

The complete chloroplast genome sequence of *Myricaria wardii* Marquand 1929 (Tamaricaceae): a shrub species endemic to the Tibet Plateau

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ABSTRACT

Myricaria wardii Marquand 1929, endemic to the Tibet Plateau, is a perennial shrub with important medicinal and ecological values. In this study, the complete chloroplast (cp) genome of *M. wardii* was assembled, and the phylogenetic tree was reconstructed to evaluate the phylogenetic location of the species. The results showed that the cp genome size of the *M. wardii* was 155,299 bp, which contained a pair of inverted repeat (IR) regions (26,150 bp), a large single copy (LSC) region (84,715 bp), and a small single copy (SSC) region (18,284 bp). The total GC content of the cp genome was 36.30%. A total of 128 genes were annotated, consisting of 83 protein-coding genes, 37 tRNA genes and 8 rRNA genes. The phylogenetic analysis showed that *M. wardii* was closely related to *M. prostrata*. This study provides useful information for the conservation of this species and the phylogenetic analysis of Tamaricaceae.

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Myricaria wardii; complete chloroplast genome; sequencing; phylogenetic relationship

Introduction

Myricaria wardii Marquand 1929, a perennial shrub species of Tamaricaceae, is narrowly distributed in sandy river banks with high altitudes of 3000~4000 m in the Tibet Plateau (Yang and Gaskin 2007), including Nyingchi, Lhasa and Shigatse of Tibet Autonomous Region, Qilian, Jiuzhi and Guide county of Qinghai province, Mianyang, Ya'an and Ngawa of Sichuan province, Tianshui and Lanzhou of Gansu province and Yulong county of Yunnan province (PPBC, <http://ppbc.iplant.cn/sp/23032>; CVH, <https://www.cvh.ac.cn/spms/list.php?&taxonName=Myricaria%20wardii>). The plant has strong sand-fixing capacity because of its developed roots (Zhang and Zhang 1984). Meanwhile, *M. wardii* is a traditional Tibetan medicine with the efficacy for heat-clearing and detoxifying (La et al. 2010). However, the number and size of *M. wardii* populations are decreasing sharply due to increasing human interferences in recent decades (Wei et al. 2022). Molecular information is necessary to gain insight into the systemic evolution of the plant. In this study, we reported the complete chloroplast (cp) genome of *M. wardii* and reconstructed the phylogenetic relationship within *Myricaria* genus, which will provide baseline genomic information of the plant.

Materials and methods

The fresh sample of *M. wardii* was collected from Nyingchi City, Tibet Autonomous Region, China (94°19'40.49" E, 29°41'47.52" N), with an elevation of 3007 m (Figure 1). Then, the fresh leaves were immediately dehydrated in silica gel. The voucher specimen was logged in the Herbarium of Wuhan Botanical Garden, CAS (HIB0231114, Yuanyuan Chen, yychen@wbgcas.cn). The whole genome DNA was extracted from about 0.3 g dried leaves following the CTAB method (Doyle and Doyle 1987). The DNA quality was detected by NanoDrop 2000 micro spectrophotometer. The purified DNA was used to build the sequencing library by the Illumina NovaSeq 6000 platform. Totally, we obtained 5.68 Gb raw data and 5.56 Gb clean data. The complete chloroplast genome of *M. wardii* was assembled with the software GetOrganelle v1.7.1a (Jin et al. 2020).

The resultant genome was annotated by PGA (Qu et al. 2019) with the chloroplast genome sequence of *M. prostrata* (NC_046761) and *Frankenia laevis* (NC_041277) as references, and the result was manually adjusted by the program Geneious Prime (Tillich et al. 2017). Then, the cis- and trans-splicing genes were detected by the program CPGView (Liu et al. 2023). Using the same software, the circular gene map of the *M. wardii* plastid genome was visualized. The complete

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Figure 1. Photos of *Myricaria wardii*. (A) The plants of *M. wardii* in its natural habitat; (B) The flowers of *M. wardii*. The species reference images were taken by Yuanyuan Chen from the sampling location in this study.

