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PLASTOME REPORT

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Complete chloroplast genome of *Artabotrys hexapetalus* (L.f.) Bhandari 1965 (Annonaceae)

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ABSTRACT

Artabotrys hexapetalus (L.f.) Bhandari, 1965, an evergreen climbing shrub of significant value, is prominent in Chinese history and culture. The whole-gene sequencing of its chloroplast genome using Illumina pair-end sequencing data is conducted during this research. The complete chloroplast genome was determined to be 178,457 bp in size, separated by a large single copy (LSC) and a small single copy (SSC) region of 90,803 and 3,066 bp, respectively. A total of 134 genes were identified, including 90 protein-coding genes, 36 tRNA, and eight rRNA genes. Phylogenetic analysis revealed a close relationship between *A. hexapetalus* and *Artabotrys pilosus*, forming a sister branch with 100% support. The study suggests that the chloroplast genome of *A. hexapetalus* provides valuable insights into its evolutionary history and will contribute to the conservation efforts of this species.

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Annonaceae; *Artabotrys hexapetalu*; chloroplast genome; phylogenetic analysis

Introduction

Artabotrys hexapetalus (L.f.) Bhandari, 1965, is an evergreen climbing shrub belonging to the genus *Artabotrys R. Br.* in the Annonaceae Juss. family. *A. hexapetalus* is native to India and widely distributed in southern China (Puri 2020). Referred to as 'Eagle's Claw' owing to the distinctive hook-like features on its involucral pedicel (Figure 1C), this plant serves both as an ornamental and trellis plant while also bearing significant economic value as a source of raw material for hemp ropes, paper, high-quality perfumes, soaps, and cosmetics. Further, it possesses medicinal attributes with applications in treating malaria and scrofula (Bajaj et al. 2018). Moreover, it holds a prominent place in Chinese cultural heritage. As depicted by the Chinese proverb, 'Before there was the Hoi Tong Monastery (a venue for foreign affairs activities during the period of the Canton System (1757-1842) in China), there was the eagle's claw'. Despite substantial research into the chemical composition of *Artabotrys hexapetalus* (Xi et al. 2016, 2017; Bailly and Hénichart 2022), the studies of its chloroplast (cp) genome still need to be expanded. It is understood that chloroplast, essential organelles in plants housing a distinct genome, have been extensively employed in plant phylogenetic analysis (Cheng et al. 2013). This study

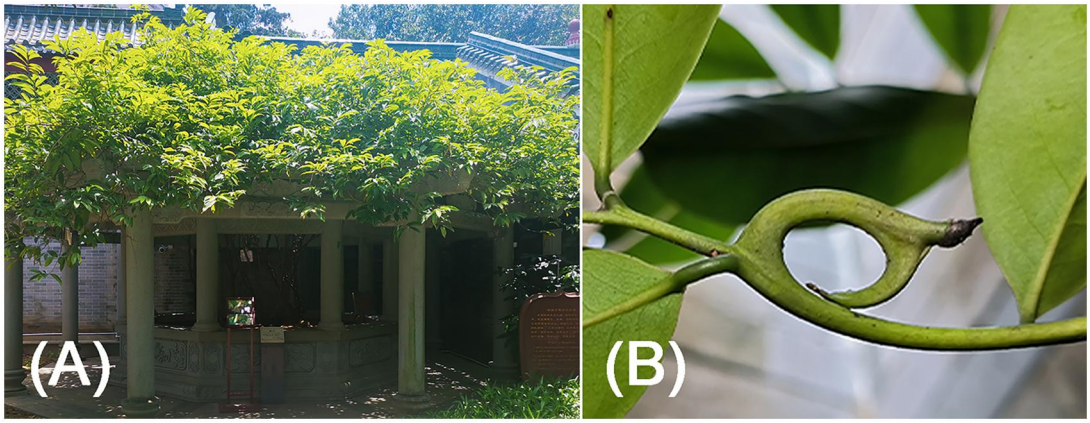

Figure 1. Field photos of *Artabotrys hexapetalus*. (A) Habitat; (B) Twig; (C) Hook. All photos were taken by Kai Zhu in Hoi Tong Monastery, Guangzhou, Guangdong Province, China (23°06′41″N, 113°15′55″E). core features: *A. hexapetalus* is an evergreen climbing shrub. The distinctive hook-like features on its involucral pedicel.

