

The complete chloroplast genome sequence of *Rhamnella wilsonii* Schneid (Rhamnaceae)

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ABSTRACT

Rhamnella wilsonii Schneid 1914 is a member of the *Rhamnaceae* and endemic to China. In this study, the complete chloroplast genome of *R. wilsonii* was sequenced and assembled. The complete chloroplast genome was 160,049 bp in length, including a pair of inverted repeats (IRs) of 26,502 bp, one large single copy (LSC) region of 88,274 bp and one small single copy (SSC) region of 18,771 bp. The genome contained 129 genes, including 8 rRNA genes, 37 tRNA genes and 84 protein-coding genes. The overall GC content of the complete chloroplast genome was 37.15%. The phylogenetic analysis demonstrated that *R. wilsonii* is closely related to *R. martinni*. This study provides basic information for further studies on the identification and evolution of *R. wilsonii* and *Rhamnella* from genomic perspective.

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Introduction

The *Rhamnella* genus is a small group within the Rhamnaceae family, which includes around 61 other genera (Hauenschild et al. 2016, Richardson et al. 2000). Within this genus, there are 11 species of small trees, climbers, and evergreen shrubs (Chen and Schirarend 2007; Hauenschild et al. 2016). *Rhamnella* is morphologically similar to *Berberis*, the drupes of both genera turn purple-black or black when ripe. One of these species is *Rhamnella wilsonii*, which is only found in Western Sichuan and Eastern Tibet in China, with a small population on the Qinghai-Tibet Plateau at elevations between 2000–3000 m (Chen and Schirarend 2007). Due to its restricted distribution and population size, it is imperative to formulate conservation strategies for *R. wilsonii*. Chloroplast is an essential organelle in plant, its genome sequence can be used for phylogeny construction, taxonomic classification and comparative genomic analysis (Ravi et al. 2008). To date, the chloroplast genome of *Rhamnella* has not been reported. Here, we present the complete chloroplast genome of *R. wilsonii*, offering additional information for future population genetic studies and the conservation of *R. wilsonii*.



Materials and methods


The fresh leaves of *R. wilsonii* were collected from an individual in Danba, Sichuan Province, China (30°38'02" N, 102°03'48" E; Figure 1), and a specimen has been deposited in the Herbarium of State Key Laboratory of Plateau Ecology

and Agriculture, Qinghai University under voucher number 1813s-2017-LZ06-25_H3TLGDMXX (contact person: Gaini Wang, wanggaini07@163.com). Genomic DNA was isolated using the modified CTAB method (Doyle and Doyle 1987). Subsequently, sequencing of the total genomic DNA was performed on the Illumina HiSeq 2500 platform, yielding approximately 8.88 gigabase pairs (Gbp) of raw paired-end reads. These reads were assembled by NOVOPlasty 4.3.1 (Dierckxsens et al. 2020), and the assembled genome was annotated using CPGAVAS2 (Shi et al. 2019). The genome map was generated using CPGView (<http://www.1kmpg.cn/cpgview>). The annotated complete chloroplast genome sequence of *R. wilsonii* was submitted to GenBank (accession number: OM876863). For phylogenetic analysis, the cp genome sequences of other 13 species were obtained from NCBI. Multiple sequence alignments were generated utilizing MAFFT (Kato and Standley 2013). Maximum likelihood (ML) trees were constructed using IQ-TREE v1.6.12 (Nguyen et al. 2015) with 5000 bootstrap replicates.

Results and discussion

The complete chloroplast genome of *R. wilsonii* was 160,049 bp in length (Figure 2), comprising one large single copy (LSC) region of 88,274 bp, one small single copy (SSC) of 18,771 bp and a pair of inverted repeat regions (IRs) of 26,502 bp each. The genome contained 129 genes, of which 112 were unique, including 84 protein-coding genes (78

