

## The complete plastome of *Thalictrum elegans* Wall. ex Royle and its phylogenetic analysis

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### ABSTRACT

*Thalictrum elegans* Wall. ex Royle, a species within the family Ranunculaceae, is mainly distributed along forest margins and grassy slopes at altitudes 2700–4000 m on the Qinghai-Tibetan Plateau. Despite its wide distribution in alpine ecosystems, its genetic diversity remains poorly understood. In this study, we assembled and characterized the complete chloroplast genome of *T. elegans*, addressing a significant gap in our understanding of its genetic composition. The chloroplast genome is 155,864 base pairs long and contains 131 genes, including 86 protein-coding genes, 37 tRNA genes, and 8 rRNA genes. Phylogenetic analysis of 15 representative *Thalictrum* species revealed that the genus can be classified into three clades. *T. elegans* along with another nine other species formed the largest monophyletic clade and is most closely related to *T. petaloideum* and *T. foliosum*. These findings enhance our understanding of the genetic diversity of *T. elegans* and contribute to its conservation and utilization.

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Ranunculaceae; chloroplast genome; phylogeny; Qinghai-Tibetan Plateau; *Thalictrum elegans*



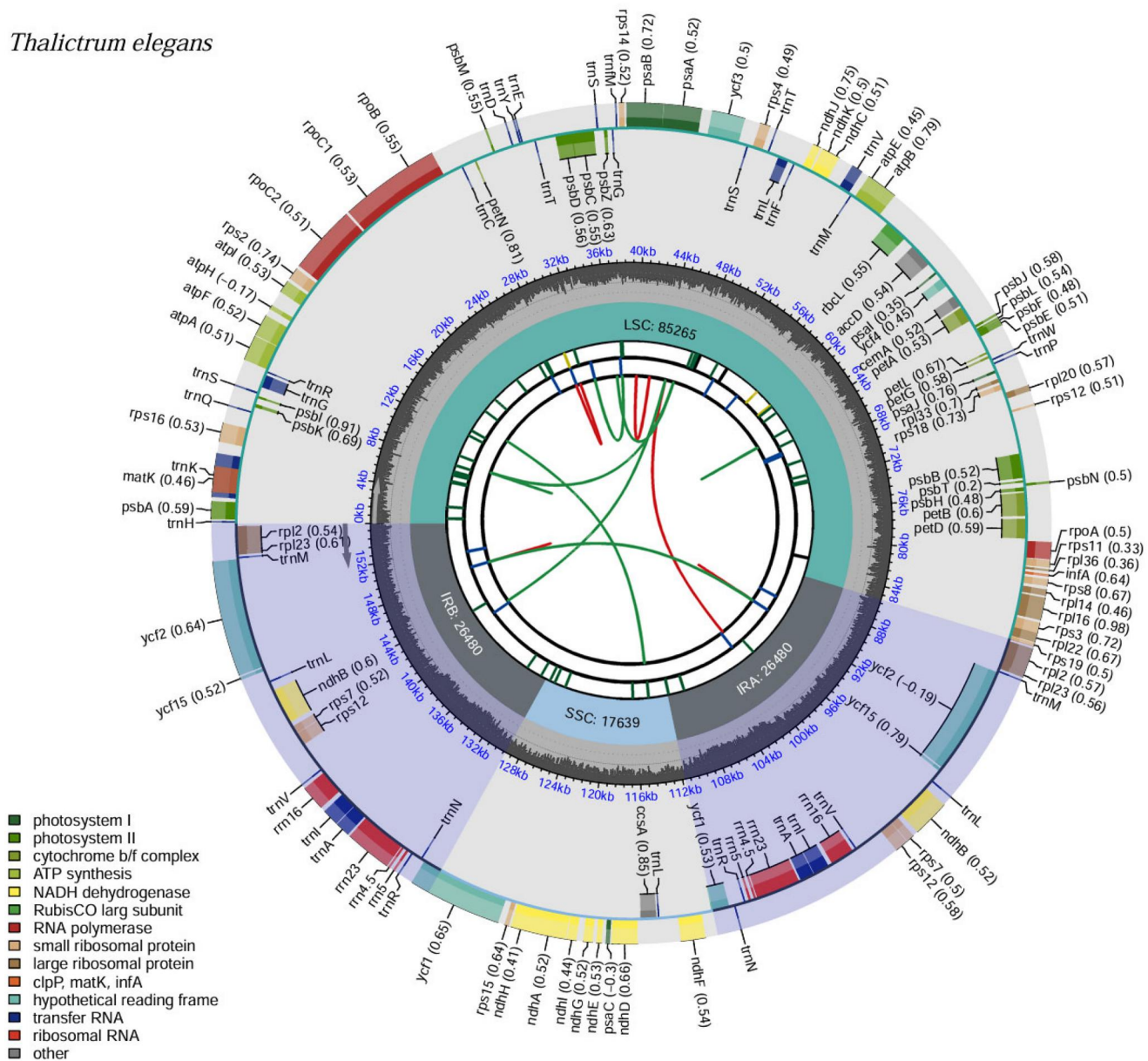
**Figure 1.** *Thalictrum elegans* photographed by Ruijing Cheng (Nyalam county, Tibet). (A) the whole plant, (B) compressed achenes with narrow and lateral wings, (C) leaves with small leaflets.