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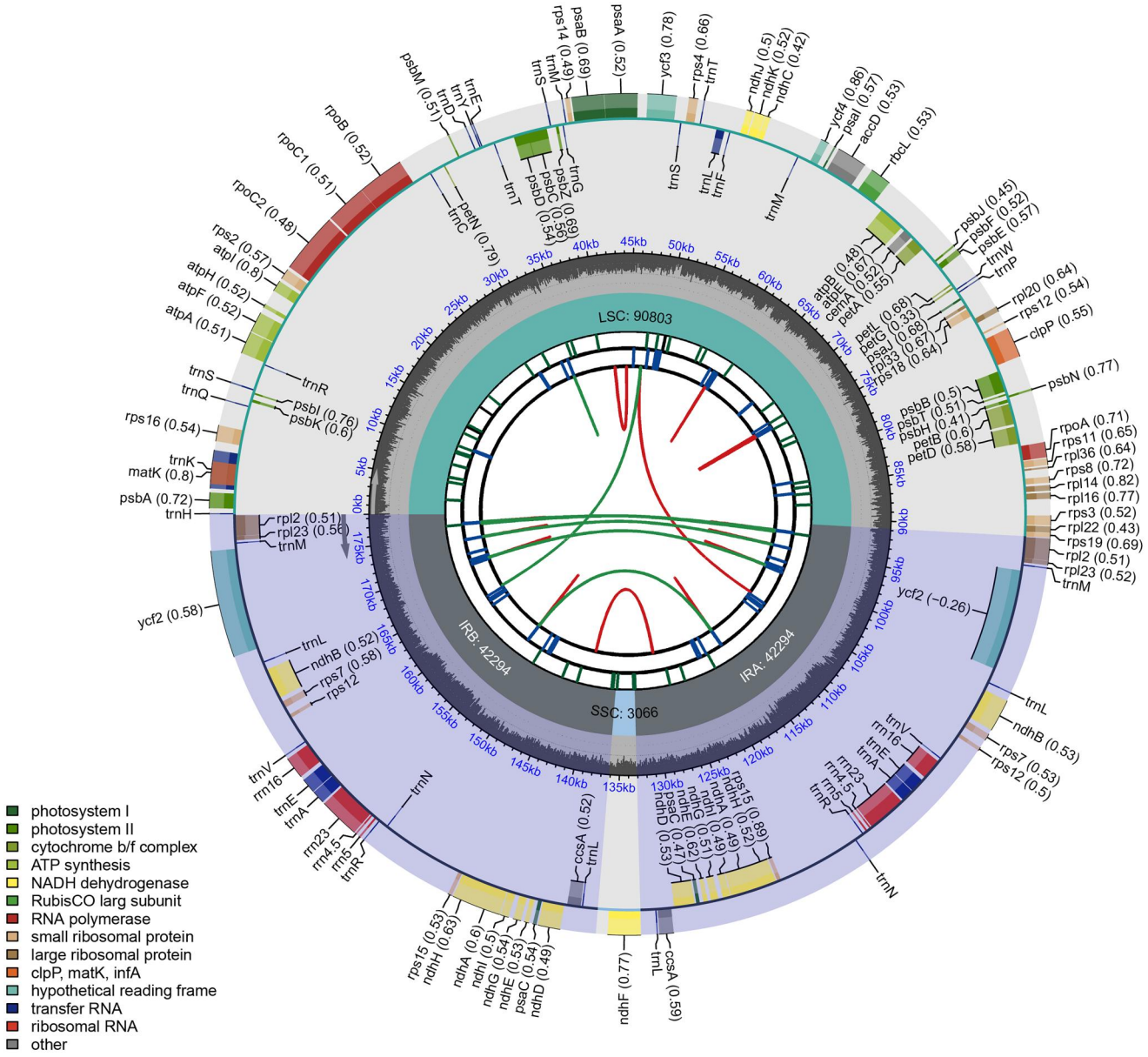

