woody species endemic to Yunnan and south

Sichuan (Hsue 1984; Tang et al. 2007). *F. major* was classified as the second-class national protected plants on the “list of rare and endangered plants in China” (State Environmental Protection Administration and Institute of Botany 1987). Subsequently, the “China Plant Red Data Book: Rare and Endangered Plants” recorded that the wild plants of *F. major* was hard to be found in the wild, or might be extinct due to natural vegetation has been heavily damaged in central Yunnan (Fu and Chin 1992). Furthermore, *F. major* was considered as extinction in the wild on the “IUCN Red List of Threatened Species” (Sun 1998). Fortunately, around 400 wild individuals of *F. major* were rediscovered in the National Nature Reserve of Panzhihua Cycad (Wang 2001). In 2017, > 2000 wild individuals of *F. major* had been found in Ninglang (ca. 1000 individuals), Yulong (ca. 1000 individuals), and Yuanmou (11 individuals) in central and northwest Yunnan (http://lijiang.yunnan.cn/html/2017-08/25/content_4920733.htm). In the present paper, we report a complete chloroplast genome of *F. major*. The plastid genome will contribute to develop protection measures for this endangered species.

A fresh leaf was collected from a single individual of *F. major* in the National Nature Reserve of Panzhihua Cycad, then it was dried using silica gel. Genomic DNA was isolated using Tiangen Plant Genomic DNA Kits (Tiangen Biotech, Beijing). The 150 bp pair-end reads were generated using the Illumina Hi-Seq 2500. There were total 19.82 million reads for both ends. De novo assembling the chloroplast genome used GetOrganelle pipeline (<https://github.com/Kinggerm/GetOrganelle>). The procedure was described in other studies (Jiang et al. 2017; Song et al. 2017). For the genome annotation, we used CpGAVAS (Liu et al. 2012) to annotate the chloroplast genes automatically, then adjusted and confirmed the annotated genes using Geneious 9.1.7

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(Kearse et al. 2012). We generate the plastome map of *F. major* (Fig. 1) using OGDRAW (Lohse et al. 2013).

The complete chloroplast genome of *F. major* was a quadripartite circular and 161,302 bp in length, comprising a large single copy (LSC) of 90,178 bp and a small single copy (SSC) of 20,038 bp, separated by two inverted repeat (IR) regions of 25,543 bp (Fig. 1). It contained 132 genes, including 87 protein-coding, 8 ribosomal RNA, and 37 tRNA genes. The plastome contained 114 unique genes, 18 genes duplicated in the IR regions. Among annotated genes, 24 genes contained a single intron, and three genes had two introns (i.e., *clpP* CDS, *ycf3* CDS, and *trnK-UUU* tRNA). The base compositions of *F. major* chloroplast genome were uneven (31.1% A, 18.6% C, 18.3% G, 32.0% T), with an overall GC content of 36.9%, and

the corresponding values of the LSC, SSC and IR regions reaching 34.7, 31.3 and 42.9%, respectively.

To ascertain phylogenetic position of *F. major* in the family Malvaceae, we selected 13 published plastomes of Malvaceae and *Aquilaria sinensis* (Lour.) Spreng. (Thymelaeaceae) as outgroup. The LSC, SSC and one IR regions of the 15 plastomes were aligned using MAFFT 7.310 (Katoh and Standley 2013). The maximum likelihood tree was reconstructed by RAxML 8.2.9 (Stamatakis et al. 2008). The result indicated that *F. major* is sister to *Tilia* spp. (Fig. 2). The complete chloroplast genome reported in this paper, which provided informative data for population genomic studies and conservation genetics on *F. major*, as well as for future phylogenetic studies in Malvaceae.