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*Thalictrum elegans*

**Figure 2.** The complete chloroplast genome map of *T. elegans* generated using CPGview. The map contains six tracks by default. From the center outward, the first track shows the dispersed repeats. The second track shows the long tandem repeats as short blue bars. The third track shows the short tandem repeats or microsatellite sequences as short bars with different colors. The small single-copy (SSC), inverted repeat (IRA and IRb), and large single-copy (LSC) regions are shown on the fourth track. The GC content along the genome is plotted on the fifth track. The genes are shown on the sixth track. The transcription directions for the inner and outer genes are clockwise and anticlockwise, respectively. The functional classification of the genes is shown in the bottom left corner.

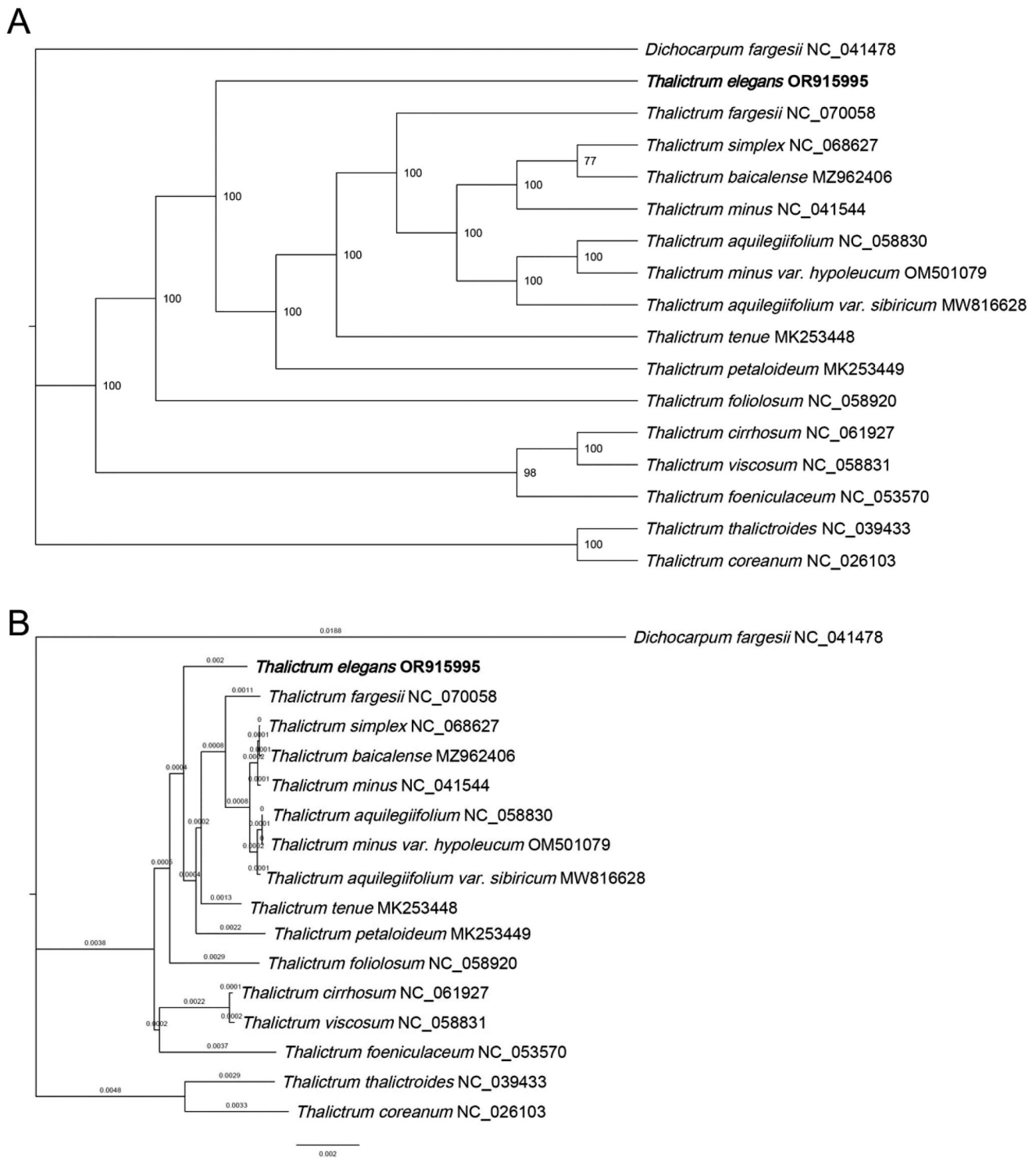
## Introduction

The genus *Thalictrum* (Ranunculaceae) encompasses approximately 200 species in temperate regions, are utilized in Chinese folk medicine and are popular as garden plants (Wu et al. 1998; Wang and Xiao, 1979). *Thalictrum elegans* Wall. ex Royle is characterized by small leaflets and compressed achenes with narrow, lateral wings. It is primarily distributed in forest margins and grassy slopes at altitudes between 2700 m and 4000 m on the Qinghai-Tibetan Plateau and the adjacent regions (Wang et al. 2001). According to morphological characters, this species was classified into section *Thalictrum* series *Virgata*, showing close relationship with *T. foeniculaceum*, and *T. viscosum*. However, results of molecular

phylogeny studies on *Thalictrum* do not align well with morphological classification (Soza et al. 2012, 2013; Xiang et al. 2022). The phylogenetic position of *T. elegans* is of lack molecular evidence. To fill this knowledge gap, we assembled and characterized the complete chloroplast genome of *T. elegans*. Combining with other 15 published *Thalictrum* chloroplast genomes, we aim to uncover the phylogenetic position of *T. elegans*. Our study provides new genetic resources of *T. elegans* and shed light on its conservation and utilization.

## Materials and methods

The fresh leaves used for sequencing were collected from Nyalam Town, Nyalam County, Shigatse City, Tibet



