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PLASTOME REPORT

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The complete chloroplast genome sequence of *Ampelopsis delavayana* Planchon. ex Franch 1886 (Vitaceae)

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ABSTRACT

Ampelopsis delavayana Planchon. ex Franch 1886 is a plant with significant pharmacological effects and ornamental importance. This research unveiled the complete chloroplast (cp) genome sequence of *A. delavayana*. The study highlights that the cp genome of *A. delavayana* possesses a distinct tetrameric structure spanning 162,497 base pairs, comprising a small single-copy (SSC) region of 18,902 base pairs, a large single-copy (LSC) region of 90,441 base pairs, and two inverted-repeat regions (IRs), each 26,577 base pairs in length. The GC content of the SSC, LSC, and IR regions of the genome was 31.80%, 35.16%, and 42.82%, respectively, culminating in an overall GC content of 37.27%. The genome comprised 130 genes, which included eight rRNAs, 36 tRNAs, and 86 protein-coding genes. Through phylogenetic analysis utilizing the maximum-likelihood method, it was established that *A. delavayana* was closely related to *Ampelopsis glandulosa* var. *brevipedunculata*, positioning it as a sister species. This report not only provides a scientific reference for understanding the phylogeny of the family Vitaceae but also enriches our genetic information of *Ampelopsis*.

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Ampelopsis delavayana; genome structure; phylogenetic analysis

Introduction

Ampelopsis delavayana Planchon. ex Franch 1886, a species of woody vines within the *Ampelopsis* genus in family Vitaceae, is predominantly found in southern China (Editorial Committee of Flora of China 2007). Its roots and root bark have been utilized in traditional Chinese medicine for their reputed abilities to clear heat, remove toxins, dispel wind, and activate collaterals (Chinese Pharmacopoeia Commission 2005). Recent pharmacological research on *A. delavayana* has highlighted its richness in flavonoids, especially dihydromyricetin, suggesting its potential efficacy in mitigating high-fat, high-sugar, and related diseases (Zhang et al. 2024).

In this study, we employed high-throughput sequencing technology to assemble and annotate the chloroplast (cp) genome of *A. delavayana*. Our investigation focused on analyzing the structural characteristics of its cp genome and predicting its affiliations with closely related species in family Vitaceae. The aim was to provide a theoretical foundation for its classification within the family and elucidate its evolutionary relationships.

Materials and methods

The fresh leaf samples of *A. delavayana* utilized in this study were gathered from Enshi, Hubei, China (109°29′E, 30°17′N, altitude: 433 m) (Figure 1). The plant collection was authorized by the College of Forestry and Horticulture at Hubei Minzu University. Specimens were preserved in the Key Laboratory Building of the College of Forestry and Horticulture at Hubei Minzu University (https://www.hbmzu.edu.cn/linxy/, contact person: Qun Hu, email: 2811316096@qq.com) with the voucher number 20230507001. After collection, total cp genome DNA from the fresh leaves of *A. delavayana* was extracted using the modified cetyltrimethylammonium bromide (CTAB) method (Spadoni et al. 2019). Subsequently, the cp genome was sequenced on the Illumina HiSeq 2500 platform (San Diego, CA), generating 3.40 GB of raw data. The Biological Project Accession number for the original sequence data, maintained by NCBI SRA, is SRR26076561. Following quality control, the initial 20,000,000 reads were utilized to assemble the *A. delavayana* cp genome using the GetOrganelle software (Jin et al. 2020). The raw data underwent mapping to the cp genome sequence using HSAT2 software, and the resulting depth of coverage was depicted in Supplemental Figure S1 (Kim et al. 2019). The cp genome of *Ampelopsis glandulosa* (NC_072280.1) served as a reference to annotate the *A. delavayana* cp genome sequence. This annotation process was carried out using the online software CPGAVAS2 (http://47.96.249.172:16019/

