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PLASTOME REPORT

The complete chloroplast genome of *Stachyurus himalaicus*

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ABSTRACT

The complete chloroplast genome of *Stachyurus himalaicus* Hook. f. et Thoms. ex Benth. was sequenced and analyzed. The circular cp genome of *S. himalaicus* is 162,642 bp in length, consisting of inverted repeats (IRs; 26,437 bp), small single-copy (SSC; 18,762 bp), and large single-copy regions (LSC; 91,006 bp). The genome contains 131 genes (86 protein-coding, 37 tRNA, 8 rRNA) with 37.0% GC content. Phylogenetic analysis revealed the monophyly of Stachyuraceae, and *S. himalaicus* was sister to the other 3 *Stachyurus* species examined. Our results provide useful genetic resources for further studies on the evolution and phylogeny of Stachyuraceae.

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Stachyurus himalaicus; complete chloroplast genome; phylogenetic analysis

Introduction

Stachyuraceae includes only the genus *Stachyurus* Siebold & Zucc., with 16 species and 8 varieties, which is endemic to East Asia (Chen 1981; Kimoto and Tokuoka 1999; Su et al. 2020). *Stachyurus himalaicus* Hook. f. et Thoms. ex Benth. 1861, a deciduous shrub (Figure 1), is mainly found in mountain shrubs or forests at an altitude of 400-3000 meters in southwestern China (Chen 1981; Wei and Yang 2001). The pith of the stem is white and can be used in traditional Chinese medicine 'ricepaper pith'. It has been used as a galactopoietic, diuretic, and for the treatment of dropsy and gonorrhea for a long time (Chen 1981; Wei and Yang 2001; Peng et al. 2004; Yang et al. 2011).

The familial status of Stachyuraceae has been accepted by most classification systems (Su et al. 2020). However, similar morphological characteristics and scarce molecular data have posed challenges for researchers to investigate the relationship between Stachyuraceae and other families such as Actinidiaceae, Dilleniaceae, Theaceae, Ochnaceae, Crossosomataceae and Staphyleaceae (Jin and Wei 2002; Wei et al. 2002; Zhu et al. 2006). Nandi et al. (1998) reported Crossosomataceae as the closest relative of Stachyuraceae based on morphological analysis and *rbc*L sequences. However, the embryological and pollenological evidence did not support this conclusion, suggesting that Staphyleaceae and Stachyuraceae form a sister group (Jin and Wei 2002; Wei et al. 2002). To date, the phylogenetic relationship of Stachyuraceae remains controversial. Here, we sequenced and analyzed the whole chloroplast genome sequence of *S. himalaicus*, which will provide important evidence on the relationships between these families and a deeper understanding of plant taxonomy and evolutionary studies.

Materials and methods

Plant material collection and DNA extraction

Fresh leaves were collected from the Kunming Botanical Garden (Yunnan, China; coordinates: 25°07′21″N, 102°44′29″E). The voucher specimen of *S. himalaicus* was identified by Yuan Huang, and deposited in the herbarium of Yunnan Normal University (Kunming, China; Jianlin Hang, hjlynnu@163.com) under the accession number Y-30. Total genomic DNA was extracted using a modified CTAB method (Porebski et al. 1997).

Genome sequencing, assembly and annotation

A short-insert pair-end (2×150 bp) library was constructed using Illumina TruSeq DNA Sample Prep Kit (Illumina Inc., USA), according to the manufacturer's protocol. The Illumina Hiseq X Ten platform was used to sequence the DNA of *S. himalaicus* with a read length of 150 bp. The raw data (4.73 GB) was filtered using fastp v.0.23.2 software (https://github.com/OpenGene/fastp) to remove low quality sequences, resulting in 4.67 GB clean data. We obtained 31,385,636 filtered reads and then assembled the cp genome using NOVOPlasty v2.7.2 software (Dierckxsens et al. 2017) with *Stachyurus chinensis* (GenBank accession number MT584419.1) as the reference genome. The annotation of the assembled chloroplast genome was based on the comparison with *S. chinensis* using Geneious V2020.1.1 software (Kearse et al. 2012). The circular gene map of the chloroplast genome