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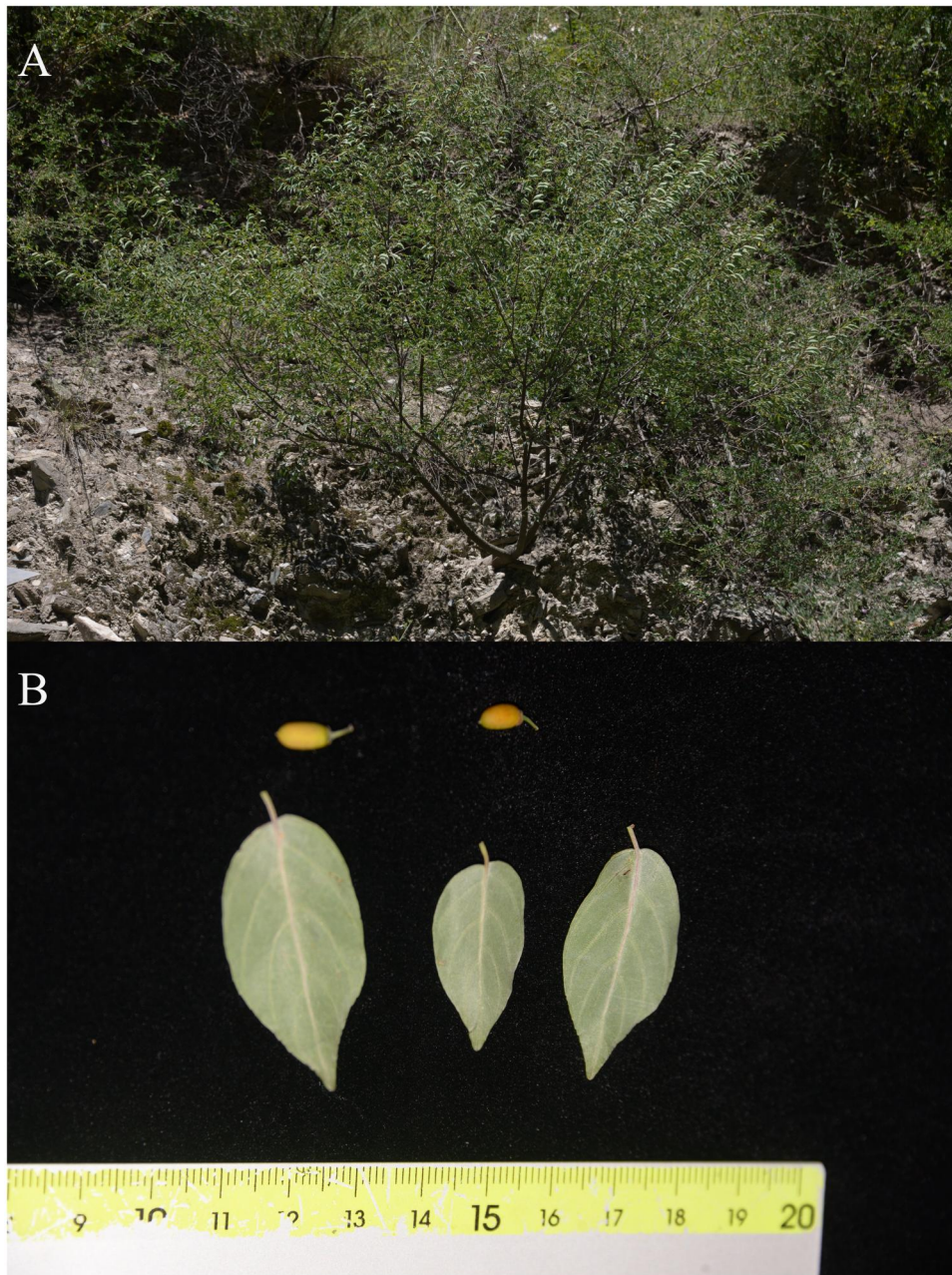


Figure 1. The species reference photographs of *R. wilsonii*. (A) The habitats of *R. wilsonii*; (B) the leaves and seeds of *R. wilsonii*. The photographs were taken by Gaini Wang from Danba, Sichuan Province, China (30°38'02" N, 102°03'48" E). Main identifying features: shrubs or small tree, petiole 3–7mm. The leaves are glabrous on both surfaces with 3–5 lateral veins on each side. Drupe is nearly cylindrical, 6–8mm in length, with a pedicel of 3–4mm.

