

The complete chloroplast genome of *Hiptage benghalensis* (L.) Kurz and its phylogenetic analysis in malpighiaceae

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ABSTRACT

Hiptage benghalensis (L.) Kurz 1874, an economically significant woody liana in the Malpighiaceae family, lacks a reported chloroplast genome, hindering its phylogenetic analysis. To address this, we sequenced and assembled the complete chloroplast genome of *H. benghalensis*, spanning 160,719 bp with an overall GC content of 36.7%, and containing 132 genes (87 protein-coding, 37 tRNA, and 8 rRNA). Phylogenetic analysis revealed that *H. benghalensis* exhibits the closest evolutionary relationship with species from the genus *Aspidopterys*. This study provides the first complete chloroplast genome for *H. benghalensis*, enhancing our understanding of its evolution and potential applications within the Malpighiaceae family.

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Introduction

Hiptage benghalensis (L.) Kurz 1874 is a shrub or twining liana belonging to the genus *Hiptage* within the family Malpighiaceae. It is mainly found in dense valley forests and roadsides along ravines at an altitude of 100–1900 meters in tropical regions of Asia (Ren 2015). *H. benghalensis* is a multi-purpose species with industrial, pharmaceutical, and ornamental value. Its seeds yield ricinoleic acid (12-hydroxy-9-octadecenoic acid), a hydroxylated fatty acid extensively utilized in industrial applications (Tian et al. 2019). Leaf extracts demonstrate potent reactive free radical scavenging activity and have exhibited notable hepatoprotective effects in rat models (Maheshwari et al. 2012). Acetonic extracts from root bark show potential as insecticidal agents, effectively controlling the reproduction of malaria vectors including *Anopheles* spp. and *Aedes aegypti* mosquitoes (Lalrotluanga et al. 2012). It has mirror-image flowers, with white, intensely fragrant petals, and exhibit significant ornamental value.

Although previous studies have investigated the molecular phylogenetic relationships of *H. benghalensis* within the genus *Hiptage* using ITS gene (Tan et al. 2019), the chloroplast genome of this species and its evolutionary position in the order Malpighiales remain unreported. Chloroplast genomes are highly conserved in most angiosperms and serve as robust markers for reconstructing phylogenetic trees and resolving evolutionary relationships among taxa, thus being widely utilized in phylogenomic studies (Daniell et al. 2016). In this

study, we assembled and annotated the complete chloroplast genome of *H. benghalensis*, providing essential plastid genomic data to further elucidate its phylogenetic placement within Malpighiales.

Materials and methods

Leaf samples of *H. benghalensis* were collected from South China Botanical Garden, CAS, Guangdong, China (111°36'71.96" E longitude, 23°18'17.97" N latitude) (Figure 1), and were stored in the Medical Research Center, Sun Yat-sen Memorial Hospital, Sun Yat-sen University (contact Yabin Guo, guoyb9@mail.sysu.edu.cn, voucher no. GYBL-20241210). Total DNA was extracted from fresh young leaves using a modified CTAB protocol (Allen et al. 2006), followed by library preparation and paired-end sequencing (150 bp × 2) on the DNBSEQ-T7 platform at Kindstar Sequenon (Wuhan, China), generating approximately 10 Gb of raw data. Raw reads were processed through quality control using fastp v0.20.1 with default parameters, including adapter removal and quality filtering (Chen 2023).

The chloroplast genome was *de novo* assembled using GetOrganelle v1.7.7.1 (Jin et al. 2020), with circular structure validation performed via Bandage v0.9.0 (Wick et al. 2015). The structural orientation was confirmed using CPStools (Huang et al. 2024) and annotated through CPGAVAS2 (Shi et al. 2019) with *Aspidopterys cavaleriei* (PQ112544) (Liao

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Figure 1. Photographs of *H. benghalensis*. A. Entire habit of *H. benghalensis*, a lianoid shrub. B. Flowers. Raceme exhibiting white petals with yellow basal spots. C. Fruits. Samara showing an elliptic or obovate-lanceolate median wing. D. Leaves and stem. Leaves are oblong with an acuminate apex and broad base; stem is right-twining. All photographs were taken by Ya-Bin guo at the South China Botanical Garden in Guangdong.

