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PLASTOME REPORT

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The complete chloroplast genome and phylogenetic analysis of *Bauhinia glauca* subsp. *hupehana* (Craib) T. C. Chen 1988

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ABSTRACT

Bauhinia glauca subsp. *hupehana* (Craib) T. C. Chen 1988, a member of the *Leguminosae* family, *Cercidoideae* subfamily, and *Bauhinia* genus, has a rich history of traditional usage in Chinese medicine. Renowned for its analgesic properties, it is commonly employed for managing inflammation and pain. This study aimed to sequence the complete chloroplast genome of *B. glauca* subsp. *hupehana* using Illumina paired-end sequencing data. The chloroplast genome spans 156,967 bp and consists of four main regions: the large single-copy (LSC) region (89,185 bp), the small single-copy (SSC) region (19,146 bp), and a pair of inverted repeats (IRs) (24,318 bp). The overall GC content of the chloroplast genome is 36.19%, with specific values of 33.99%, 29.79%, and 42.76% for the LSC, SSC, and IR regions, respectively. A total of 128 genes were annotated in the chloroplast genome, including 83 protein-coding genes, 37 tRNA genes, and eight rRNA genes. Phylogenetic analysis revealed that *B. glauca* subsp. *hupehana* is closely related to *Bauhinia racemose*, indicating a sister taxon relationship between the two species. This study significantly contributes to the chloroplast genomic resource for *Bauhinia*, laying the groundwork for future phylogenetic investigations within the genus.

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Introduction

The *Bauhinia* genus encompasses approximately 300 species, with its nomenclature derived from distinct leaf morphology resembling either a cow's paw or hoof (Cechinel Filho 2009; Šerá et al. 2021). *Bauhinia* is a perennial evergreen, including trees, shrubs, and woody vines with tendrils. It is widely distributed in tropical and subtropical regions across Africa, Asia, and America (Meng et al. 2014). With robust climbing capabilities and a preference for sunlight, this species demonstrates remarkable resilience to environmental pollution, making it an ideal choice for pergola greening due to its foliage reminiscent of cloven hooves. Analysis of species within the *Bauhinia* genus has revealed a diverse array of constituents, including dihydrodibenzoxepins, flavonoids, phenolic acids, steroids, and lectins (Pettit et al. 2006; Boonphong et al. 2007). Empirical evidence supports the bioactivities of these compounds, showcasing anti-malarial, antifungal, antibacterial, anti-HIV, and cytotoxic properties (Fang et al. 2010; Annegowda et al. 2012). In traditional Chinese medicine, *Bauhinia glauca* subsp. *hupehana* (Craib) T. C. Chen 1988 is a botanical entity that is acknowledged for its analgesic efficacy (Clark et al. 2017).

The roots, stems, and leaves of *B. glauca* subsp. *hupehana* are attributed to multifaceted functions, including dispelling wind, promoting blood circulation, alleviating pain, and detoxification. As a result, it has found applications in the therapeutic management of inflammatory conditions and various pain-related disorders, such as back pain, hemorrhage-induced swelling and pain, and rheumatoid arthritis (Xu et al. 2015). Ongoing research endeavors suggest that the *B. glauca* subsp. *hupehana* holds promise as a source for innovative non-opioid plant-derived pharmaceuticals, exhibiting potential analgesic properties (Cechinel Filho 2009). However, the chloroplast genome of *B. glauca* subsp. *hupehana* remains inadequately characterized, with a limited investigation conducted so far. Furthermore, there is a lack of phylogenomic evidence to support the species' taxonomic placement within the broader phylogenetic framework. This study aims to address these knowledge gaps by presenting, for the first time, the complete sequence of the chloroplast genome of *B. glauca* subsp. *hupehana*. The acquired genomic data not only fill a critical void in our understanding but also provide invaluable insights into the taxonomic classification, phylogenetic relationships, and population genetics of *Bauhinia* genus.