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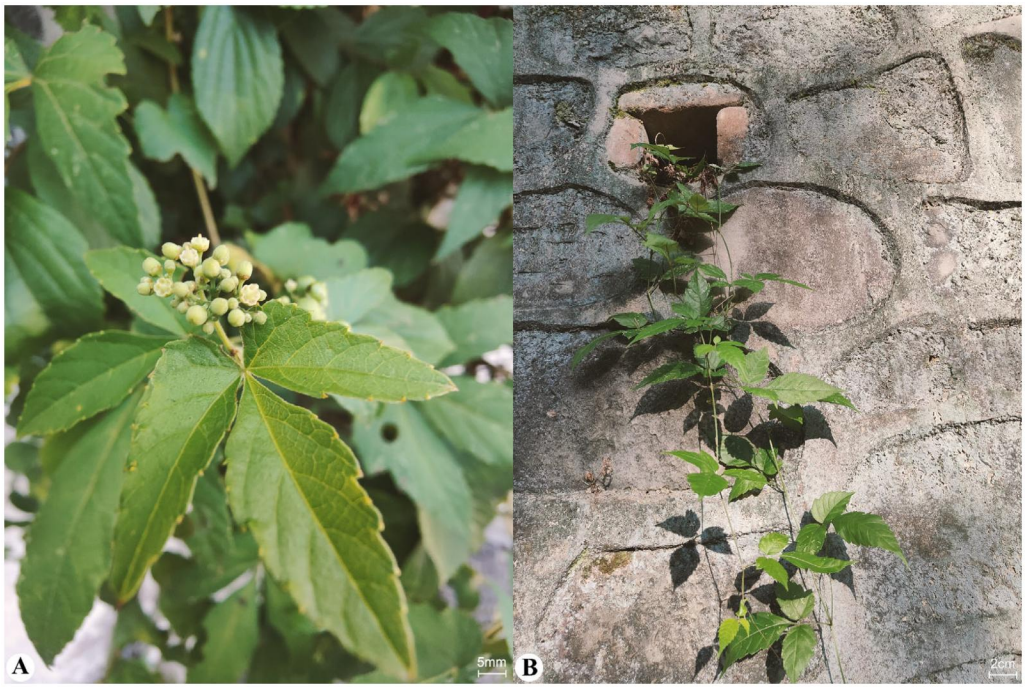

Figure 1. Species reference images of *A. delavayana*. (A) Morphological features of *A. delavayana* leaves. (B) Whole plant of *A. delavayana*. Core features: Terete branches, three-lobed leaves with elliptic-lanceolate leaflets, and dichasial cymes. This image, taken by Jiaqi Wu, is confirmed for our use with his permission.

analyzer/home) as described by Shi et al. (2019). Subsequent to manual review and adjustments, the annotation results were submitted to NCBI GenBank (OR540411). To ascertain the phylogenetic position of *A. delavayana* within the family Vitaceae, cp genome sequences of 23 other species from family were chosen to construct a maximum-likelihood (ML) phylogenetic tree. *Rosa cymosa* (MT471268) and *Berchemia berchemiifolia* (MG739656) were utilized as outgroups. The sequences for this analysis were obtained from NCBI GenBank. After multiple comparisons and alignments of 68 protein-coding gene sequences shared among the 26 sequences, sequence concatenation and optimization were carried out using MAFFT v7.313 (Katoh et al. 2019). An ML-based phylogenetic tree was then constructed for the optimized 26 sequences using IQ-TREE v.1.6.8. The GTR + F + I + G4 model was identified as the best fit by BIC (Ronquist and Huelsenbeck 2003; Nguyen et al. 2015).

Results

The cp genome of *A. delavayana* spanned 162,497 bp and was segmented into four distinct regions: a small single-copy (SSC) region measuring 18,902 bp, a large single-copy (LSC) region spanning 90,441 bp, and two inverted-repeat regions (IRs), each 26,577 bp in length (IRA, IRB) (Figure 2). The GC content of the SSC, LSC, and IR regions was 31.80%, 35.16%, and 42.82%, respectively, resulting in an overall GC content of 37.27%. Within the genome were 130 genes, encompassing eight rRNAs, 36 tRNAs, and 86 protein-coding genes. Both IR regions duplicated seven tRNAs (*trn*M-CAU, *trn*N-GUU, *trn*I-GAU, *trn*R-ACG, *trn*A-UGC, *trn*V-GAC, and *trn*L-CAA), four rRNAs (*rrn*16S, *rrn*23S, *rrn*5S, and *rrn*4.5S), and seven protein-coding genes (*ycf*15, *ycf*2, *rps*7, *rps*12, *ndh*B, *rpl*23, and *rpl*2). The genome contained introns in five tRNAs and 13 protein-coding genes, with the *clp*P, *ycf*3, and *rps*12 genes each containing two introns (Supplemental Table S1). The structures of the 16 proteins encoding *trans* and *cis* spliced genes were depicted in Supplemental Figure S2, with *rps*12 being identified as the *trans* spliced gene. A total of 74 simple sequence repeats (SSRs) were detected in the cp genome of *A. delavayana*, comprising 66 mononucleotides (T/A) and eight dinucleotides (TA/AT) (Supplemental Table S2). The ML tree results indicate the clustering of all six species of *Ampelopsis* (Figure 3). Within the genus *Ampelopsis*, *A. delavayana* and *A. glandulosa* var. *brevipedunculata* are positioned on a single branch, implying a closer relationship between them.

