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COMPLETE PLASTOME SEQUENCE OF CASTANOPSIS HAINANENSIS MERR.1922: AN ENDEMIC SPECIES IN HAINAN

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ABSTRACT

This *Castanopsis hainanensis* Merr.1922, a tree species native to Hainan province, possesses desirable characteristics such as firmness, density, and resistance to water and humidity. It is commonly used in the construction of beams, columns, floors, furniture, and agricultural tools. In this study, we present a comprehensive analysis of the complete plastome of *C. hainanensis*. The total length of the plastome is 160,826 bp, which includes a Large Single-Copy (LSC) region, a Small Single-Copy (SSC) region, and two Inverted Repeats (IRs). The lengths of the IR regions are 90,471 bp, 18,957 bp, and 25,699 bp, respectively. The plastome contains a total of 261 genes, including 130 protein-coding genes (with sixteen genes duplicated in the IR), 37 tRNA genes (with seven genes duplicated in the IR), and eight rRNA genes (5S rRNA, 4.5S rRNA, 16S rRNA, and 23S rRNA). The overall G/C content in the plastome of *C. hainanensis* is 35.9%. This comprehensive analysis provides valuable insights into the genetic composition and organization of the plastome of *C. hainanensis*, contributing to our understanding of the evolutionary and functional aspects of this species.

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INTRODUCTION

Castanopsis hainanensis Merr.1922 is a tree species found in the low-altitude forests of Hainan province. Its leaves are obovate, obovate-elliptic, ovate-elliptic, or broadly ovate in shape, with a thick papery to nearly leathery texture. The abaxial surface of the leaves often turns grayish with age. The leaf base is acute to broadly cuneate, while the margin is serrate except for the basal portion, which is entire. The apex of the leaves is rounded to mucronate (Figuer 1). This species is known for its slightly firm and heavy material with a dense structure. It has excellent resistance to bursting after drying and is tolerant to water and humidity. As a result, it is highly suitable for various applications such as beams, columns, floors, furniture, and agricultural tools. Despite its significance, the systematic position of *C. hainanensis* has not been extensively reported. To address this gap, we utilized the complete plastid sequence of *C. hamina* (GenBank accession number: OL581718, this study) to investigate the phylogenetic relationships. Our aim is to enhance the collection, preservation, and systematic research of *C. hainanensis* germplasm resources. By exploring the phylogenetic relationships, we hope to provide valuable insights that will contribute to a better understanding of *C. hainanensis* and facilitate future studies on its genetic resources.

MATERIALS AND METHODS

The sampling of *C. hainanensis* was conducted in Ledong county, Hainan province, China (108.79° E, 18.69° N) as part of this study. A voucher specimen (voucher code: H. Xiu, Y.-F. Quan, T.-T. Fu S2, HUTB) was collected and deposited in the Herbarium of the Institute of Tropical Agriculture and Forestry (herbarium code: HUTB) at Hainan University in Haikou, China. For DNA extraction, dried leaf tissue was utilized, and a modified cetyltrimethylammonium bromide (CTAB) method was employed, involving chloroform:isoamyl alcohol separation and isopropanol precipitation at -20°C, following the protocol by Doyle and Doyle (1987).

The experimental procedure employed in this study closely followed the method described in Wang *et al*. (2021). Each individual sample underwent sequencing, generating approximately 6 Gb of clean data. Adapter sequences and low-quality reads with a Q-value ≤ 20 were removed from the dataset. The clean reads were then assembled against the reference plastome of Castanopsis hainanensis (NC_037389.1) using MITObim v.1.8 (Hahn *et al*., 2013). Subsequently, the plastome was annotated using Geneious R8.0.2 (Biomatters Ltd., Auckland, New Zealand).

RESULTS

The result of our study indicates that the complete length of the plastome of *C. hainanensis* possesses 160,826 bp with the typical quadripartite structure of angiosperms. This plastid contains an LSC region of 90,471 bp and an SSC region of 18,957 bp, two IRs of 25,699 bp. The plastome contains 261 genes, consisting of 130 protein-coding genes (sixteen of which are duplicated in the IR), 37 tRNA genes (seven of which are duplicated in the IR), and eight rRNA genes (5S rRNA, 4.5S rRNA, 16S rRNA, and 23S rRNA). The overall G/C content in the plastome of *C. hainanensis* is 35.9%, which the corresponding value of the LSC, SSC, and IR region were 34.6%, 30.9%, and 42.8%, respectively (Figure 2).

Figure 1. Photograph of *Castanopsis hainanensis*; note Petals 5, ovate or narrowly ovate, Stamens exserted. Xiaofeng Zhang took it at Ledong (The copyright of the picture belongs to us).

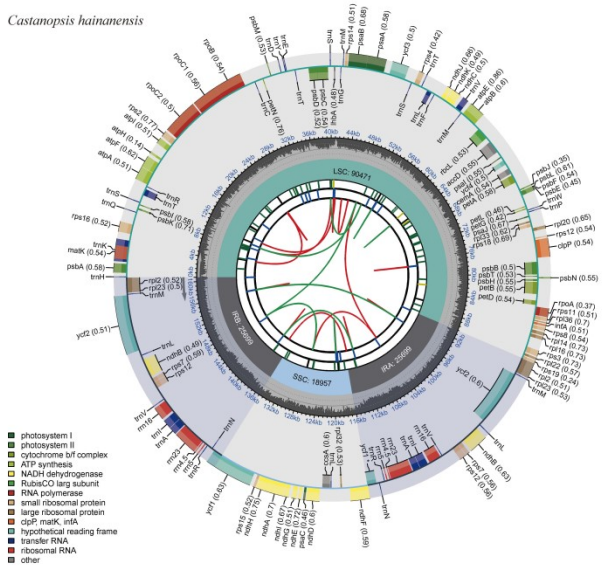

Figure 2. Illustrates features of C. hainanensis chloroplast genome using CPGView (http://www.1kmpg.cn/cpgview/). It consists of four circles. Starting from the center, the first circle displays distributed repeats connected by red (forward) and green (reverse) arcs. The next circle shows tandem repeats as short bars. The third circle represents LSC, SSC, IRa, and IRb regions. Microsatellite sequences are depicted as short bars on the circle. The fourth circle shows color-coded genes based on functional groups. Outer genes are transcribed clockwise, while inner genes are transcribed anticlockwise. Functional classification is indicated at the bottom left.