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Materials and methods

Fresh leaf tissue samples were collected from Jiangxi Agricultural University (Nanchang, China; coordinates: 28.7597 N, 115.8352 E) (Figure 1). These samples are assigned the voucher number KQnum20220505008 and have been securely stored at the College of Bioscience and Engineering, Jiangxi Agricultural University (http://shenggong.jxau.edu.cn/, Licao Cui, email: cuilicao@jxau.edu.cn). Genomic DNA extraction employed a modified CTAB method applied to the freshly harvested leaves (Doyle and Doyle 1987). Subsequently, a sequencing library with an insert length of 400 bp was prepared. Paired-end reads of 150 bp were generated using the Illumina NovaSeq 6000 system, provided by Personalgene in Nanjing, China, specifically for chloroplast genome analysis.

After removing adaptor contamination and low-quality reads, a total of 26,776,580 clean reads were obtained for the complete DNA sequence. *De novo* assembly of the chloroplast genome was performed using Fast-Plast v.1.2.8 software, which utilizes a reference index of over 1000 whole chloroplast genomes from GenBank, with Fabales as the reference. Annotation of the chloroplast genome was carried out using the web application Geseq (https://chlorobox.mpimp-golm.mpg.de/geseq.html) (Tillich et al. 2017), with default parameters, to identify transfer RNAs (tRNAs), protein-coding genes, and ribosomal RNAs (rRNAs) using default parameters. The complete chloroplast genome sequence and annotation files of *B. glauca* subsp. *hupehana* were deposited in GenBank under accession no. OR750454. Sequencing depth was assessed by aligning clean reads with the chloroplast genome through BWA v0.7.17-r1188 (Li and Durbin 2010) and Samtools v1.12 (Li et al. 2009). To visualize the chloroplast genome, Chloroplast Genome Viewer (CPGView) (Liu et al. 2023) was utilized, which generated a circular map along with schematic representations of *cis*- and *trans*-splicing genes.

To determine the phylogenetic position of *B. glauca* subsp. *hupehana*, the complete chloroplast genome sequences of 18 additional species from the subfamily *Cercidoideae* were retrieved from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/), with *Cadellia pentastylis* F. Muell. 1860 (MN709816) serving as outgroup. Multiple sequence alignments of the full-length chloroplast genome sequences were performed using MAFFT v.7.313 (Katoh and Standley 2013). A maximum-likelihood phylogenetic tree was constructed using RAxML v.8.2.12 (Stamatakis 2014) under the GTRCAT model, and the statistical support for the tree topology was assessed using 1000 bootstrap replicates.

Results and discussion

The chloroplast genome of *B. glauca* subsp. *hupehana* showed a length of 156,967 bp, with an overall GC content of