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Figure 1. Images of *S. himalaicus*. Photographed by Yuan Huang in Kunming Botanical Garden (25°07′21″N, 102°44′29″E). (A) The whole plant, 3-5 m tall, bark smooth, brown or dark brown. (B) Leaf, hard papery to thin leathery, lanceolate to oblong lanceolate. (C) Inflorescence, spike, ovary ovate-oblong.

was generated using CPGView software (http://www.1kmpg.cn/cpgview/) (Liu et al. 2023). Finally, the chloroplast DNA sequence with complete annotation information was submitted to GenBank under accession number OP380385.1.

Phylogenetic tree construction

To explore the phylogenetic relationships within related families and genera, the complete chloroplast genome of *S. himalaicus* and 25 related chloroplast genomes downloaded from GenBank were aligned for phylogenetic analysis using the MAFFT v7.47 software (Katoh and Standley 2013). The 25 species included 5 Staphyleaceae species, 3 Stachyuraceae species, 3 Ochnaceae species, 3 Dilleniaceae species, 7 Theaceae species, 4 Actinidiaceae species, and *Saurauia tristyla* was selected as an outgroup. The maximum likelihood (ML) phylogenetic tree was then constructed using IQ-TREE v1.6.10 software (Nguyen et al. 2015) under the TVM + F + R2 best-fit model (Kalyaanamoorthy et al. 2017). Branch support was tested with 10,000 replicates using ultrafast bootstrap (UFBoot) (Hoang et al. 2018) and SH-like approximate likelihood ratio test (SH-aLRT) (Guindon et al. 2010). The resulting phylogenetic tree was visualized and edited using FigTree v1.4.4 (https://github.com/rambaut/figtree).

Results

Characteristics of chloroplast genome

The chloroplast genome sequence of *S. himalaicus* was 162,642 bp in length with an average coverage of 3549.8 (Supplemental Figure S1). It exhibited a typical tetrad structure, consisting of a small single-copy region (SSC; 18,762 bp), a large single-copy region (LSC; 91,006 bp) and two inverted repeat regions (IR; 26,437 bp) (Figure 2). The total GC content of the genome was 37.0%. A total of 131 genes were annotated in the chloroplast genome, including 86 protein-coding genes, 37 tRNA genes and 8 rRNA genes. Among these genes, 15 genes (*rpl*2, *rpl*16, *rps*16, *atp*F, *rpo*C1, *ndh*A, *ndh*B, *pet*B, *pet*D, *trn*A-UGC, *trn*I-GAU, *trn*L-UAA, *trn*G-UCC, *trn*V-UAC and *trn*K-UUU) contain one intron, and three genes (*ycf*3, *rps*12 and *clp*P) contain two introns (Supplemental Figure S2). In total, one trans-splicing gene (*rps*12) and 11 cis-splicing genes (*rps*16, *atp*F, *rpo*C1, *ycf*3, *clp*P, *pet*B, *pet*D, *rpl*16, *rpl*2, *ndh*A, *ndh*B) were identified.

Phylogenetic relationships

To determine the phylogenetic status of *S. himalaicus* with other related species, a total of 25 additional chloroplast genomes were obtained from the GenBank database for phylogenetic analysis. The phylogenetic results showed that Stachyuraceae forms sister groups with Staphyleaceae, which diverged later than the other related families (Figure 3). Stachyuraceae is monophyletic and is divided into two clades. *S. chinensis*, *S. yunnanensis* and *S. retusus* are clustered in one branch, and form sister groups with *S. himalaicus*. The monophyly of Ochnaceae, Dilleniaceae, Theaceae, Actinidiaceae is supported in the phylogenetic tree.