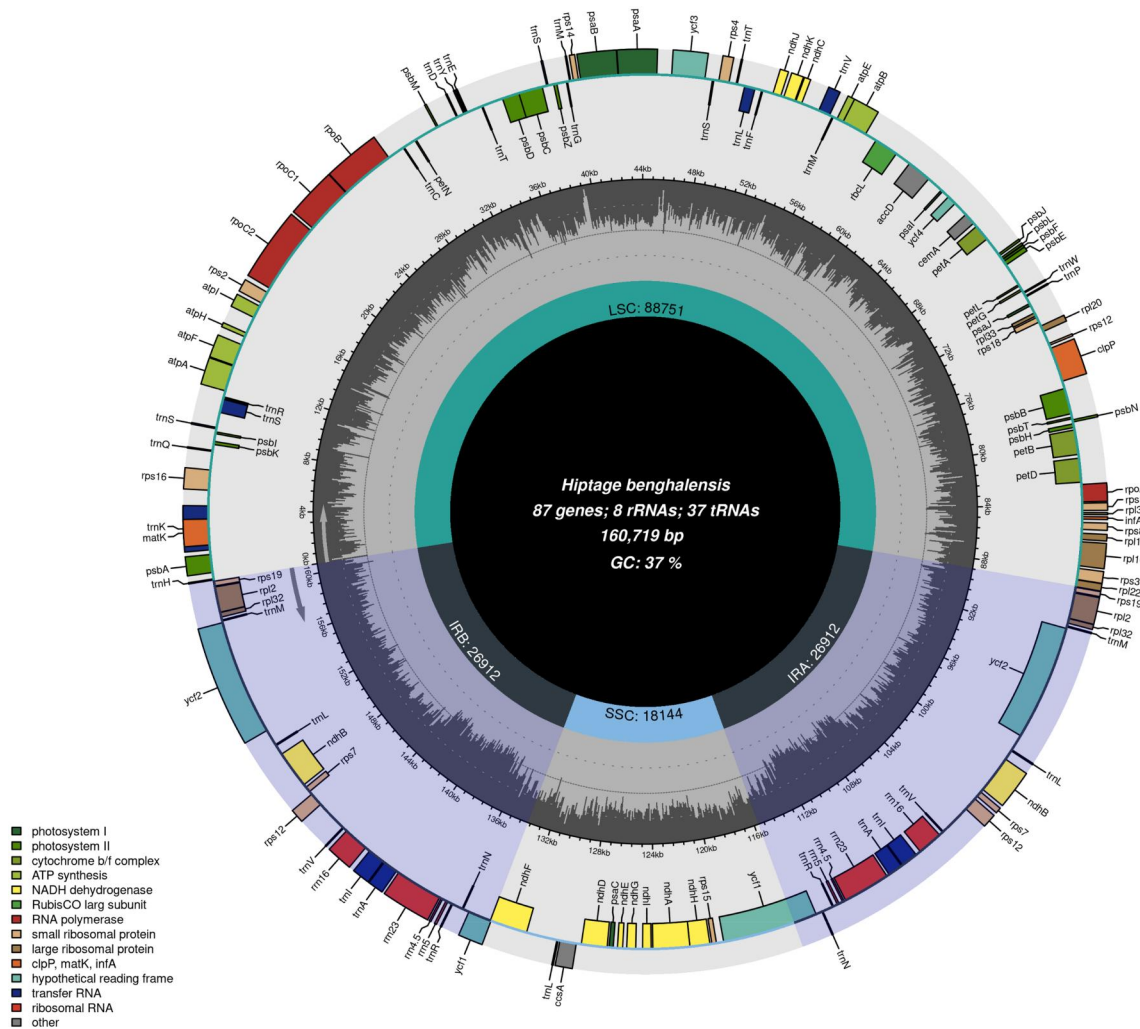


Figure 2. The chloroplast genome map of *H. benghalensis*. Arrows indicate the transcription directions of genes on the inner and outer circles, corresponding to clockwise and counterclockwise orientations, respectively. Genes are color-coded based on their functional categories. The inner circle delineates the chloroplast structure: large single copy (LSC), small single copy (SSC), and inverted repeat (IR) regions.