Myricaria wardii

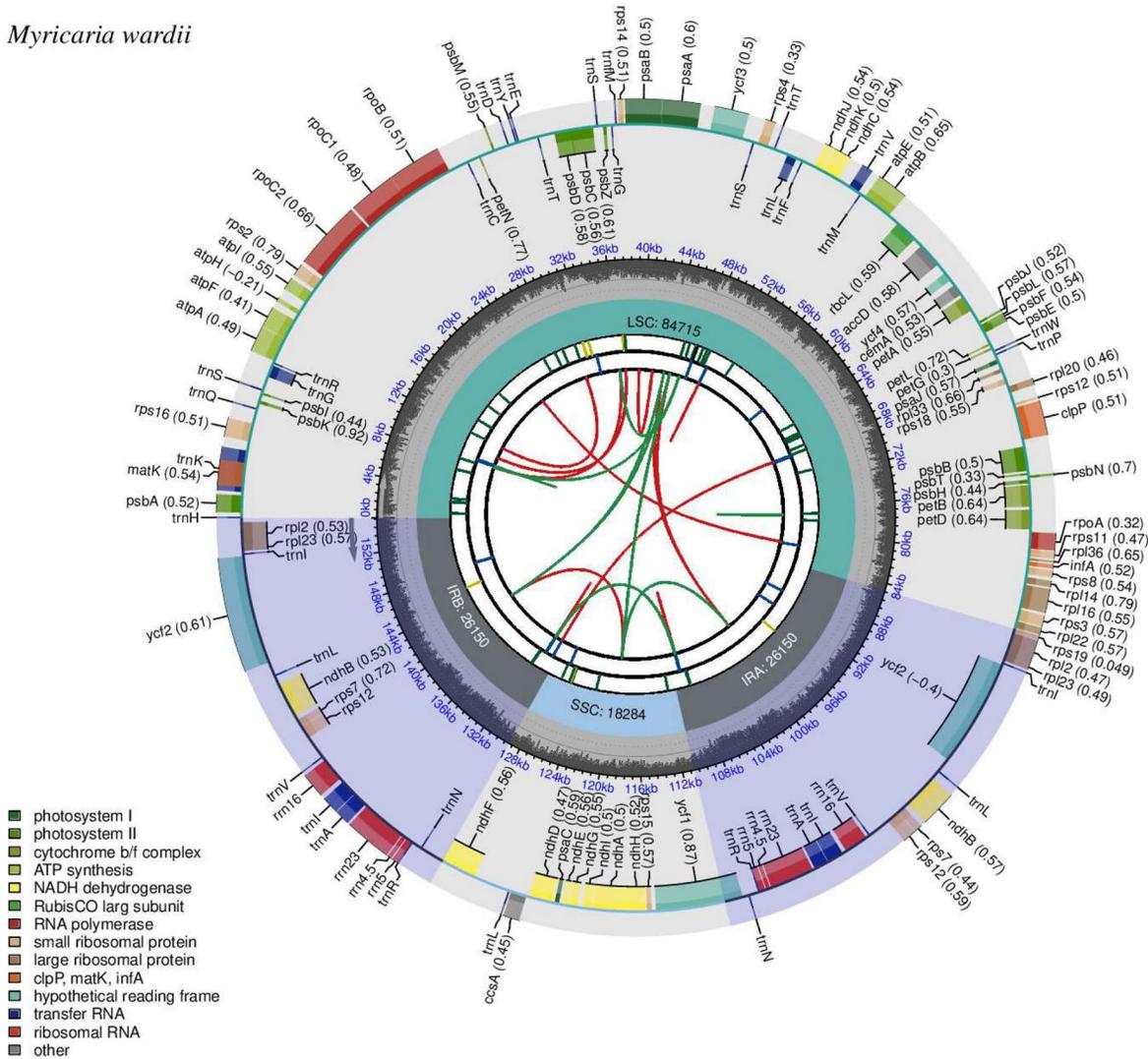


Figure 2. Gene map of the *Myricaria wardii* plastid genome. The map consists of six circles, each with the following information from the center outward: the circle closest to the center was indicated by red and green arcs for forward and reverse repeats, respectively. The second and third circles are indicated by short bars for tandem repeats and microsatellite sequences, respectively. The fourth circle indicates the positions of the LSC, SSC, IRA, and IRB regions, respectively. The fifth circle indicates the GC content. The outer circle indicates the function of the gene. Different colors are used to show different functional categories, as shown in the lower left of the picture.