were unique), 8 rRNA genes (4 were unique), and 37 tRNA genes (30 were unique). The genome assembly had a high coverage of 10,530 (Figure S1). Among the annotated genes, 9 protein-coding genes contained two introns (*atpF*, *nadA*, *nadB*, *petB*, *petD*, *rpl16*, *rpl2*, *rpoC1* and *rps16*), and 3 genes had three introns (*clpP*, *rps12* and *ycf3*). The gene structure of 13 cis-splicing genes and trans-splicing gene *rps12* were displayed in Figure S1 and Figure S2 (Supplementary Material), respectively. The base compositions of the chloroplast genome were uneven, with a content of 31.08% A, 18.91% C, 18.24% G, and 31.76% T, and an overall GC content of 37.15%, with corresponding values of 34.99%, 31.63%, and 42.72% for the LSC, SSC, and IR regions, respectively. The

phylogenetic analysis based on the complete chloroplast genome sequence of *R. wilsonii* and other Rhamnaceae species showed that *R. wilsonii* was a sister of *R. martinni*, and *Rhamnella* was closely related to *Berchemia* (Figure 3). The results were consistent with the results of phylogenetic analysis based on nuclear ITS sequences of Rhamnaceae (Lu and Sun 2020).

Conclusion

This newly reported chloroplast genome sequence of *R. wilsonii* provides a valuable genetic resource for future population genomic studies, which could be useful for the

