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PLASTOME REPORT

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The complete chloroplast genome of *Semiaquilegia danxiashanensis* (Ranunculaceae), a rare species endemic to Danxia landform in Guangdong, China

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ABSTRACT

Semiaquilegia danxiashanensis is currently known only from the type locality, Danxia Mountain, characterized by its spectacular red sandstone cliffscape. In this study, we assembled the complete chloroplast genome sequence of *S. danxiashanensis* and inferred its phylogenetic relationships. Total length of the chloroplast genome was 160,548 bp, with an overall GC content of 39%. The chloroplast genome had typical quadripartite structure and contained one LSC region (89,882 bp) and one SSC region (17,386 bp), which were separated by two IRs regions (26,640 bp, respectively). It comprised 133 genes, including 84 protein coding genes, 41 tRNA genes and eight rRNA genes. The maximum likelihood phylogenetic analysis indicated that *S. danxiashanensis* was sister to *S. adoxoides*; meanwhile, *Semiaquilegia* was closely related to both *Urophysa* and *Aquilegia* in Ranunculaceae. This study sheds light on the evolutionary history of *Semiaquilegia* and provides preliminary data for future comparative analysis of chloroplast genomes.

Introduction

The genus *Semiaquilegia* Makino currently includes four species, which are mainly distributed in China as well as Korea and Japan and adapted to distinct habitats (Qin et al. 2020). Three species of them have extremely narrow distributions, of which *Semiaquilegia danxiashanensis* L. Wu, J.J. Zhou, Q. Zhang & W.S. Deng 2019 (Zhou et al. 2019) is restricted to Danxia mountain in Guangdong, China, *S. guangxiensis* Yan Liu & Y.S. Huang (Huang et al. 2017) is endemic to Guilin and Liuzhou in Guangxi, China, and *S. quelpaertensis* D.C. Son & K.H. Lee (Son et al. 2017) is confined to Jeju-do, Korean. *Semiaquilegia adoxoides* (DC.) Makino, however, is widely distributed in China, Korea and Japan (Fu and Orbélia 2001). Furthermore, the three narrowly distributed species are habitat specialists, with *S. danxiashanensis* adapted to Danxia red sandstones, *S. guangxiensis* to karst limestones under forests and *S. quelpaertensis* to granite under broadleaf forests. Whereas, *S. adoxoides* colonizes variable habitats, primarily in soils and to a lesser extent in limestone and sandstone rocks, found in both open and forested areas. Therefore, the habitat adaptations maybe have prompted the diversification and speciation. The genetic and genomic information of *Semiaquilegia* is needed to evaluate how the habitat adaptations have driven speciation. In addition, the phylogenetic relationships within *Semiaquilegia* have only been investigated based on the internal transcribed spacer (ITS) and *trn*L-F sequences (Zhou et al. 2019), which also needs to be tested at the genome level. Here, we report the complete chloroplast genome sequence of *S. danxiashanensis*, and reveal phylogenetic relationships of this species in *Semiaquilegia* and the genus in tribe Isopyreae.