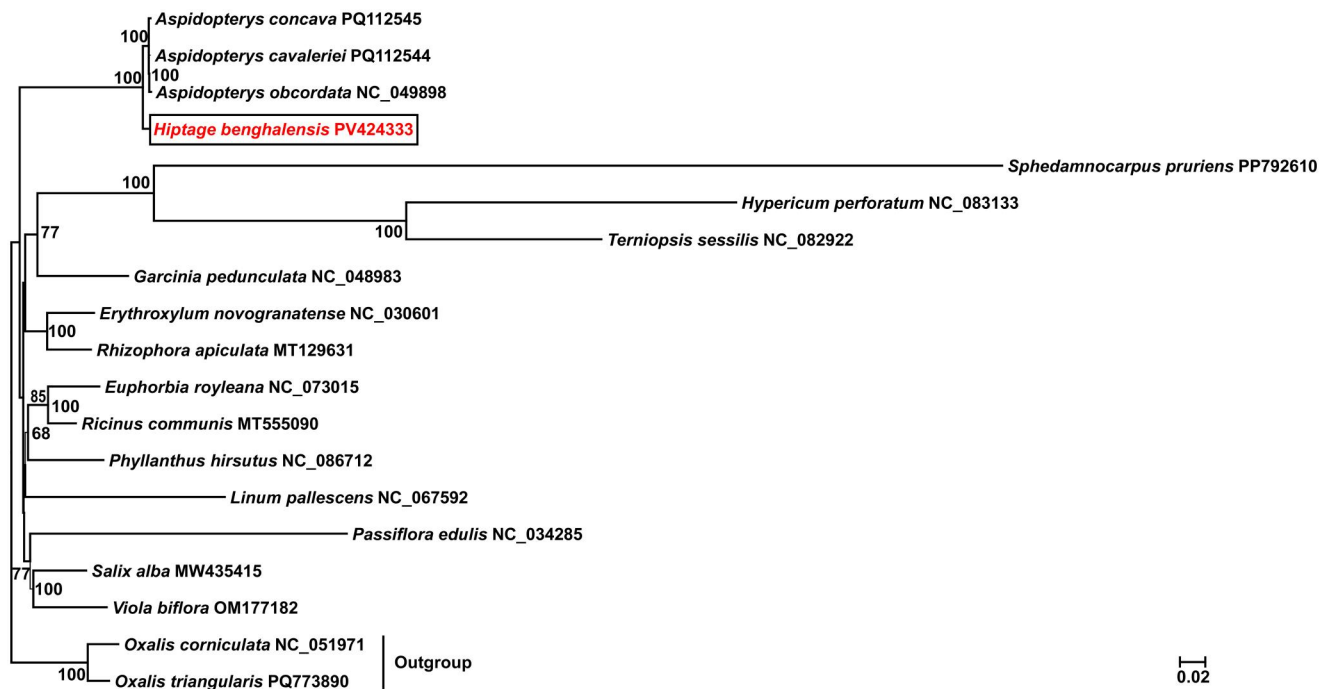


Figure 3. Phylogenetic tree illustrating the evolutionary relationships of *H. benghalensis* within malpighiales. The tree was constructed based on complete chloroplast genomes using MAFFT for sequence alignment, followed by maximum likelihood (ML) analysis in IQTREE with 1,000 bootstrap replicates. Bootstrap support values are indicated at each node, with only values above 60 being displayed. The following GenBank accession numbers correspond to the taxa included: *Hiptage benghalensis* (PV424333, in this study), *aspidopterys cavaleriei* (PQ112544) (Liao et al. 2025), *aspidopterys obcordata* (NC_049898) (Gong et al. 2020), *aspidopterys concava* (PQ112545) (Liao et al. 2025), *erythroxylum novogranatense* (NC_030601), *euphorbia royleana* (NC_073015), *garcinia pedunculata* (NC_048983) (Yang et al. 2019), *hypericum perforatum* (NC_083133), *linum pallescens* (NC_067592), *passiflora edulis* (NC_034285) (Cauz-Santos et al. 2017), *phyllanthus hirsutus* (NC_086712), *rhizophora apiculata* (MT129631) (Zhang et al. 2023), *ricinus communis* (MT555090) (Muraguri et al. 2020), *salix alba* (MW435415) (Wagner et al. 2021), *spedamnocarpus pruriens* (PP792610), *terniopsis sessilis* (NC_082922), and *viola biflora* (OM177182). *Oxalis corniculata* (NC_051971) (Lubna et al. 2020) and *oxalis triangularis* (PQ773890) are as the outgroup.

et al. 2025) as the reference genome, followed by manual curation. The circular genome map was visualized using Chloroplot (Zheng et al. 2020). The complete chloroplast genome sequence has been deposited in NCBI GenBank under accession number PV424333.

A total of 18 complete chloroplast genomes, including 16 representative Malpighiales species, were obtained from NCBI GenBank for phylogenetic reconstruction. To ensure the correctness of the root and the reliability of the results, the related genus *Oxalis* (*O. corniculata* and *O. triangularis*) was used as the outgroup. Multiple sequence alignment was performed using MAFFT v7.525 (Katoh and Standley 2013), by the 'auto' parameter. Then we used Trimal to modify the low aligned regions via the 'automated' strategy (Capella-Gutiérrez et al. 2009). To determine its phylogenetic position, we used IQ-TREE to build the phylogenetic tree using the maximum likelihood method (ML) (Minh et al. 2020). We set the parameter '-m MFP -bb 1000' to automatically find the best model and performed 1000 bootstrap tests. According to Bayesian Information Criterion (BIC), the best fit nucleotide model is 'GTR+F+I+R3', which was identified through the ModelFinder (Kalyanamoorthy et al. 2017).