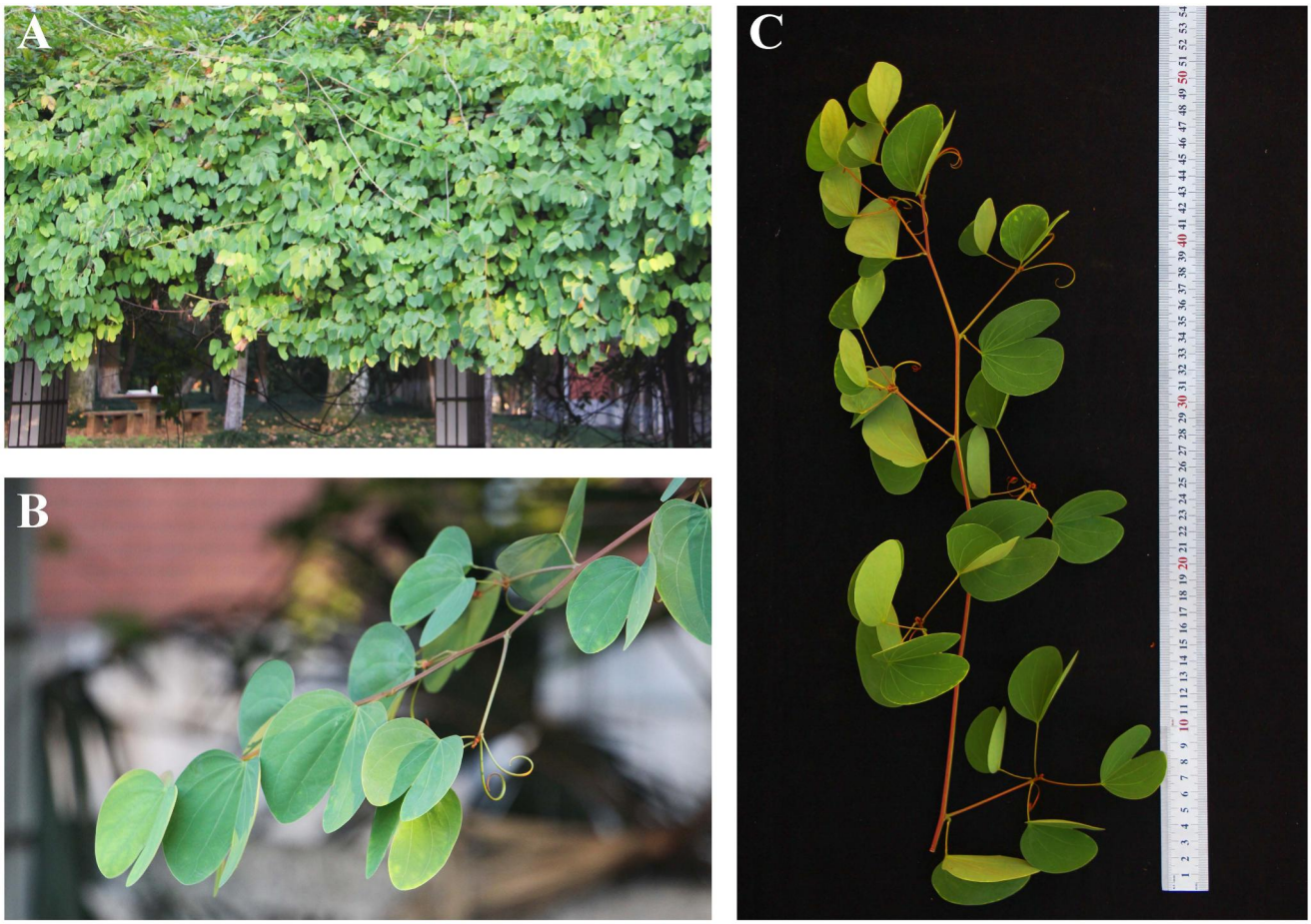

Figure 1. Photographs of *B. glauca* subsp. *hupehana* captured by the authors at the Jiangxi Agricultural University, Nanchang, China (coordinates: 28.7597 N, 115.8352 E). (A) The climbing habit of *B. glauca* subsp. *hupehana*; (B) close-up of *B. glauca* subsp. *hupehana* plant leaves; (C) specimen utilized in this study. Species images were captured by the corresponding author, Yi-Han Li, in Jiangxi province, China.

36.19% (Figure 1). The sequencing depth across the assembled genome ranged from 276.50× to 7962.13×, with an average of 923.08× (Supplementary Figure 1). Structurally, the chloroplast genome followed the typical quadripartite organization, consisting of a large single-copy (LSC) region spanning 89,185 bp, a small single-copy (SSC) region of 19,146 bp, and a pair of inverted repeats (IRs) totaling 24,318 bp (Figure 2). The GC content of the LSC, SSC, and IR regions was 33.99%, 29.79%, and 42.76%, respectively (Supplemental Table 1). A total of 128 genes were annotated in the chloroplast genome, comprising 83 protein-coding genes, eight rRNA genes, and 37 tRNA genes (Supplementary Table 2). Among these genes, 13 underwent *cis*-splicing (Supplemental Figure 2), with 11 (*rpoC1*, *atpF*, *rps16*, *matK*, *ndhA*, *rpl2*, *rpl16*, *petD*, *petB*, and two *ndhB*) containing a single intron, and two (*ycf3* and *clpP*) containing two introns. Furthermore, the *rps12* underwent *trans*-splicing (Supplemental Figure 3), characterized by two introns.