Material and methods

Plant material, DNA extraction and sequencing

The leaves of *S. danxiashanensis* were collected from Danxia Shan, Renhua, Guangdong, China (25°1′41.15″N, 113°44′1.16″E) (Figure 1). The voucher specimen of *S. danxiashanensis* was deposited in Guangxi Institute of Botany (http://www.gxib.cn/spIBK/, contact person: Chun-Rui Lin, Email: chunruilin@tom.com) with a register number IBK00458906. The genomic DNA was extracted from the leaves by using Plant Genomic DNA Kit according to the instructions. Then, the paired end (PE150) sequencing was performed on NovaSeq 6000 instrument (Novogene, Tianjin, China). Finally, about 3 Gb of raw data was obtained.
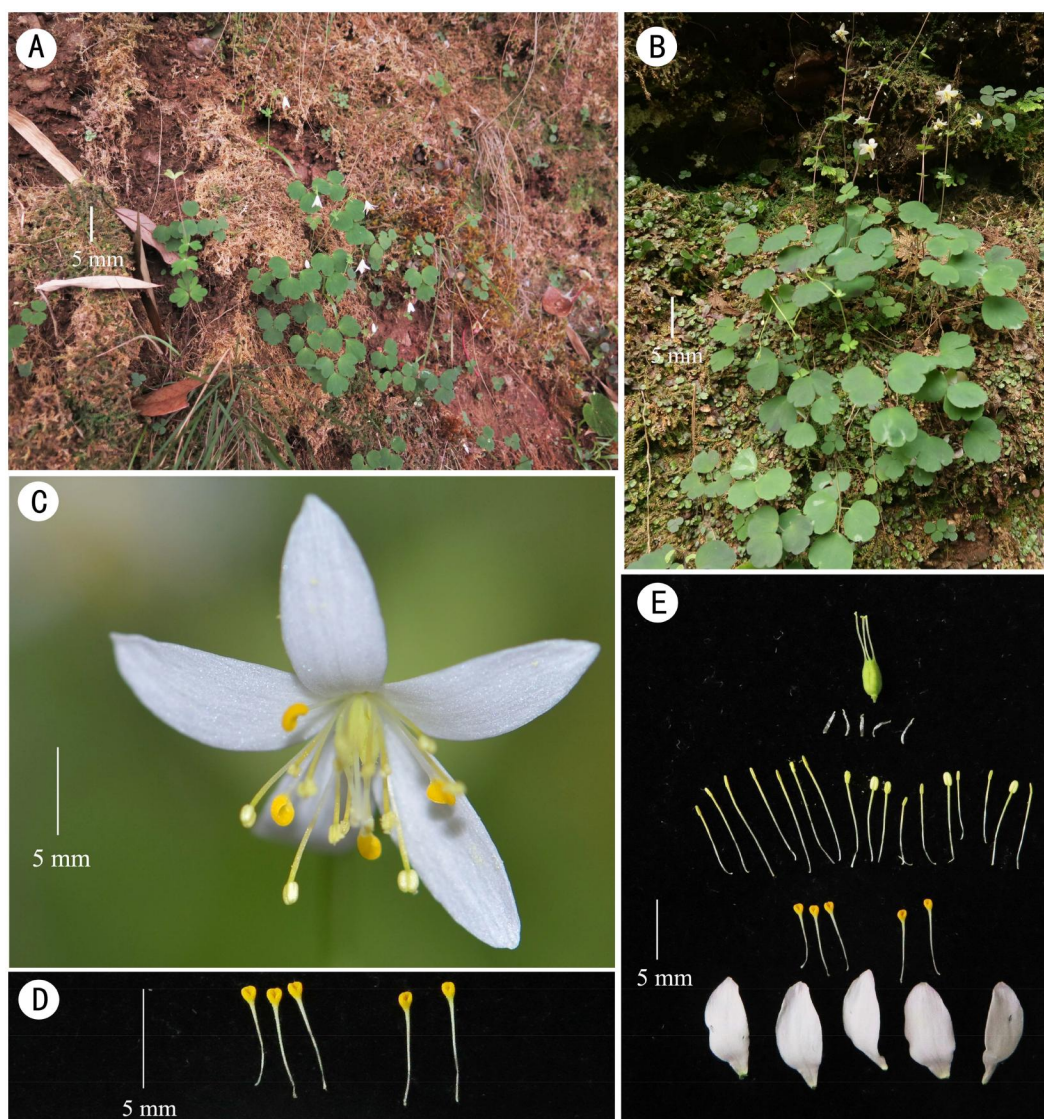
Figure 1. The morphology of *Semiaquilegia danxiashanensis*. A. Habit; B. Plant; C. Flower; D. Petals; E. Anatomy of the flower: from bottom to top are sepals, petals, stamens, staminodes and pistils. The distinctive feature that distinguishes *S. danxiashanensis* from other species in this genus is the clawed petals with golden-yellow suborbicular limbs and claws significantly longer than limbs. The photo was captured by Xinmei Qin in Danxia Shan, Renhua, Guangdong, China.

Chloroplast genome assembly and annotation

The chloroplast genome of *S. danxiashanensis* was assembled using NOVOPlasty (Dierckxsens et al. 2017) with *S. guangxiensis* (NC_057495) as the reference. Subsequently, the raw reads were mapped to the assembled genome sequence using BWA-MEM (Li 2013), and the results were visualized using IGV (Robinson et al. 2023) for inspection and correction based on sequencing depth. The chloroplast genome was annotated using CPGAVAS2 (Shi et al. 2019) and PGA (Qu et al. 2019) referring to *S. guangxiensis* (NC_057495) and *S. adoxoides* (MK569498). The annotated chloroplast genome was submitted to GenBank under the accession number PP541616. The chloroplast genome map, cis-splicing genes, and trans-splicing genes were generated using CPGview (Liu et al. 2023). The coverage depth of each base was calculated by mapping all the raw reads to the assembled chloroplast genome using BWA-MEM (Li 2013) and SAMtools (Danecek et al. 2021), and then ggplot2 (Wickham 2016) was used to produce the coverage depth map.