Discussion and conclusion

In this study, the complete chloroplast genome sequence of *S. himalaicus* was reported for the first time. The genome was 162,642 bp in length and contained a total of 131 predicted genes. The chloroplast genome structure and gene

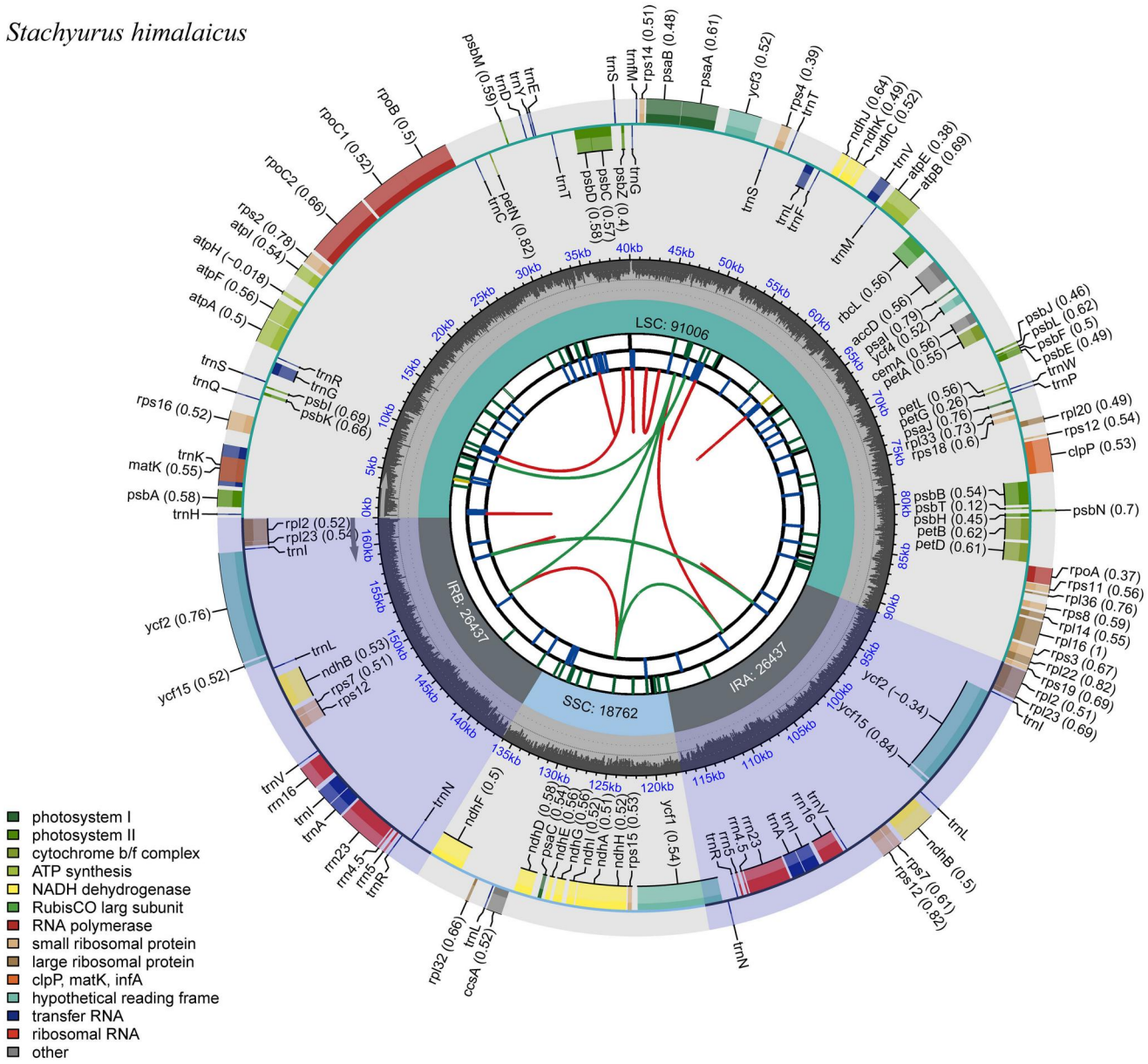

Figure 2. The complete chloroplast genome map of *S. himalaicus*. The map contains six tracks from the center outward. The first track represents the dispersed repeats, the red arcs represent the direct repeats and the green arcs represent the palindromic repeats. The second track shows the long tandem repeats as short blue bars. The third track shows the short tandem repeats or microsatellite sequences as colored bars. The small single-copy (SSC), inverted repeat (IRs), 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 last track shows the coding genes categorized by function. The optional codon usage bias is displayed in the parenthesis after the gene name. The transcription direction for the inner and outer genes is clockwise and anti-clockwise, respectively.