**Figure 3.** Maximum-likelihood (ML) phylogenetic tree inferred by IQ-TREE (v2.2.0) based on *Thalicttrum elegans* (bold font) and 15 *Thalicttrum* species. *Dichocarpum fargesii* was used as the outgroup. A total of 1000 bootstrap replicates were computed and the bootstrap support values are shown at the branches of the cladogram (a). The corresponding phylogram is shown in panel B. Sequences used for tree construction were as follows: *Dichocarpum fargesii* (NC\_041478, Zhai et al. 2019), *T. elegans* (OR915995, this study), *T. fargesii* (NC\_070058), *T. simplex* (NC\_068627), *T. baicalense* (MZ962406), *T. minus* (NC\_041544, Zhai et al. 2019), *T. aquilegiifolium* (NC\_058830), *T. minus* var. *hypoleucum* (OM501079), *T. aquilegiifolium* var. *sibiricum* (MW816628), *T. tenue* (MK253448, He et al. 2019), *T. petaloideum* (MK253449, He et al. 2019), *T. foliolosum* (NC\_058920), *T. cirrhosum* (NC\_061927), *T. viscosum* (NC\_058831), *T. foeniculaceum* (NC\_053570), *T. thalictroides* (NC\_039433), *T. coreanum* (NC\_026103, Park et al. 2015).

Autonomous Region, China (85.8525 E, 28.2153 N) (Figure 1) on September 06, 2023, and a specimen collected by the author has been deposited in the Sichuan University Herbarium (contact person: Xiaoting Xu, [xiaotingxu@scu.edu.cn](mailto:xiaotingxu@scu.edu.cn)) under the collection number CFC40. The modified Cetyltrimethylammonium

bromide (CTAB) method (Porebski et al. 1997) was used to extract genomic DNA. The quality of the extracted genomic DNA was determined using Qubit 4.0 (Invitrogen, Q3326). The total genomic DNA extracts were fragmented and then sequenced with DNBSEQ-T7 (Shenzhen, China) in paired-end

2 × 150 bp sequencing mode. After the quality control process, roughly 6.96 GB clean paired-end reads were assembled using GetOrganelle v1.7.7.0 (Jin et al. 2020). The complete circular chloroplast genome was annotated using CPGAVAS2 (Shi et al. 2019) with the *T. aquilegiifolium* chloroplast genome (NC\_058830) as a reference and manually adjusted in Geneious Primer (v2021.1.1.0). Finally, the annotated chloroplast genome was submitted to GenBank using Bankit and assigned the accession number OR915995. The CPGview (Liu et al. 2023) online tool was utilized to exhibit the circular genome map of the novel chloroplast genome.