Figure 2. The chloroplast genome map of *A. hexapetalus* (L.f.) Bhandari chlobroplast genome. The length of the genome is presented in the inner circle. Large single-copy (LSC), short single-copy (SSC), and inverted repeat regions (IRA and IRB) are marked. A total of 134 annotated genes are presented in the outer circle, consisting of 90 protein-coding genes, eight rRNA genes, and 36 tRNA genes. Genes were classified by their function in colors. From the center outward, the first track shows the dispersed repeats. The dispersed repeats consist of direct (D) and palindromic (P) repeats, connected with red and green arcs. 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 colors, the type of repeat they represent, and the description of the repeat types are as follows. Black: c (complex repeat); green: p1 (repeat unit size = 1); yellow: p2 (repeat unit size = 2); purple: p3 (repeat unit size = 3); blue: p4 (repeat unit size = 4); orange: p5 (repeat unit size = 5); red: p6 (repeat unit size = 6). 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. Genes are color-coded by their functional classification. 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.

analyses the complete cp genome of *A. hexapetalus* using high-throughput sequencing. It aims to provide fundamental genetic information for understanding the species' genetic diversity, functional genome, and genetic engineering studies, thereby establishing a foundation for further exploration of its biological properties and related research.

Materials and methods

Fresh sample leaves of *A. hexapetalus* (L.f.) Bhandari were collected from Hoi Tong Monastery, Guangzhou, Guangdong Province, China (Longitude: 113.265451 E, Latitude: 23.111474 N) for genomic DNA extraction (Figure 1). The voucher specimens were deposited in the herbarium of South China Agricultural University with the voucher number 33207 (contact: Zheng Ming-Xuan, zhengmx@scau.edu.cn).

Sequencing was performed using the Illumina Novaseq6000 high-throughput sequencing platform with a PE150 (Pair-End 150) sequencing strategy and a minimum of 2 Gb sequencing data. cp genome sequences were assembled using SPAdes v. 3. 5. 0 (Lapidus et al. 2014) software and then annotated using CpGAVAS (Liu et al. 2012) and ORF Finder. The cp genome was annotated using CpGAVAS (Liu et al. 2012) and ORF Finder.