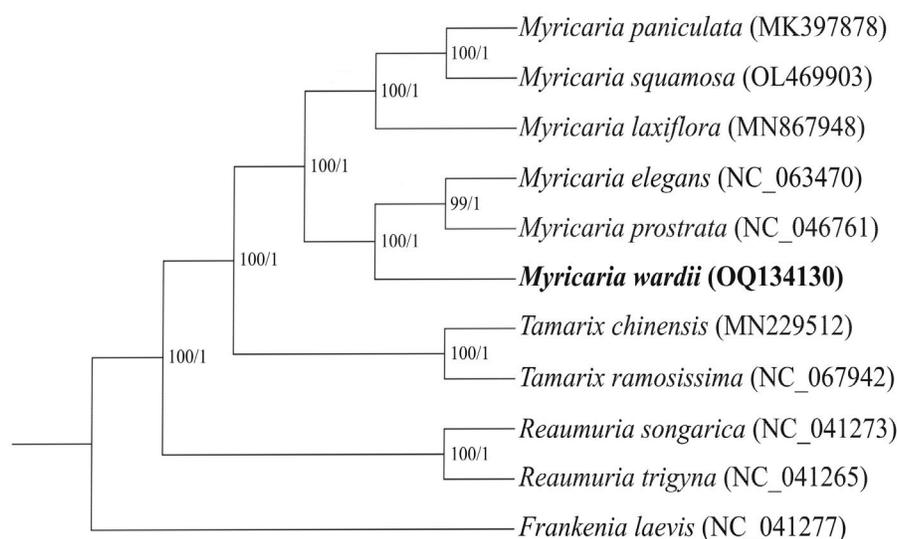


Figure 3. Maximum-likelihood (ML) and Bayesian inference (BI) phylogenetic trees based on 11 plastomes with *Frankenia laevis* as an outgroup. The numbers near the nodes were the bootstrap values for ML and posterior probabilities for BI tree of each clade, respectively. The following sequences were used: *Myricaria paniculata* MK397878, *Tamarix chinensis* MN229512, *Tamarix ramosissima* NC_067942, *Reaumuria songarica* NC_041273, *Reaumuria trigyna* NC_041265 and *Frankenia laevis* NC_041277 (Yao et al. 2019), *Myricaria laxiflora* MN867948 (Wang et al. 2020), *Myricaria elegans* NC_063470 (Han et al. 2021), *Myricaria prostrata* NC_046761 and *Myricaria squamosa* OL469903 (Chi et al. 2019).

cp genome sequence and the annotation of *M. wardii* have been submitted to GenBank with the accession number of OQ134130.

The maximum likelihood (ML) and Bayesian Inference (BI) phylogenetic trees were constructed based on 11 complete chloroplast genomes, including 10 species in Tamaricaceae and *F. laevis* (NC_041277) as an outgroup. Firstly, the cp sequences of 11 species were concatenated by BioEdit (Hall 1999). Then, the concatenated file was aligned by program MAFFT (Katoh and Daron 2013). The best model of ML tree (TVM+I+G) was computed by jModelTest 2 (Darriba et al. 2012). Then the ML phylogenetic tree was constructed by PhyML v.3.0 (Guindon et al. 2010). The BI tree was built by the program MrBayes (Ronquist et al. 2012) with 20,000 generations and sampling every 10 generations. The first quarter of all trees was regarded as ‘burn-in’ and discarded. Then, the posterior probabilities of Bayesian analysis were calculated based on the remaining trees.

Results and discussion

The total length of the chloroplast genome of *M. wardii* was 155,299 bp, including one large single copy (LSC) region (84,715 bp), one small single copy (SSC) region (18,284 bp), and two inverted repeat (IR) regions (26,150 bp). The mean sequencing coverage depth of the plastid genome was 972 (ranged from 270 to 1456), indicating the reliable genome assembly (Figure S1). The total GC content of the chloroplast genome was 36.30%, with 34.10%, 29.60% and 42.40% for the LSC, SSC and IR region, respectively (Figure 2). Totally, 128 genes were annotated, including 83 protein-coding genes, 37 tRNA genes and 8 rRNA genes. Among them, 17 genes were duplicated, including 6 protein-coding genes (*rpl2*, *rpl23*, *ycf2*, *ndhB*, *rps12* and *rps7*), 7 tRNA genes (*trn-CAU*, *trnL-CAA*, *trnV-GAC*, *trnI-GAU*, *trnA-UGC*, *trnR-ACG* and

trnN-GUU) and 4 rRNA genes (*rrn5*, *rrn4.5*, *rrn23* and *rrn16*). Meanwhile, 13 cis-splicing genes and one trans-splicing gene (*rps12*) were identified, and the structures of them were shown in the Supplementary Figures S2 and S3, respectively. Both ML and BI phylogenetic trees consistently showed a close relationship between *M. wardii* and *M. prostrata* (Figure 3). Compared with the *Reaumuria*, the *Myricaria* was the sister clade to *Tamarix* genus (Park et al. 2016). The complete cp sequence in the present study provides useful information for the conservation of this species and the phylogenetic analysis of Tamaricaceae.

Ethics approval and consent to participate

The collection of plant material was carried out complying with the Regulations of the People’s Republic of China on Wild Plants Protection and Wetlands Conservation Law of the People’s Republic of China.

Authors’ contributions

YC and XF were involved in the conception and design; YC contributed the sample collection; HL and GW performed the analysis and interpretation of the data; HL and XF contributed the drafting of the paper; YC and XL revised it critically for intellectual content. All authors were involved in the final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/nuccoreOQ134130.1/> under the accession no. OQ134130. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA939399, SRR23653047, and SAMN33537681, respectively.

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