Phylogenetic analysis

To uncover the phylogenetic positions of *S. danxiashanensis* and *Semiaquilegia*, we downloaded the complete chloroplast genomes of 31 species from the NCBI GenBank database, including 30 species in tribe Isopyreae and a species *Trollius chinensis* Bunge as the outgroup according to the larger-scale phylogenetic studies in Ranunculaceae (Zhai et al. 2019). Firstly, the sequences were aligned using MAFFT (Katoh and Standley 2013). Then, the 'maskSegment' and 'degap' functions in the R package alignmentFilter (Zhang et al. 2023) were used to mask ambiguously aligned or overly divergent segments (with stringency-controlling parameter *prob* set to 0.001) and remove sites with more than 50% gaps, respectively. Finally, the phylogenetic tree was reconstructed using maximum likelihood (ML) in the software IQ-TREE v2 (Minh et al. 2020), with GTR + F+R5 substitution model selected by ModelFinder (an embedded package of IQ-TREE) and 5000 ultrafast bootstraps to obtain nodal support.
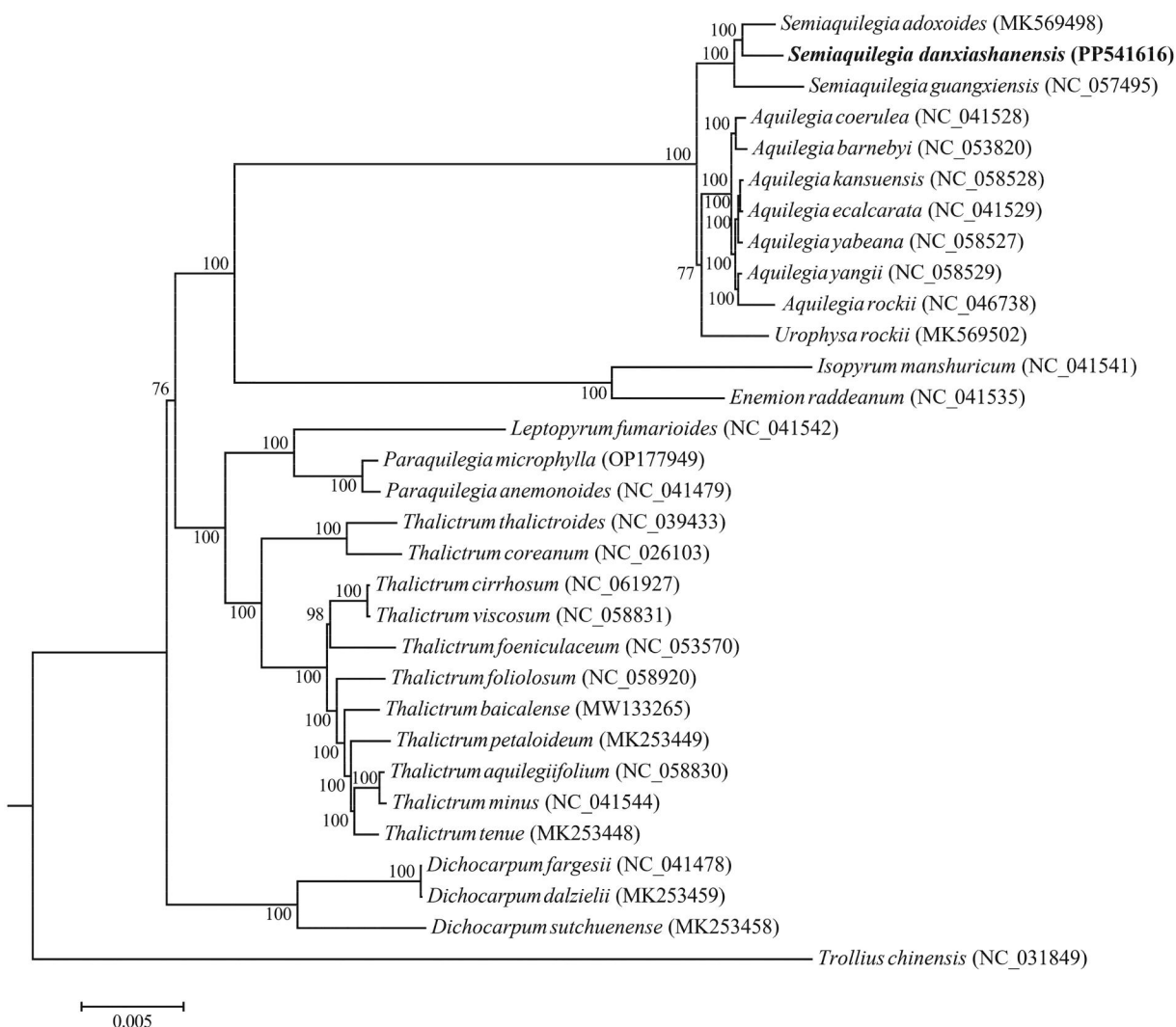
Results