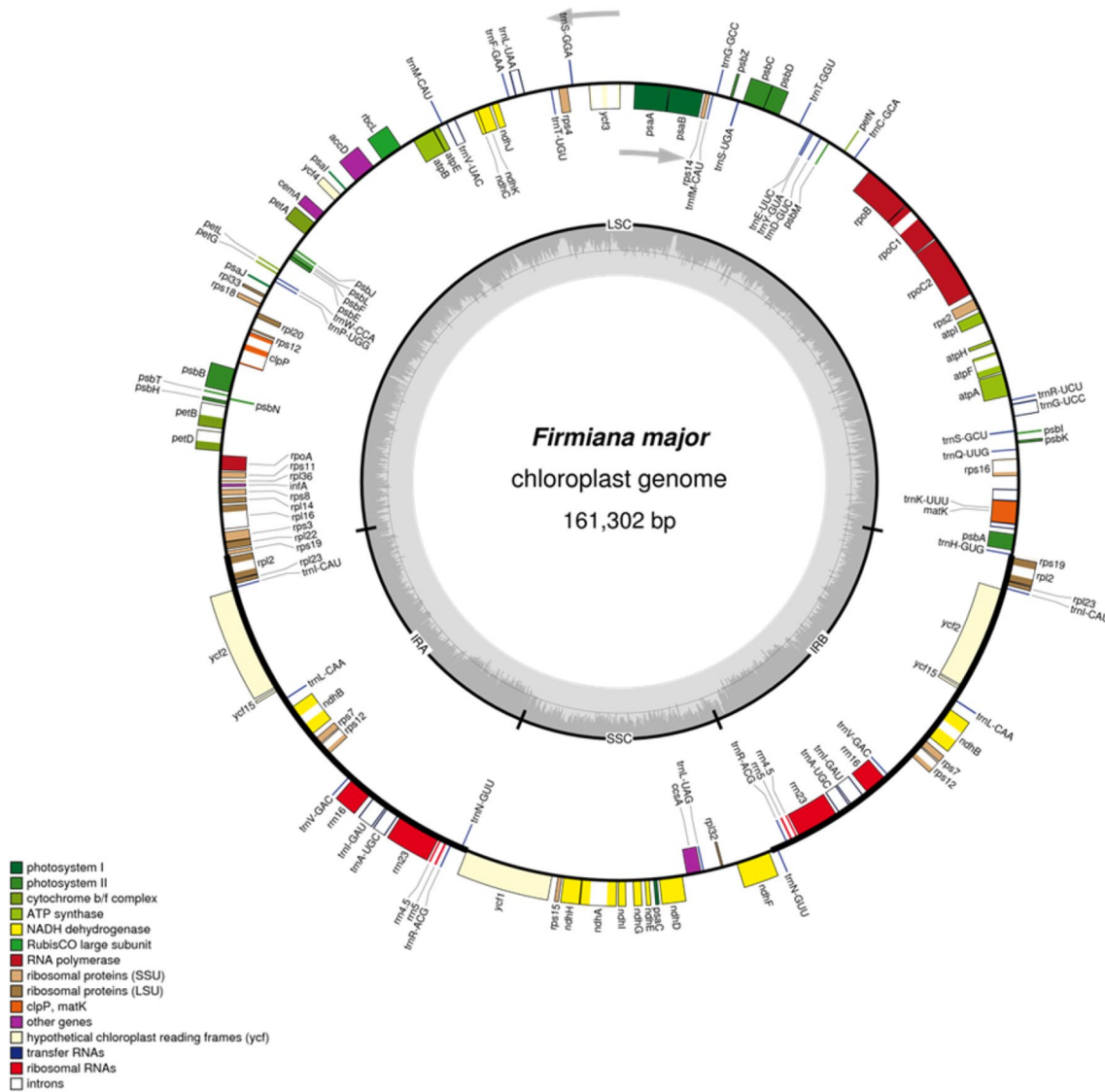
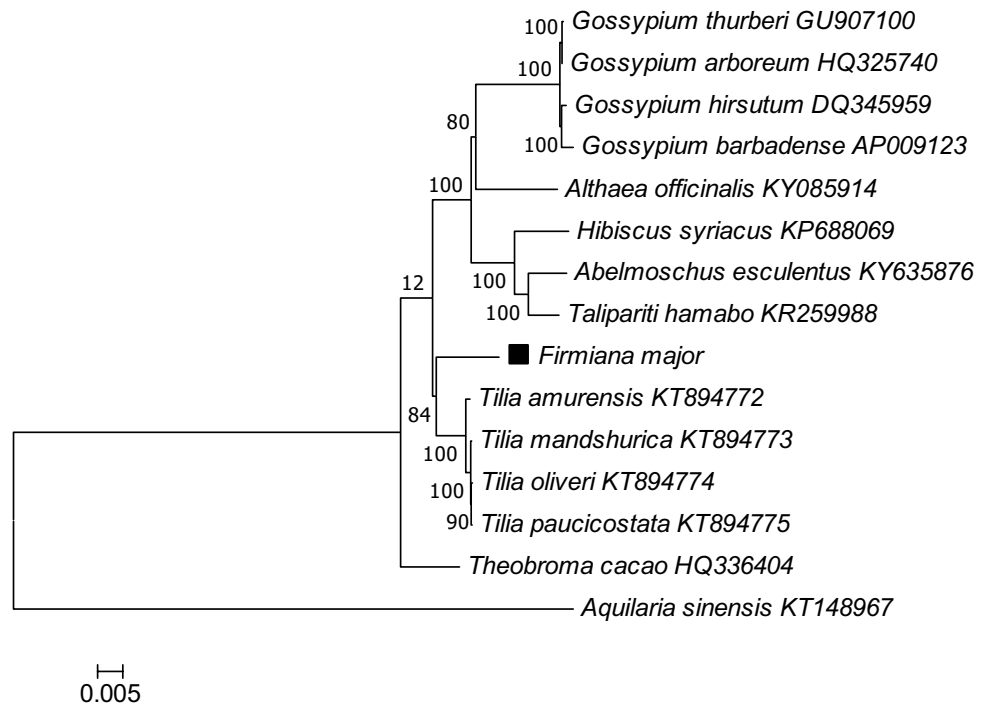