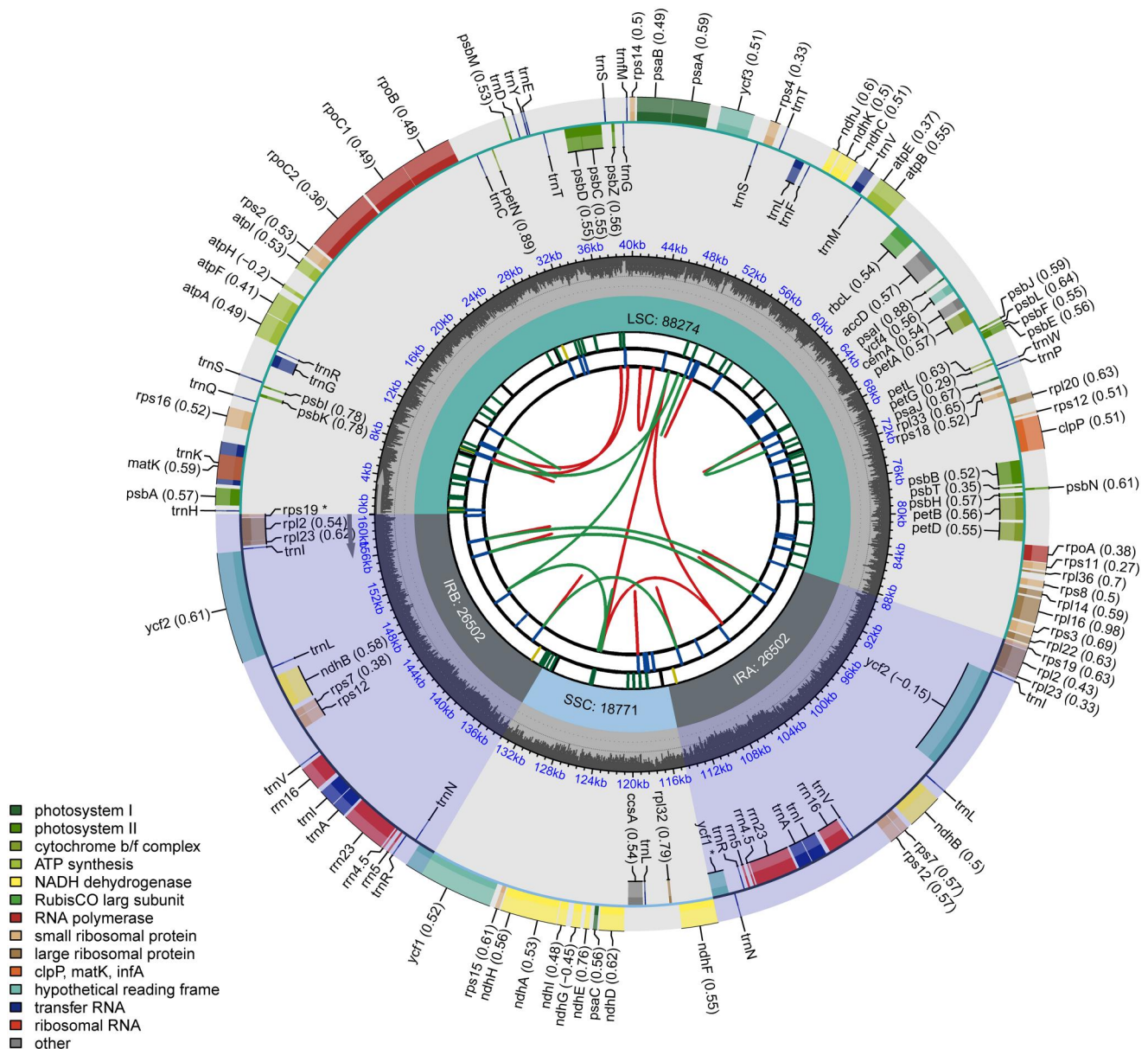


Figure 2. The complete chloroplast genome map of *R. wilsonii*. The map consists of six tracks from outside to inside. The outermost track exhibits variably sized and colored boxes, symbolizing genes and their respective lengths. The outer and inner boxes within this track denote genes transcribed in a clockwise and counter-clockwise direction. The second track provides a depiction of the genomic GC content. The third track illustrates the structural composition of the chloroplast genome, including the LSC region, SSC region, and two IR regions. The fourth track shows short tandem repeats or microsatellite sequences through colored bars. The fifth track reveals long tandem repeats represented as concise blue bars. The innermost track indicates the sequence of forward and reverse repeats, connected by red and green arcs.

development of conservation and management strategies for this species. In addition, this information is fundamental for further research into the genetic variation and evolutionary history of Rhamnaceae using chloroplast genomes.

Ethical approval

Rhamnella wilsonii Schneid is not a protected plant, and our research did not damage the population of *R. wilsonii*. Therefore, no special permission was needed.

Disclosure statement

No potential conflict of interest was reported by the author(s). The authors report no potential conflicts of interest. The authors alone are responsible for content and writing of the paper.

Authors' contributions

Jianping Si conceived and designed the experiments. Gaini Wang collected the sample. Gaini Wang and Jilong An analyzed the data and drafted the manuscript. Jianping Si and Gaini Wang revised the manuscript. All authors read and approved the final manuscript.

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Data availability statement

The data that support the findings of this study are openly available in NCBI GenBank at <https://www.ncbi.nlm.nih.gov> under accession number:

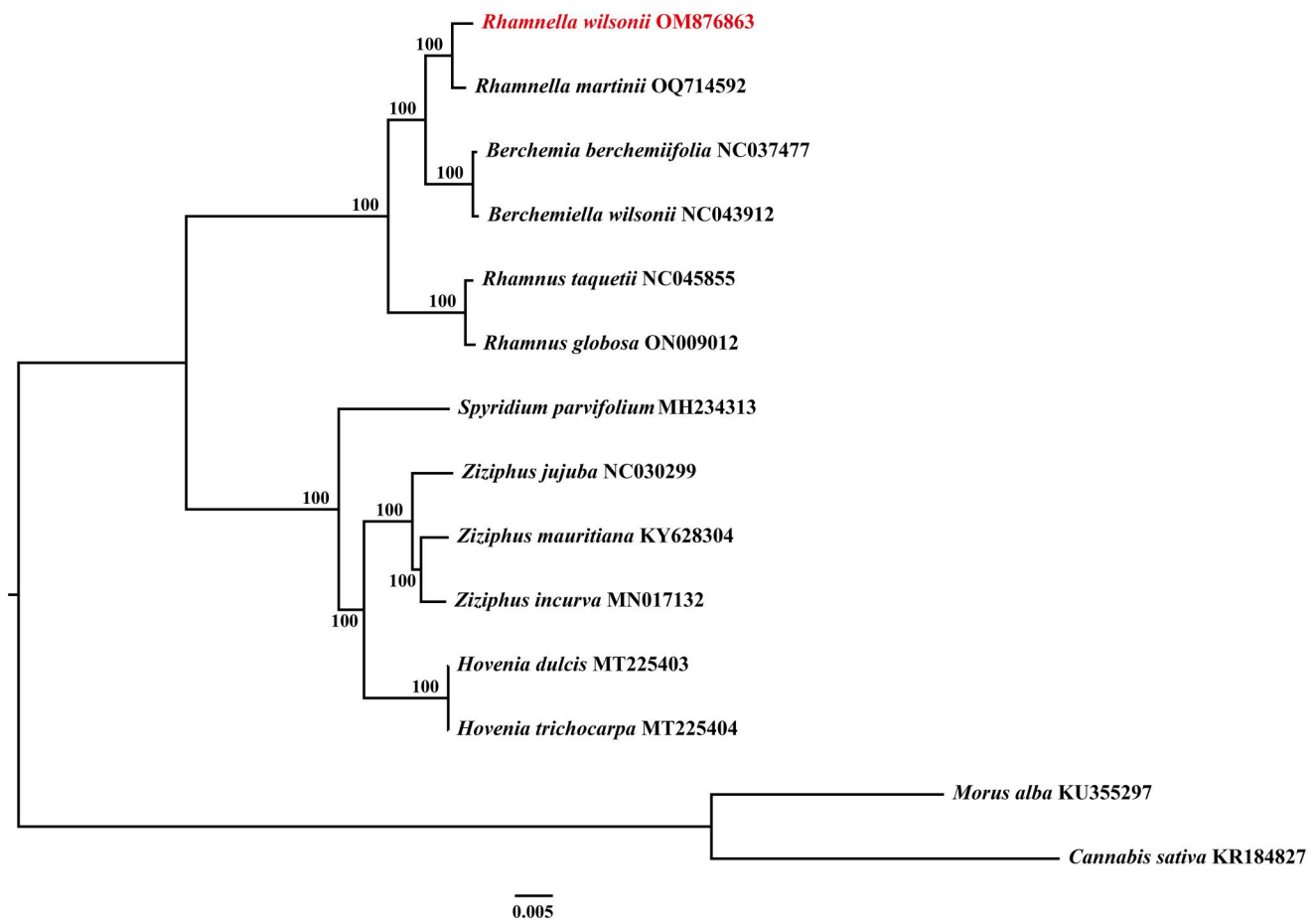


Figure 3. The ML tree based on the complete chloroplast genome of *R. wilsonii* and other 11 species of rhamnaceae, with *Cannabis sativa* and *Morus Alba* as outgroups. The number above the branch is bootstrap value. The following sequences were used: *Berchemia berchemiifolia* (NC037477) (Cheon et al. 2018); *Berchemiella wilsonii* (NC043912) (Li et al. 2019); *Cannabis sativa* (KR184827) (Oh et al. 2016); *Hovenia dulcis* (MT225403) (Li et al. 2020); *H. trichocarpa* (MT225404) (Li et al. 2020); *Morus Alba* (KU355297) (Li et al. 2018); *Rhamnella rubrinervis* (ON881505); *Rhamnus globosa* (ON009012) (Xie et al. 2020); *Rhamnus taquetii* (NC045855) (Jin et al. 2020); *Spyridium parvifolium* (MH234313) (Clowes et al. 2018); *Ziziphus jujuba* (NC030299) (Ma et al. 2017); *Z. mauritiana* (KY628304) (Huang et al. 2017); *Z. incurva* (MN017132) (Wang et al. 2019).

OM876863. The associated BioProject, SRA, and BioSample numbers are PRJNA821616, SRR21151097, and SAMN27116178, respectively.

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