Discussion and conclusions

The cp genome structure and GC content of *A. delavayana* closely align with those of other *Ampelopsis* species (Raman and Park 2016; Luo 2023). Notably, the LSC and IR regions of *A. delavayana* expanded, while the SSC region contracted compared to those in *Vitis* species (Guo 2021; Zhang et al. 2022). This variation may contribute to the differences observed in genome lengths. The SSRs in cp genomes exhibit high polymorphism and genetic stability, establishing them as valuable markers for genetic studies to elucidate evolutionary relationships among species (Cato and Richardson 1996). These findings are crucial for species identification and the development of new cultivars.

The phylogenetic results indicate a closer relationship between the genera *Ampelopsis* and *Nekemias*, suggesting that they may be sister genera. *Ampelopsis cordata* and *Nekemias arborea* are the first branches of their respective genera, indicating that they may have an older evolutionary history compared to other species in their genera. These

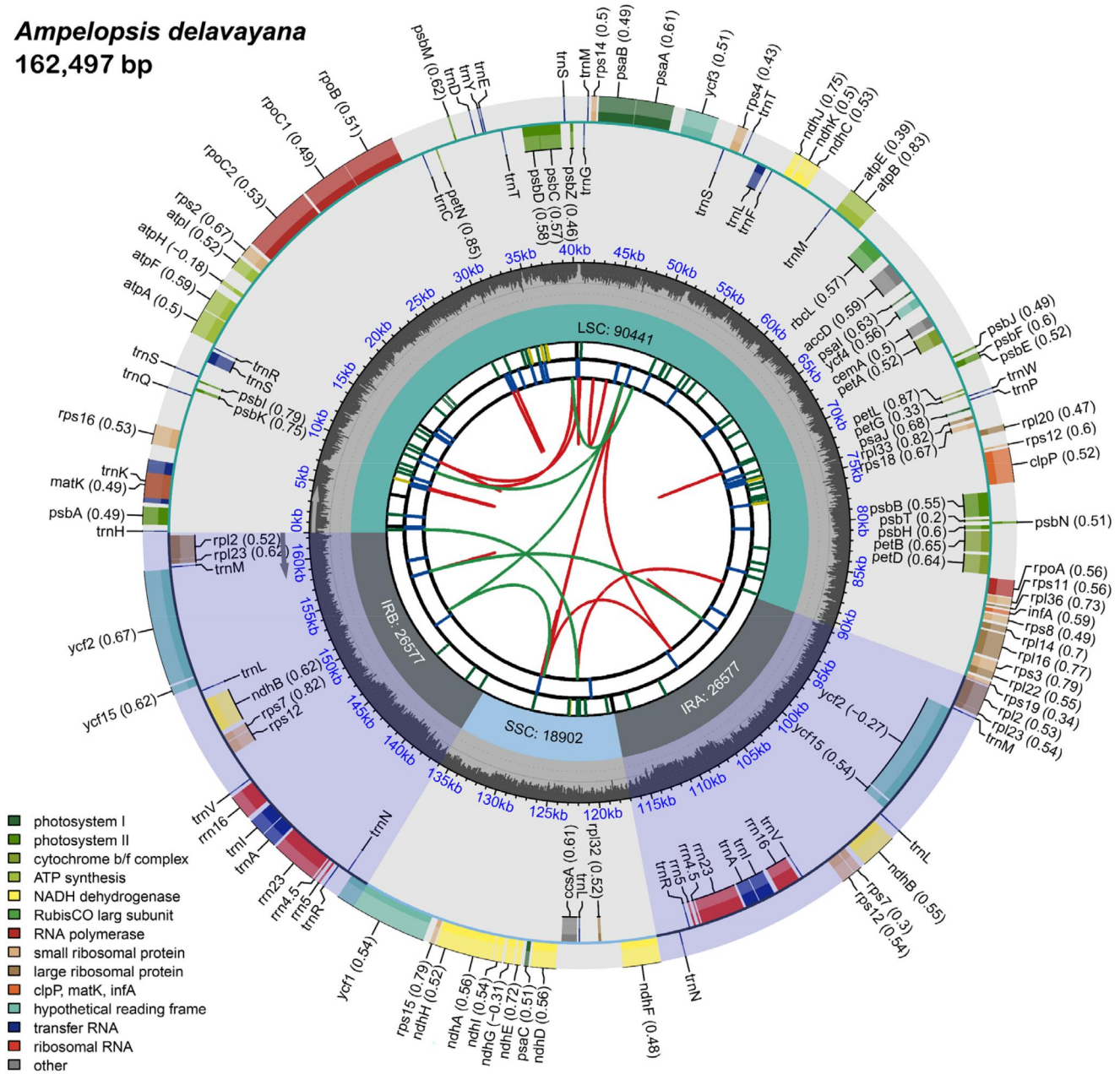

Figure 2. Genetic map of the cp genome of *A. delavayana*. The map is depicted in six concentric circles, progressing from the outermost to the innermost: gene distribution; GC content at each locus; length and distribution of IRA, IRB, LSC, and SSC; short tandem repeat sequences depicted with various colored bands; long tandem repeat sequences represented by blue bands; and forward and reverse repeat sequences connected by red and green arcs, respectively. Genes in the inner circle are transcribed in a clockwise direction, while those in the outer circle are transcribed counterclockwise.

findings align with previous research (Luo 2023; Zhang et al. 2024). This study partially reveals the evolutionary relationships between *A. delavayana* and its closely related species. However, due to limitations in the available data, the phylogenetic relationships within the *Ampelopsis* genus could not be fully explored. Future research should focus on a more comprehensive integration of the cp genome of *Ampelopsis* genus to fully elucidate phylogenetic system.

In summary, this report offers a detailed analysis of the cp genome of *A. delavayana* and clarifies its phylogenetic position, providing essential data for future phylogenetic and genetic studies.

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Author contributions

In this research, Qun Hu designed and executed the experiments, completed the data analysis, and drafted the initial version of the paper. Yongjian Luo and Qing Li contributed to experimental design and analysis of experimental results. Zhijun Deng and Jun Liu supervised the experimental design, data analysis, and contributed to writing and