Fig. 1 Plastome map of *F. major*. Dashed area in the inner circle indicates the GC content. Arrows indicate the direction of transcription

Fig. 2 The plastome phylogeny of Malvaceae. Maximum Likelihood bootstrap of nodes showed next the node. *F. major* was indicated by a filled square



Acknowledgements We are grateful to the Germplasm Bank of Wild Species in Southwest China for supporting NGS; to Jing Yang, and Ji-Xiong Yang, for their assistance in molecular experiments. This study is supported by Grants from the National Key Basic Research Program of China (2014CB954100), the Major International Joint Research Project of National Natural Science Foundation of China (31320103919), and the Visiting Scholar Fellowships of Chinese Academy of Sciences and China Council Scholarship.

References

- Fu L-K, Chin C-M (eds) (1992) China plant red data book. Rare and Endangered Plants Science Press, Beijing
- Hsue H-H (1984) Sterculiaceae. In: Feng K-M (ed) Flora reipublicae popularis sinicae, vol 49. Science Press, Beijing, pp 112–189. http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=10850
- Jiang N, Zhou Z, Yang J-B, Yu W-B (2017) Complete chloroplast genome of *Anemoclema glaucifolium* (Ranunculaceae), a vulnerable and threatened species endemic to the Hengduan Mountains. *Conserv Genet Resour*. doi: 10.1007/s12686-12017-10874-12682
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30:772–780. doi:10.1093/molbev/mst010
- Kearse M et al (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. doi:10.1093/bioinformatics/bts199
- Liu C, Shi L, Zhu Y, Chen H, Zhang J, Lin X, Guan X (2012) CpGAVAS, an integrated web server for the annotation, visualization, analysis, and GenBank submission of completely sequenced chloroplast genome sequences. *BMC Genom* 13:715. doi:10.1186/1471-2164-13-715
- Lohse M, Drechsel O, Kahlau S, Bock R (2013) OrganellarGenomeDRAW—a suite of tools for generating physical maps of plastid and mitochondrial genomes and visualizing expression data sets. *Nucleic Acids Res* 41:W575–W581. doi:10.1093/nar/gkt289
- Song Y et al (2017) Evolutionary comparisons of the chloroplast genome in Lauraceae and insights into loss events in the Magnoliids. *Genom Biol Evol* 9:2354–2364. doi:10.1093/gbe/evx180
- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. *Syst Biol* 57:758–771. doi:10.1080/10635150802429642
- State Environmental Protection Administration, Institute of Botany (1987) List of the protected rare and endangered plants in China. *Bull Biol* 7:23–28
- Sun W (1998) *Firmiana major*. IUCN Red List Threat Species 1998:e.T32349A9700122. doi:10.2305/IUCN.UK.1998.RLTS.T32349A9700122.en
- Tang Y, Gilbert MG, Dorr LJ (2007) Sterculiaceae. In: Wu Z-Y, Raven PH, Hong D-Y (eds) *Flora of China*, vol 12, Missouri Botanical Garden & Science Press, St. Louis & Beijing, pp 302–330
- Wang D-S (2001) Discovering wild population of *Firmiana major* in Panzhihua. *Sichuan Plants* 2001:4–5