The phylogenetic analysis demonstrated that *B. glauca* subsp. *hupehana*, *B. blakeana*, *B. brachycarpa*, *B. purpurea*, *B. racemosa*, and *B. variegata* var. *variegata* formed a strongly supported monophyletic clade with high bootstrap values (Figure 3). Our phylogenetic tree structure is closely aligned with previous studies (Gu et al. 2019; Xiao et al. 2022; Zeng et al. 2022). Additionally, the analysis indicated a sister relationship between *B. glauca* subsp. *hupehana* and *B. racemosa* in the current taxonomic sampling. Notably, *B. racemosa* is a versatile medicinal plant with a comparable genome size of 155,501 bp comparable to that of *B. glauca* subsp. *hupehana*, shared identical numbers of rRNA genes, tRNA genes, and protein-coding genes. However, a distinctive feature in *B. racemosa* was the presence of an additional pseudogene

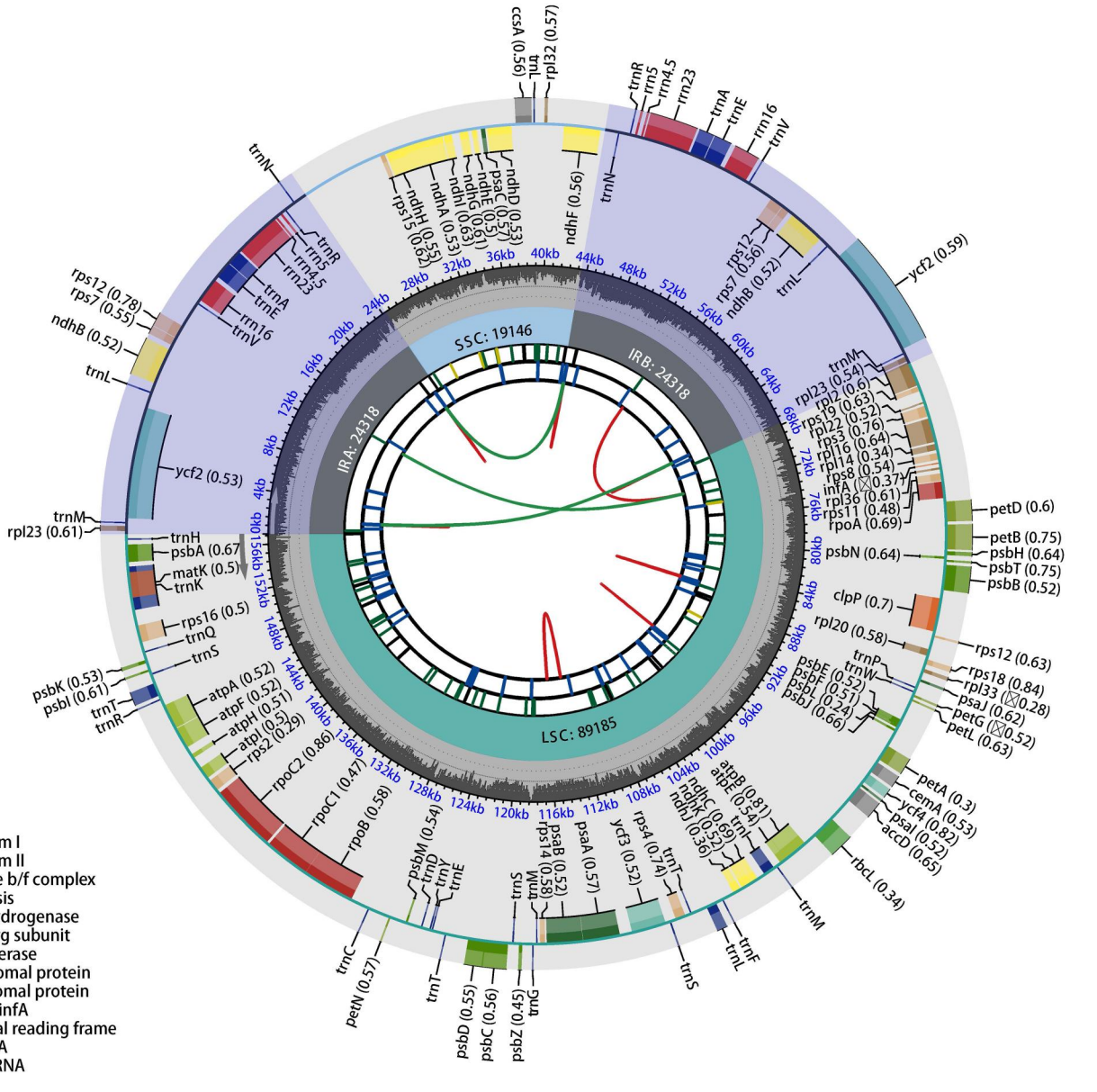

Figure 2. Visualization of the chloroplast genome of *B. glauca* subsp. *hupehana* using CPGView. In the outermost circle, genes are color-coded based on functional groups. The second circle displays the percentage of GC content and genomic positions in kilobase pairs. The third circle delineates the large single-copy (LSC), small single-copy (SSC), and inverted repeat (IR) regions. Short tandem repeats or microsatellite sequences are represented as colored bars in the fourth circle, while the fifth circle illustrates long tandem repeats with short blue bars. The innermost circle indicates dispersed repeats.