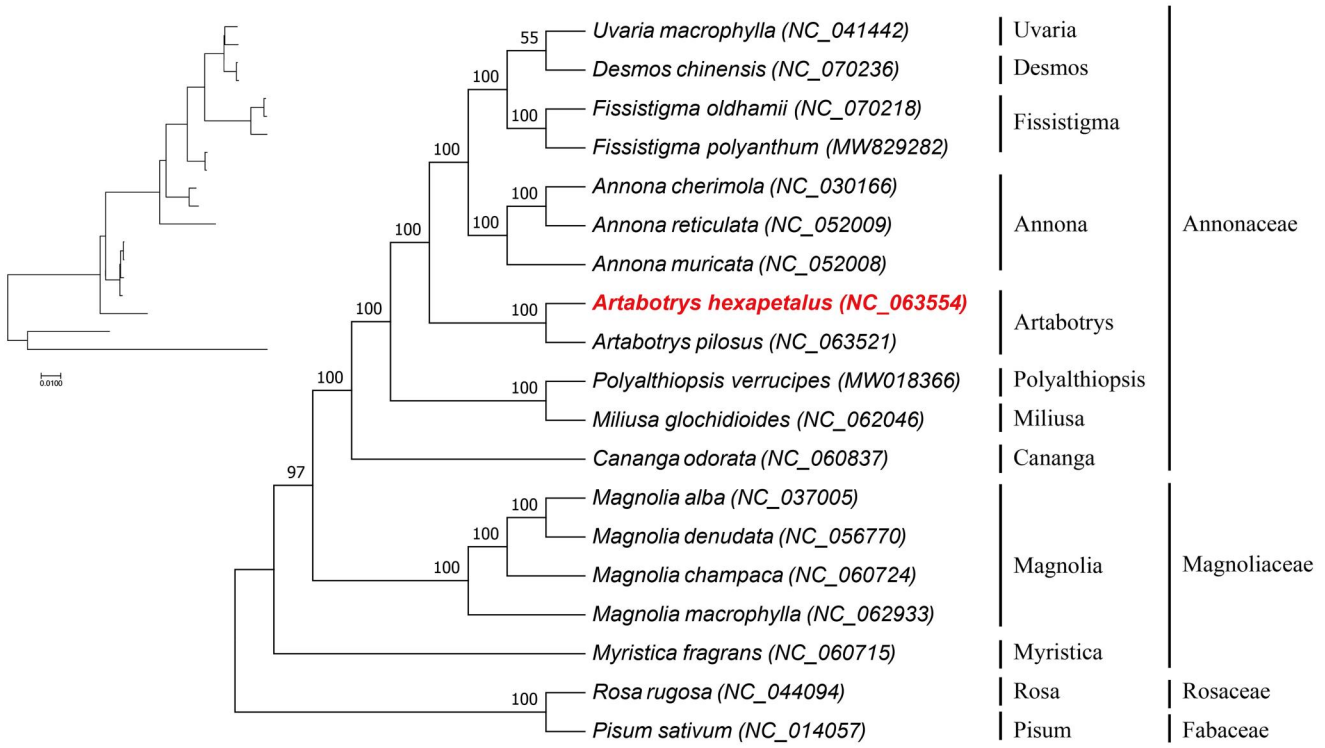

Figure 3. The phylogenetic position of *A. hexapetalus* was inferred from maximum likelihood (ML) based on 19 complete chloroplast genomes, with *Rosa rugosa* and *Pisum sativum* as outgroups. Statistical support values were shown on nodes. The complete chloroplast genomes used for constructing the phylogenetic tree contain *Annona cherimola* (Talavera et al. 2023) (NC_030166), *Annona muricata* (Niu et al. 2020) (NC_052008), *Annona reticulata* (Niu et al. 2020) (NC_052009), *Artabotrys hexapetalus* (MZ936420), *Artabotrys pilosus* (Liang et al. 2022) (NC_063521), *cananga odorata* (Suryani et al. 2022) (NC_060837), *desmos chinensis* (NC_070236), *Fissistigma oldhamii* (MW829281), *Fissistigma polyanthum* (MW829282), *Magnolia Alba* (Hinsinger and Strijk 2017)(NC_037005), *Magnolia champaca* (NC_060724), *Magnolia denudata* (NC_056770), *Magnolia macrophylla* (NC_062933), *miliusaglochidioides* (NC_062046), *myristica fragrans* (NC_060715), *Pisum sativum* (NC_014057), *polyalthiopsis verrucipes* (MW018366), *Rosa rugosa* (Kim et al.2019) (NC_044094), *uvaria macrophylla* (Wu et al. 2019) (NC_041442). The type of sequences used for building the phylogenetic tree are the complete chloroplast genomes downloaded from NCBI (https://www.ncbi.nlm.nih.gov/).

tRNA annotation was performed using ARWEN (Laslett and Canbäck 2008), and the reference sequence was re-validated in conjunction with predictions from tRNAscan-SE 2.0 (Lowe and Chan 2016).

To investigate the evolutionary relationship of *A. hexapetalus* (L.f.) Bhandari, we retrieved the sequences of 18 other cp genomes sequences, including *Rosa rugosa* and *Pisum sativum* from NCBI (https://www.ncbi.nlm.nih.gov). Multiple sequence alignments were performed using MUSCLE v.3.8.31 (Edgar 2004), and a maximum likelihood tree was constructed using RAxML 8.1.5 (Alexandros 2006) with jModel Test 2.1.7 (Posada 2008) (https://code.google.com/p/jmodeltest2/) and 1000 bootstrap replicates.