Genome structure analysis

The minimum and average read mapping depth of the assembled genome was 1650× and 4349.27× (Figure S1). The chloroplast genome sequence of *S. danxiashanensis* had a total length of 160,548 bp, with an overall GC content of 39% (Figure 2). The chloroplast genome contained a large single-copy (LSC) region (89,882 bp) and a small single-copy (SSC) region (17,386 bp), which were separated by two inverted repeat (IR) regions (26,640 bp, respectively). It had a typical quadripartite structure. The chloroplast genome encompassed 133 genes, including 84 protein coding genes, 41 tRNA genes and eight rRNA genes. There were 13 cis-splicing genes that contained introns (Figure S2), and one trans-splicing gene *rps12* with three exons (Figure S3).

Phylogenetic analysis

The phylogenetic tree (Figure 3) showed that *S. danxiashanensis* was sister to *S. adoxoides* with strong support (BS = 100%). meanwhile, *Semiaquilegia* was closely related to a clade (BS = 77%) consisting of *Urophysa* Ulbrich and *Aquilegia* Linnaeus in Ranunculaceae (BS = 100%).

Discussion and conclusion

This study was at the first time to assemble and annotate the chloroplast genome of *S. danxiashanensis*. It was found that *S. danxiashanensis* was sister to *S. adoxoide*, which was consistent with the study of Zhou et al. (2019). *Semiaquilegia*, *Urophysa* and *Aquilegia* formed a clade, suggesting that these three genera were the closest, which were consistent with other studies

Figure 2. A schematic illustration representing overall features of *Semiaquilegia danxiashanensis* chloroplast genome depicted in a six-tracks map. The map includes presentations of dispersed repeats, long tandem repeats, short tandem repeats or microsatellite sequences, genomic regions (SSC, IRa, IRb, LSC), GC content, and gene organization with optional codon usage bias details in brackets. Additionally, it specifies the transcription directions for inner and outer genes as clockwise and anticlockwise, respectively.

Figure 3. A maximum likelihood (ML) phylogenetic tree was constructed using complete chloroplast genome sequences of 30 species in the tribe Isopyreae. Bootstrap support values are presented nearby the nodes of the tree. Details of the sequences and their references can be found in Table S1.

(Li 2006; Wang and Chen 2007). The findings of this study contribute to future comparative and evolutionary studies of *Semiaquilegia*. For instance, the process of dispersal among different habitats can be traced based on phylogenomic tree, and the plastomic variations underpinning adaptations to different (such as forested versus open) habitats might be uncovered by comparative analyses with more taxa or accessions sampled.

Authors' contributions

X.-M.Q.: Identified the plant specimens, guided the data analysis and wrote the manuscript. X.-Y.M. and Z.-J.Z.: collected and analyzed data. Y.Y. and H.-Y.J.: collected materials and sent samples for sequencing. H.L.: designed the work and modified the manuscript. All authors have read and agreed to publish the manuscript.

Ethics statement

The sample collection was approved by Danxia Mountain National Nature Reserve, Shaoguan, Guangdong province, China.

Disclosure statement

No potential conflict of interest was reported by the authors.

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 PP541616. The associated BioProject, SRA, and BioSample numbers are PRJNA1092291, SRR28465664, and SAMN40622768, respectively.

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