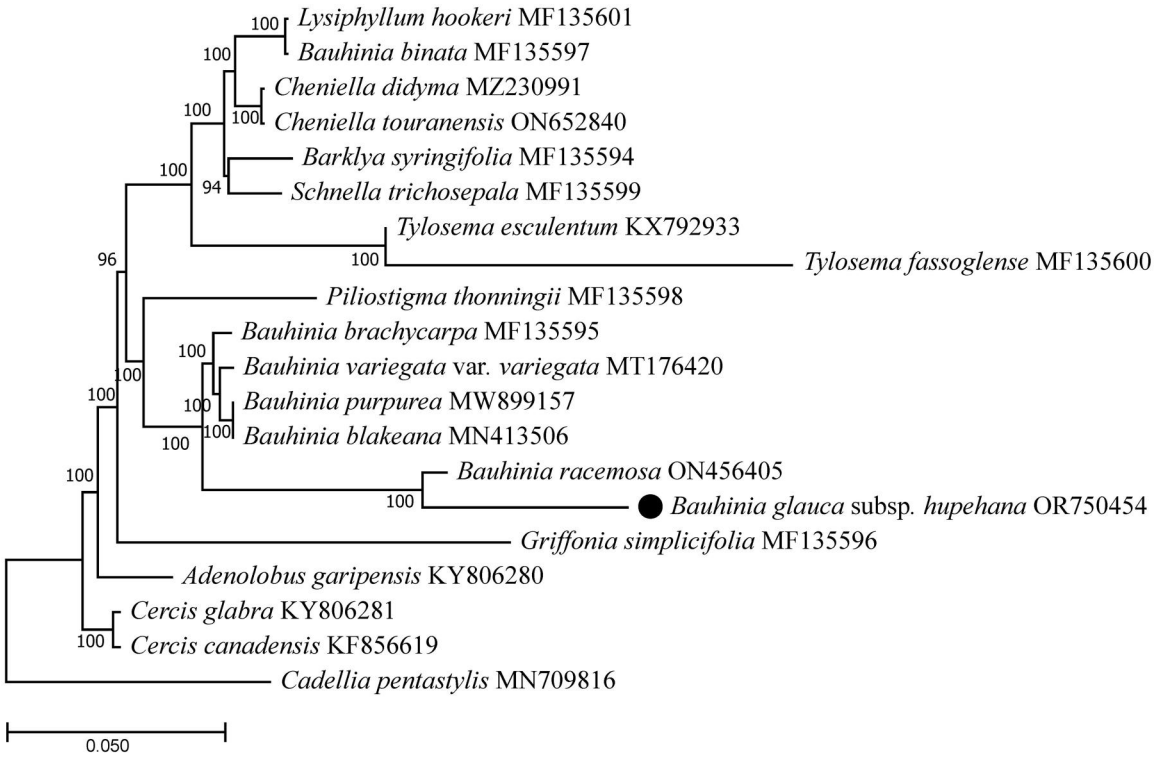

Figure 3. A maximum-likelihood phylogenetic tree was constructed using RAxML v.8.2.12, incorporating the 20 complete chloroplast genome sequences. Bootstrap support values (from 1000 replicates) are indicated on the branches. The sequences employed for the construction of the phylogenetic tree are detailed as follows: *Adenolobus garipensis* (KY806280) (Wang et al. 2017), *Barklya syringifolia* (MF135594) (Wang et al. 2018), *Bauhinia binata* (MF135597) (Wang et al. 2018), *Bauhinia blakeana* (MN413506) (Gu et al. 2019), *Bauhinia brachycarpa* (MF135595) (Wang et al. 2018), *Bauhinia purpurea* (MW899157) (Wang et al. 2018), *Bauhinia racemosa* (ON456405) (Xiao et al. 2022), *Bauhinia variegata* var. *variegata* (MT176420) (Gu et al. 2020), *Cercis canadensis* (KF856619) (Wang et al. 2018), *Cercis glabra* (KY806281) (Wang et al. 2017), *Cadellia pentastylis* (MN709816) (Zhang et al. 2020), *Cheniella didyma* (MZ230991) (Zeng et al. 2022), *Cheniella touranensis* (ON652840), *Griffonia simplicifolia* (MF135596) (Wang et al. 2018), *Lysiphyllum hookeri* (MF135601) (Wang et al. 2018), *Piliostigma thonningii* (MF135598) (Wang et al. 2018), *Schnella trichosepala* (MF135599) (Wang et al. 2018), *Tylosema esculentum* (KX792933) (Kim and Cullis 2017), and *Tylosema fassoglense* (MF135600) (Wang et al. 2018).

(*rps19*). This comparative analysis highlights both the similarities and differences in the structure of closely related species, providing valuable insights into their phylogenetic relationships.

Conclusions