Results

The cp genome of *A. hexapetalus* (L.f.) Bhandari exhibited a circular structure of 178,457 bp and a GC content of 38.77% (Figure 2). It consisted of a large single copy region (LSC) of 90,803 bp, a small single copy region (SSC) of 3,066 bp, and two inverted repeating regions (IR) of 42,294 bp. Annotation of the cp genome revealed a total of 134 genes in A. hexapetalus, including 90 protein-coding genes, eight rRNA genes, and 36 tRNA genes. Among these genes, 15 protein-coding (*ccs*A, *ndh*A, *ndh*B, *ndh*D, *ndh*E, *ndh*G, *ndh*H, *ndh*I, *psa*C, *rpl*23, *rpl*2, *rps*12, *rps*15, *rps*7 *and ycf*2), six tRNA (*trn*L-TAG, *trn*N-GTT, *trn*R-ACG, *trn*A-TGC, *trn*V-GAC and *trn*L-CAA) and four rRNA genes (*rrn*5、*rrn*4.5、*rrn*23、*rrn*16) have two copies. Additionally, 10 cis-splicing genes (*rps*16, *atp*F, *rpo*C1, *pet*B, *pet*D, *rpl*2, *ndh*B, *ndh*A, *ycf*3 and *clp*P) and one trans-splicing gene (*rps*12) were detected by CPGview (Liu et al. 2023) (http://www.1kmpg.cn/cpgview/). The *rps*12 gene consisted of one exon in the LSC region and two duplicated exons in the IR regions (Figure S1). Furthermore, 13 genes, including *rps*16, *atp*F, *rpo*C1, *pet*B, *pet*D, *rpl*2, *rpl*2, *ndh*B, *ndh*B, *ndh*A, *ndh*A, *ycf*3 and *clp*P, contain one or two introns (Figure S2). The minimal and average read mapping depths for assembled genomes were 11 × and 708.72 × (Figure S3), respectively.

RAxML8.1.5 conducted the phylogenetic analysis with 19 cp genomes. Among these, *Rosa rugosa* and *Pisum sativum* were used as outgroups. As shown in Figure 3, four clades were generated in the phylogenetic tree, including Annonaceae, Magnoliaceae, Rosaceae and Fabaceae. Our results revealed a closer relationship between *A. hexapetalus* and *Artabotrys pilosus*, both belonging to the same genus in the Annonaceae family, supported by substantial evidence.

Discussion and conclusion

The cp genome of *A. hexapetalus* (L.f.) Bhandari was characterized for the first time in this study. The cp genome characterization and phylogenetic analysis of *L. caerulea* provide helpful genetic resources and new insights for future molecular breeding. The phylogenetic analysis revealed a close

relationship between *A. hexapetalus* and *Artabotrys pilosus*, forming a sister branch with 100% support. The plastome length of *A. hexapetalus* is comparable to other Annonaceae species, e.g. *Annona muricata* (Niu et al. 2020) (NC_052008) and *Fissistigma oldhamii* (MW829281). However, it is shorter than *Annona reticulata* (Niu et al. 2020) (NC_052009) and *Annona cherimola* (Talavera et al. 2023) (NC_030166). These findings suggest establishing a theoretical basis to further understand the taxa's evolution and are anticipated to contribute to conservation endeavors.

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Author contributions

Yi Guo conceived and drafted the study. Kai Zhu contributed to the study design and sampling. Yongle Zhang and Hui Tang analyzed the experimental data. All authors provided input comments and approved the final version of the manuscript.

Ethics approval

This study includes no human, animal, or endangered plant samples, and the sample was legally collected under guidelines provided by the authors' institution and national or international regulations. Field studies complied with local legislation. No ethical approval is required for this study.

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] (https://www.ncbi.nlm.nih.gov/) under accession no. MZ936420. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA796701, SRR17629648, and SAMN24907120 respectively.

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