We used IQ-TREE v.1.6.1 (Nguyen *et al*. 2015) to establish evolutionary relationships (Kalyaanamoorthy *et al*., 2017). We inferred the phylogeny of *Castanopsis hainanensis* based on plastid alignments (Figure 3). The majority of nodes in the plastome ML trees were highly supported. The phylogenetic analysis indicates that *C. hainanensis* is closer to *C. concinna* (Champ. ex Benth.) A.DC.1863 KT793041.1 than other species in this study (Figure 3). Nowadays, the plastid sequence of *C. hainanensis* has been gradually developed and tended to be perfect and phylogenetic studies of *Castanopsis* can be explored more sufficiently.

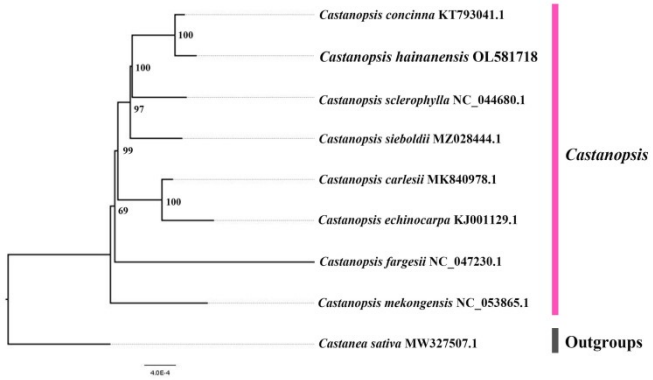

Figure 3. The best ML phylogeny recovered from 9 complete plastome sequences by RAxML. Accession numbers: *Castanopsis hainanensis* (GenBank accession number, OL581718, this study), *Castanopsis concinna*, KT793041.1, *Castanopsis sclerophylla*, NC_044680.1, *Castanopsis sieboldii*, MZ028444.1, *Castanopsis carlesii*, MK840978.1, *Castanopsis echinocarpa*, KJ001129.1, *Castanopsis fargesii*, NC_047230.1, *Castanopsis mekongensis*, NC_053865.1, *Castanea sativa*, MW327507.1.

DISCUSSION AND CONCLUSION

In addition to the previously mentioned findings, this comprehensive analysis of the plastome of *C. hainanensis* provides valuable insights into the genetic composition and organization of this species. The detailed examination of the plastome contributes to our understanding of the evolutionary history and functional aspects of *C. hainanensis*. By characterizing the complete plastome of *C. hainanensis*, we have identified and annotated a diverse range of genes, including protein-coding genes, tRNA genes, and rRNA genes. This information enhances our knowledge of the genetic repertoire and molecular processes within *C. hainanensis*. Furthermore, the organization of the plastome, with its quadripartite structure comprising the LSC, SSC, and IR regions, provides insights into the evolutionary dynamics and genomic architecture of *C. hainanensis*. Understanding the structural organization of the plastome contributes to our comprehension of gene expression, replication, and other essential cellular processes in this species. Moreover, the observed G/C content variations within different regions of the plastome shed light on the nucleotide composition and potential functional implications. These variations may have implications for the stability and adaptation of *C. hainanensis* to its environment. Overall, this comprehensive analysis significantly enhances our understanding of the genetic makeup, organization, and functional aspects of the plastome of *C. hainanensis*. These insights contribute to broader studies on the evolution, adaptation, and conservation of this species, as well as provide a valuable resource for future research on other related taxa.

Ethics Statement

The study was approved by the institutional review board of Hainan Medical University, Haikou, China. The collection of plant materials is carried out in accordance with guidelines provided by the Hainan Medical University and Hainan province regulations. Field studies comply with Hainan province field work policy drafted by Hainan Medical University (in Chinese), and the manuscript include a statement of appropriate permissions granted and licenses from related agencies of Hainan province. Voucher specimens are deposited in a public herbarium (HUTB) providing access to deposited material. Information on the voucher specimen and who identified it is included in the manuscript.

Author contributions: Qinghui Sun involved in the conception and design. Hao Xiu, Yuan Wang, Chuhan Zhang, Yaqing Miu, Meiyi Wang analyzed and interpreted the data; Hao Xiu, Yuan Wang, Chuhan Zhang, Yaqing Miu, Meiyi Wang fudrafted the paper. Qinghui Sun revised it critically for intellectual content. All authors final approved the version to be published. All authors agreed to be accountable for all aspects of the work.

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Data availability statement: The genome sequence data supporting the results of this study are publicly trustworthy in GenBank of NCBI (https://www.ncbi.nlm.nih.gov/)with registration number OL581718. Related BioProject, SRA, Biosample numbers are PRJNA780397, SRR16953195, and SAMN23139351, respectively. A specimen was deposited at Hainan University (https://ha.hainanu.edu.cn/home2020/,Q.-H. Sun and hy0211068@hainmc.edu.cn) under the voucher number S2.

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