This study represents the first characterization of *B. glauca* subsp. *hupehana* and its closely related species as a unique lineage within the *Bauhinia* genus. It substantially enhances the genomic resources available for the *Bauhinia* genus in the *Leguminosae* family, thus providing a significant contribution to future investigation in evolution, taxonomy, DNA barcoding, and molecular markers.

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Author contributions

Yi-Han Li and Chong-De Lai: conceptualization, fieldwork, writing, and editing. Qing-Lin Ke: methodology and analysis. Rui-Ying Li and Shao-Shuai Cai: annotating the chloroplast genome. Min-Qiang Tang and Li-Cao Cui: performing the data acquisition and analysis. All authors approved the published version of the manuscript, and agreed to be accountable for all aspects of the work.

Ethical approval

Specimens of *B. glauca* subsp. *hupehana* were collected from Jiangxi Agricultural University (Nanchang, China), and all experimental samples were derived from this specific planting material. The plant material utilized in this study does not include any endangered wild species. No specific permissions were necessary for sample collection in this study, and we adhered to applicable institutional, national, and international guidelines and regulations governing plant research.

Disclosure statement

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

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Data availability statement

The data supporting the conclusions of this study are publicly accessible in the GenBank of NCBI under the accession number OR750454 (https://www.ncbi.nlm.nih.gov/nuccore/OR750454.1/). Corresponding BioProject, SRA, and Bio-Sample identifiers are PRJNA1035492, SRR26664909, and SAMN38089836, respectively.

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