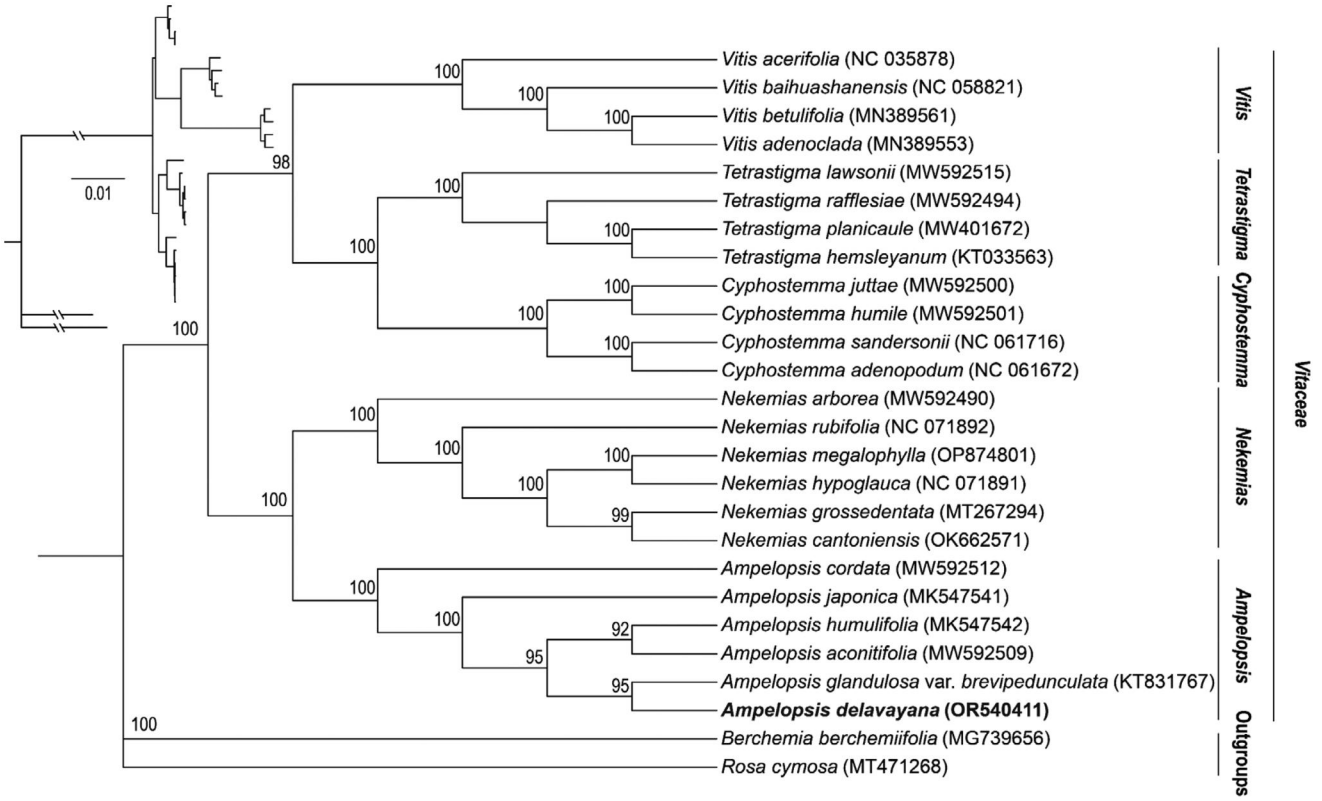

Figure 3. The phylogenetic tree was constructed using *Ampelopsis delavayana* and 23 other species from family Vitaceae based on tandem sequences of 68 shared coding genes from their cp genomes. The best-fit model was GTR + F + I + G4. *Rosa cymosa* (MT471268) and *Berchemia berchemiifolia* (MG739656) were employed as outgroups (Cheon et al. 2018; Ding et al. 2020). Branch lengths corresponded to the self-spreading values. *Ampelopsis delavayana* was highlighted in bold font. The following sequences were used: *Ampelopsis aconitifolia* (MW592509) (Luo 2023), *Ampelopsis cordata* (MW592512) (Luo 2023), *Ampelopsis glandulosa* var. *brevipedunculata* (KT831767) (Raman and Park 2016), *Ampelopsis humulifolia* (MK547542) (Yu et al. 2019), *Ampelopsis japonica* (MK547541) (Yu et al. 2019), *Nekemias arborea* (MW592490) (Luo 2023), *Nekemias cantoniensis* (OK662571) (Luo et al. 2022), *Nekemias grossedentata* (MT267294) (Luo et al. 2022), *Nekemias hypoglauca* (NC 071891), *Nekemias megalophylla* (OP874801), *Nekemias rubifolia* (NC 071892), *Vitis baihuashanensis* (NC 058821), *Vitis acerifolia* (NC 035878) (Luo 2023), *Vitis betulifolia* (MN389561) (Xu and Xu 2021), *Vitis adenoclada* (MN389553) (Xu and Xu 2021), *Cyphostemma juttae* (MW592500), *Cyphostemma humile* (MW592501), *Cyphostemma adenopodum* (NC 061672), *Cyphostemma sandersonii* (NC 061716), *Tetrastigma lawsonii* (MW592515), *Tetrastigma hemsleyanum* (KT033563) (Li et al. 2016), *Tetrastigma planicaule* (MW401672) (Huang et al. 2021), and *Tetrastigma rafflesiae* (MW592494) (Luo 2023).

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Disclosure statement

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

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Data availability statement

To support the findings of this study, the genome sequence data are openly available in GenBank of the NCBI at https://www.ncbi.nlm.nih.gov/ under accession no. OR540411. Specifically, the associated BioProject, SRA, and SubmissionID are PRJNA1018087, SRR26076561, and SAMN37419944, respectively.

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