content of *S. himalaicus* are consistent with related Stachyuraceae species (Su et al. 2020). Phylogenetic reconstruction based on 26 chloroplast genomes strongly supported the monophyly of Stachyuraceae, and *S. himalaicus* was sister to the other 3 *Stachyurus* species examined. Furthermore, Stachyuraceae formed sister relationship with Staphyleaceae, which was basically consistent with the phylogenetic results based on morphological characteristics and molecular data analysis (Jin and Wei 2002; Wei et al. 2002). Previous studies have suggested that Stachyuraceae and Crossosomataceae formed a sister group, and then formed a branch with Staphyleaceae (Nandi et al. 1998; Soltis et al. 2000; Zhu et al. 2006; Su et al. 2020). However, due to the lack of cp genomic data of Crossosomataceae plants, it is difficult to determine their phylogenetic relationship with Stachyuraceae and Staphyleaceae. Therefore, additional complete chloroplast genomes of Crossosomataceae species are required for further studies to resolve and improve the controversies and shortcomings in traditional morphological classification. In conclusion, the chloroplast genome of *S. himalaicus* will provide useful genetic resources for the studies on the classification, phylogeny, and evolutionary history of the Stachyuraceae.

Acknowledgments

Yuan Huang designed the research and revised the manuscript. Rui Li, Shubao Wang, and Shuang Xiong collected materials and performed the experiments. Yushuang Chen drafted the original manuscript and