Results

The complete chloroplast genome of *H. benghalensis* spans 160,719 bp, with an average coverage of $3567.2\times$ (Figure S1). It exhibits the conserved quadripartite structure typical of

angiosperm plastomes, consisting of a large single-copy (LSC) region (88,751 bp), a small single-copy (SSC) region (18,144 bp), and two inverted repeat (IR) regions (IRa and IRb, each 26,912 bp). The overall GC content is 36.73%, with distinct values for the LSC (34.49%), SSC (30.58%), and IR regions (42.51%). A total of 132 functional genes were annotated, including 87 protein-coding genes (PCGs), 37 tRNA genes, and 8 rRNA genes, with 111 of the unique genes (Figure 2). Among these, 15 genes (*atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpl2*, *rpl16*, *rpoC1*, *rps16*, *trnA-UGC*, *trnI-GAU*, *trnK-UUU*, *trnL-UAA*, *trnS-CGA*, and *trnV-UAC*) contain two exons, while 3 genes (*clpP*, *rps12*, and *ycf3*) possess three exons and only *rps12* exists trans-splicing (Figures S2 and S3). Most genes are present as single copies, with exceptions including duplicated genes: four rRNAs (*rrn4.5*, *rrn5*, *rrn16*, and *rrn23*), six tRNAs (*trnA-UGC*, *trnI-GAU*, *trnL-CAA*, *trnN-GUU*, *trnR-ACG*, and *trnV-GAC*), and six PCGs (*ndhB*, *rpl2*, *rpl32*, *rps7*, *rps12*, and *rps19*) (Table S1).

To determine the phylogenetic position of the newly assembled *H. benghalensis* chloroplast genome within Malpighiales, a maximum likelihood (ML) analysis was conducted using *O. corniculata* and *O. triangularis* (Oxalidaceae) as the outgroup. The ML tree placed *H. benghalensis* (Malpighiaceae) as sister to the genus *Aspidopterys* (Figure 3).

Discussion and conclusion

In this study, we present the first assembly and annotation of the complete chloroplast genome of *H. benghalensis*, which

exhibits a typical quadripartite structure (LSC, SSC, IRa and IRb) with a total length of 160,719 bp. Functional annotation identified 132 genes, including 87 protein-coding genes (PCGs), 37 transfer RNA (tRNA) genes, and 8 ribosomal RNA (rRNA) genes. Of these genes, 111 were unique. The chloroplast genome of *H. benghalensis* demonstrates a comparable length and gene content to that of its congeneric species *Aspidopterys obcordata* (Gong et al. 2020).

Phylogenetic analysis revealed that *H. benghalensis* exhibits its close evolutionary relationships with species from the genus *Aspidopterys*, likely due to their shared taxonomic classification within the Malpighiaceae family. Chloroplast phylogenomics provides critical insights into deep evolutionary relationships and diversification mechanisms (Zhai et al. 2019). Notably, species of the genus *Aspidopterys* are primarily utilized for medicinal purposes, with *A. obcordata* serving as a traditional herbal remedy among the Dai people (Gong et al. 2020). Similarly, *H. benghalensis* demonstrates pharmacological properties, particularly in liver protection (Maheshwari et al. 2012). This phylogenetic framework may facilitate the identification of additional plants with potential medicinal, industrial, and multi-purpose applications, thereby enhancing their utilization.

In summary, we report the inaugural de novo assembly and functional characterization of the complete chloroplast genome in *H. benghalensis*, filling a critical knowledge gap in Malpighiaceae plastome research. Phylogenetic analysis highlights its close affinity with *Aspidopterys*, providing a foundational framework for understanding the evolution and utilization of species within the genus *Hiptage*.

Acknowledgements

Permissions statement: Leaf sampling of *H. benghalensis* (L.), a non-IUCN-listed woody liana species, was authorized by the institution of South China Botanical Garden, CAS. Ethical approval was not required for this study, as it exclusively involved non-destructive leaf sampling and included no animal experimentation or human participants.

Author contributions

CRedit: **Jie Li:** Conceptualization, Data curation, Formal analysis, Supervision, Visualization, Writing – original draft; **Yongxian Chen:** Data curation, Formal analysis, Methodology; **Zenghong Gao:** Formal analysis, Methodology, Project administration; **Yabin Guo:** Conceptualization, Funding acquisition, Project administration, Resources, Writing – review & editing.

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Disclosure statement

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

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Data availability statement

Chloroplast genome sequence can be accessed via accession number PV424333 in NCBI GenBank. The associated BioProject, SRA, and BioSample numbers are PRJNA1256780, SRR33367786, and SAMN48197673, respectively.

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