To establish the phylogenetic relationship of *T. elegans*, the chloroplast genomes of 15 other *Thalictrum* species were downloaded from GenBank. In addition, *Dichocarpum fargesii* was selected as outgroup. The total chloroplast genome sequences were aligned using MAFFT (v7.487) (Katoh et al. 2002; Katoh and Standley, 2013) with default parameters. A maximum-likelihood (ML) phylogenetic tree was constructed using IQ-TREE (v2.2.0) (Minh et al. 2020), along with 1000 ultrafast bootstrap replicates (Hoang et al. 2018). The best-fit nucleotide substitution model was TVM + F + I + I + R4 detected by ModelFinder (Kalyanamoorthy et al. 2017) according to both AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion).

## Results

The complete chloroplast genome of the *T. elegans* is 155,864 bp in length with 38.4% GC content (Figure 2). Mapping analysis shows that the genome assembly is highly reliable (Supplementary Figure 1). It consists of the 85,265 bp large single-copy (LSC) and 17,609 bp small single-copy (SSC) regions along with two inverted repeat (IR) regions that are 26,480 bp long each, displaying a typical quadripartite structure. A total of 131 genes are predicted, including 86 protein-coding genes, 37 tRNA genes, and 8 rRNA genes (Figure 2). In the *T. elegans* chloroplast genome, 7 unique protein-coding genes (*ndhB*, *rpl2*, *rpl23*, *rps12*, *rps7*, *ycf15*, and *ycf2*), 7 unique tRNA genes (*trnA*, *trnE*, *trnL*, *trnM*, *trnN*, *trnR*, and *trnV*) and 4 unique rRNA genes (*rrn16*, *rrn23*, *rrn4.5*, *rrn5*) are located at the IR regions. Fourteen unique genes have two exons (*atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpl16*, *rpl2*, *rpoC1*, *trnA*, *trnG*, *trnI*, *trnK*, *trnL*, *trnV*), and 3 unique genes (*clpP1*, *rps12*, and *ycf3*) contain three exons. The *rps16*, *rpoC1*, *clpP1*, *petB*, *petD*, *rpl16*, *rpl2*, *ndhB*, and *ndhA* genes are cis-splicing genes (Supplementary Figure 2), while the *rps12* gene is a trans-splicing gene (Supplementary Figure 3).

Our phylogenetic analysis showed that the investigated 16 species can be classified into two monophyletic clades (Figure 3). *Thalictrum thalictroides* and *T. coreanum* formed a monophyletic clade. *T. elegans* and other 12 species formed another one. *T. elegans* is most closely related to *T. petaloideum* and *T. foliosum*.

## Discussion and conclusions

This study sequenced and characterized the chloroplast genome of *T. elegans* for the first time, which exhibits a typical

quadripartite structure with a size of 155,864 bp and 131 predicted genes. Our results agree with the division of two major clades, which might correspond to two subgenus (Soza et al. 2012, 2013; Xiang et al. 2022). These results are different from morphological division of *Thalictrum* (Tamura, 1995), suggesting a need for revising the classification of *Thalictrum*. In our phylogeny, *T. thalictroides* and *T. coreanum* form one clade. *T. elegans* clusters in a monophyletic group with other 9 species, and these species form the other clade together with *T. foliosum*, *T. cirrhosum*, *T. viscosum*, and *T. foeniculaceum*. This study contributes to the further evolutionary analysis and application of *T. elegans*, elucidates the biogeographic history of the genus *Thalictrum*, and sheds light on the evolution and adaptation of alpine plants.

## Author contributions statement

Xiaoting Xu and Xiulian Chi conceived the project, Ruijing Cheng carried out sample collection, experiments, and data analysis. Ruijing Cheng and Xiaoting Xu wrote the manuscript. Xiulian Chi revised the manuscript. All authors have read and agreed to the published version of the manuscript.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Ethical approval

This study includes no human, animal, or endangered plant samples, and the sampling site was not in the natural reserve. No permissions are needed during the collection of samples.

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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/>) under the accession number OR915995. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1051192, SRR27179068, and SAMN38766967, respectively.

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