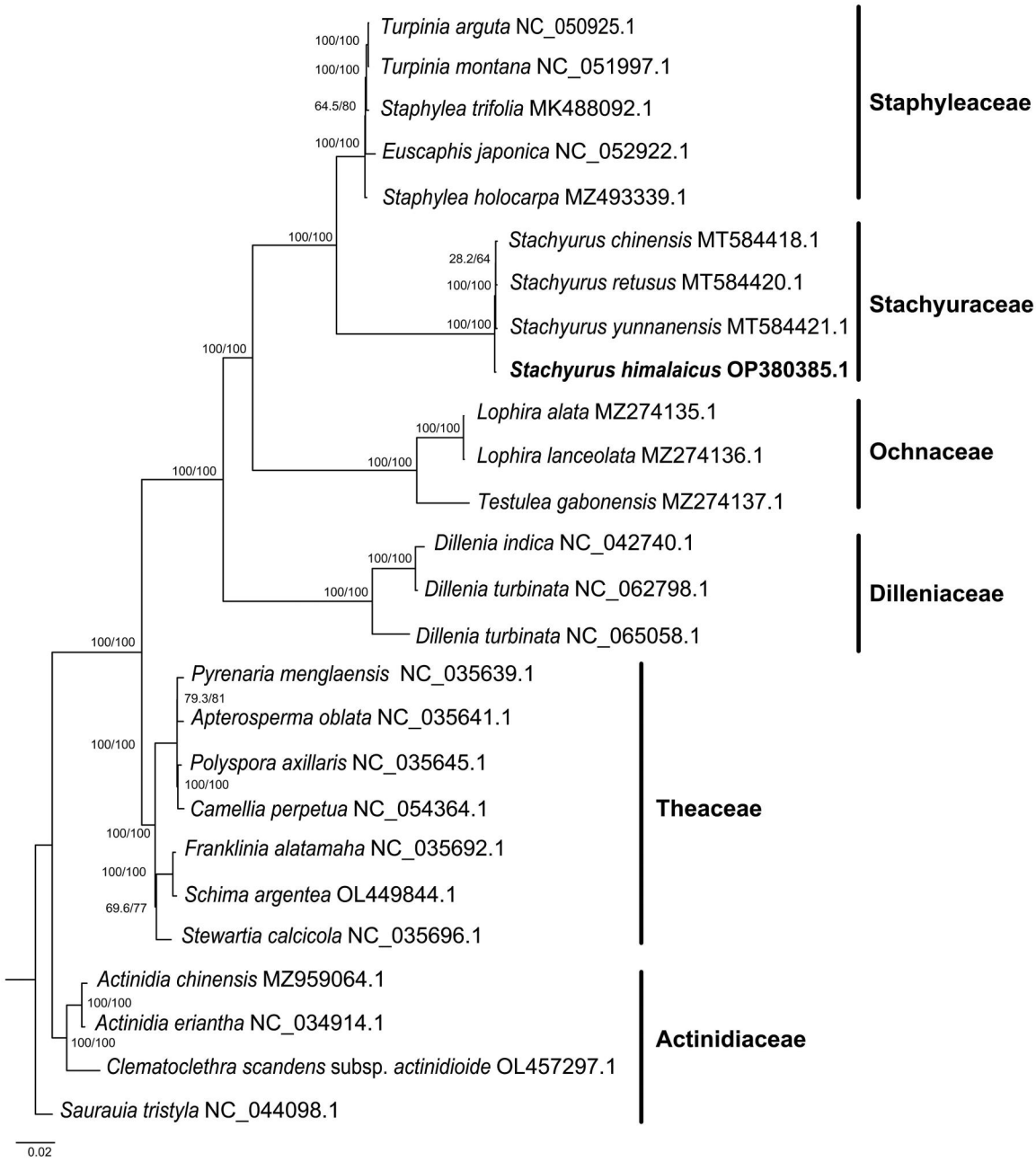

Figure 3. Maximum-likelihood phylogenetic tree was constructed based on the complete chloroplast genome sequences of *S. himalaicus* and 25 related species. *Saurauia tristyla* NC_044098.1 was selected as outgroup. Numbers at each node represent the bootstrap values. The position of *S. himalaicus* is marked in bold. The following sequences were used: *Turpinia arguta* NC_050925.1 (Cao et al. 2020), *turpinia Montana* NC_051997.1 (Sun et al. 2020), *staphylea trifolia* MK488092.1, *euscaphis japonica* NC_052922.1 (Oh and Park 2020), *Staphylea holocarpa* MZ493339.1 (Chen et al. 2024), *S. chinensis* MT584418.1, *S. retusus* MT584420.1, *S. yunnanensis* MT584421.1, *Lophira alata* MZ274135.1, *Lophira lanceolata* MZ274136.1, *Testulea gabonensis* MZ274137.1, *Dillenia indica* NC_042740.1, *Dillenia turbinata* NC_062798.1, *Tetracera sarmentosa* NC_065058.1, *pyrenaria menglaensis* NC_0356391.1, *Apterosperma oblata* NC_035641.1, *Polyspora axillaris* NC_035645.1, *Camellia perpetua* NC_054364.1 (Pei et al. 2020), *schima argentea* OL449844.1, *Franklinia alatamaha* NC_035692.1, *Stewartia calcicola* NC_0356961, *Actinidia eriantha* NC_034914.1 (Tang et al. 2019), *Actinidia chinensis* MZ959064.1 (Yao et al. 2015), *Clematoclethra scandens* subsp. *actinidioides* OL457297.1 (Zhang et al. 2022), *Saurauia tristyla* NC_044098.1.

performed the analysis of the data. All the authors read and approved the manuscript.

Ethics statement

The sample of *S. himalaicus* was collected with permission from the Kunming Botanical Garden, Yunnan Province, China, and strictly complied with local and Chinese regulations. No ethical approval is required in this study.

Author contributions

CRediT: **Yushuang Chen**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing; **Rui Li**: Data curation, Software; **Shubao Wang**: Software, Validation; **Shuang Xiong**: Data curation, Software, Visualization; **Yuan Huang**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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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 accession no. OP380385.1. The associated BioProject, SRA, and BioSample numbers are PRJNA876810, SRR21423888 and SAMN30672946, respectively.

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