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Original Research

Complete Chloroplast Genome of *Rauvolfia tetraphylla* (Gentianales: Apocynaceae) and Phylogenetic Analysis

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Abstract

Rauvolfia tetraphylla L. (1753) is an important medicinal species of Apocynaceae, widely used for its rich indole alkaloids and related bioactive compounds. We report the first complete chloroplast genome of *R. tetraphylla*. The genome exhibits a typical circular quadripartite structure of 155,667 bp, with an overall GC content of 37.8%. The genome comprises a large single-copy (LSC) region of 86,332 bp, a small single-copy (SSC) region of 17,853 bp, and a pair of inverted repeat (IR) regions of 25,741 bp each. A total of 130 unique genes are identified, including 85 protein-coding genes, 37 tRNA genes, and eight rRNA genes. Phylogenetic analysis strongly supports the close relationships among *Rauvolfia* species and indicates that *R. tetraphylla* represents an early-diverging lineage within the genus. Overall, this study enhances our understanding of the chloroplast genome characteristics and evolutionary history of *R. tetraphylla* and related taxa.

Keywords
Devil's pepper plant; medicinal shrub; monophyly lineage; plastome-scale phylogeny; *Rauvolfia tetraphylla*

1. Introduction

Rauvolfia tetraphylla is an evergreen shrub of the Apocynaceae family and is widely recognized for its medicinal importance. Native to Mexico, Central America, and the West Indies, it has been introduced across tropical Asia, including India and Southeast Asia, as an ornamental and medicinal plant [1]. Traditional healers have used *R. tetraphylla* (commonly called "devil-pepper") to treat hypertension, snakebite, skin infections, fever, and other ailments [1]. Phytochemical studies have revealed that this species is rich in monoterpenoid indole alkaloids, a class of bioactive compounds characteristic of *Rauvolfia*. Notably, *R. tetraphylla* produces numerous pharmacologically active indole alkaloids, such as ajmaline, reserpine, yohimbine, reserpiline, and related analogs [2]. These compounds contribute to the plant's antihypertensive, sedative, and other therapeutic properties, highlighting the significance of *R. tetraphylla* as a source of important natural products within the Apocynaceae.

Complete chloroplast (cp) genome sequences provide valuable data for plant genetics and systematics [3]. Comparative plastome analyses have been widely used to infer phylogenetic relationships and evolutionary history at multiple taxonomic levels [3]. The abundance of homologous genes and noncoding regions in plastomes provides a "super-barcode" for species identification and a robust framework for resolving plant phylogeny [3]. Despite the medicinal importance of *R. tetraphylla,* no complete cp genome of this species had been reported before this study. A lack of plastome data has hindered detailed comparisons with other Apocynaceae and limited phylogenetic insights into the plant group. In this study, we addressed this gap by sequencing and assembling the complete cp genome of *R. tetraphylla* and conducting a comparative analysis of its genome structure and gene content to better resolve its phylogenetic placement within Apocynaceae.

2. Materials and Methods

Fresh *R. tetraphylla* leaves were collected from Tay Ninh Province (11°25'42.1" N 106°13'39.4" E), with morphological characteristics shown in Figure 1. The specimen was deposited at the University with the voucher number UMP_2024_08-01 (contact: Minh Trong Quang, qtminh@ump.edu.vn). DNA was extracted according to the modified CTAB protocol [4]. Genome sequences were constructed using a MiSeq sequencer with a paired-end read length of 150 bp. The complete cp genome of *R. tetraphylla* was assembled *de novo* using NOVOPlasty v4.3.5 [5]. The GESEQ tool [6] was used to annotate the cp genome, which was implemented in the CHLOROBOX web toolbox (https://chlorobox.mpimp-golm.mpg.de/geseq.html). We corrected the annotation by aligning it with the cp genome homologs of *Rauvolfia verticillata* (GenBank: NC_046841) and *Rauvolfia serpentina* (GenBank: NC_047244). Annotation of transfer RNA (tRNA) genes was curated using tRNAScan-SE v2.0 [7]. Protein-coding genes (PCGs) were manually checked using BLAST

(https://blast.ncbi.nlm.nih.gov/Blast.cgi) and by alignment with homologous genes to refine start codons and exon-intron boundaries [8].

Figure 1 Morphological features of *Rauvolfia tetraphylla.* (A) A branch with opposite leaves and fruit clusters growing from the leaf axils, showing the overall appearance of the plant. The leaves are simple, smooth, and elliptic to oblong, with entire margins and a clearly visible midrib and secondary veins. (B) Close-up of the fruits (drupes) during ripening, showing the color change from green to orange-red and then to bright red at maturity. The fruits are borne on short stalks and usually occur in small clusters. (C) An axillary inflorescence with several flower buds and one open flower. The flower is small and white, with a tubular corolla and spreading lobes. (Photograph was taken using MTQ).

A total of 20 published cp genomes were retrieved from GenBank for phylogenetic reconstruction, with *Gentiana arethusae* (GenBank accession: NC_058687; Gentianaceae) designated as the outgroup. All cp PCGs were extracted and aligned using MAFFT v7.487 [9], and poorly aligned regions and gaps were removed with TrimAl. The best-fit nucleotide substitution model was selected using jModelTest v2.1.10 [10], with GTR+I+G identified as the optimal model. To infer the phylogenetic position of *R. tetraphylla*, both maximum likelihood (ML) and Bayesian inference (BI) analyses were performed. The ML tree was reconstructed in IQ-TREE [11] based on the concatenated PCG dataset, with branch support assessed using 1,000 ultrafast bootstrap replicates. BI analysis was conducted in MrBayes v3.2.7 [12] using a Markov chain Monte Carlo (MCMC) approach with 1,000,000 generations, sampling every 1,000 generations, and discarding the first 25% of trees as burn-in. The run was considered to have converged when the average standard deviation of split frequencies was below 0.01. The resulting phylogenetic trees were visualized using iTOL v7 [13].

2.1 Ethics Statement

This study did not involve human participants or animals. Plant material of *R. tetraphylla* was collected in accordance with local regulations and institutional guidelines. The species is not listed as endangered or protected, and no specific permits were required for sample collection at the study site. Voucher specimens were deposited in an institutional collection to ensure traceability and reproducibility.

3. Results

3.1 Chloroplast Genome Assembly and General Features

The assembly produced a full-length cp genome of *R. tetraphylla*, 155,667 bp, with an average coverage depth of 1,104.6× (Figure S1). The genome exhibited the typical quadripartite structure (Figure 2), comprising a pair of inverted repeat (IR) regions (IRs; 25,741 bp each) separated by a large single-copy (LSC) region of 86,332 bp and a small single-copy (SSC) region of 17,853 bp. The overall GC content was 37.8%, with values of 35.8%, 32.2%, and 43.3% in the LSC, SSC, and IR regions, respectively.

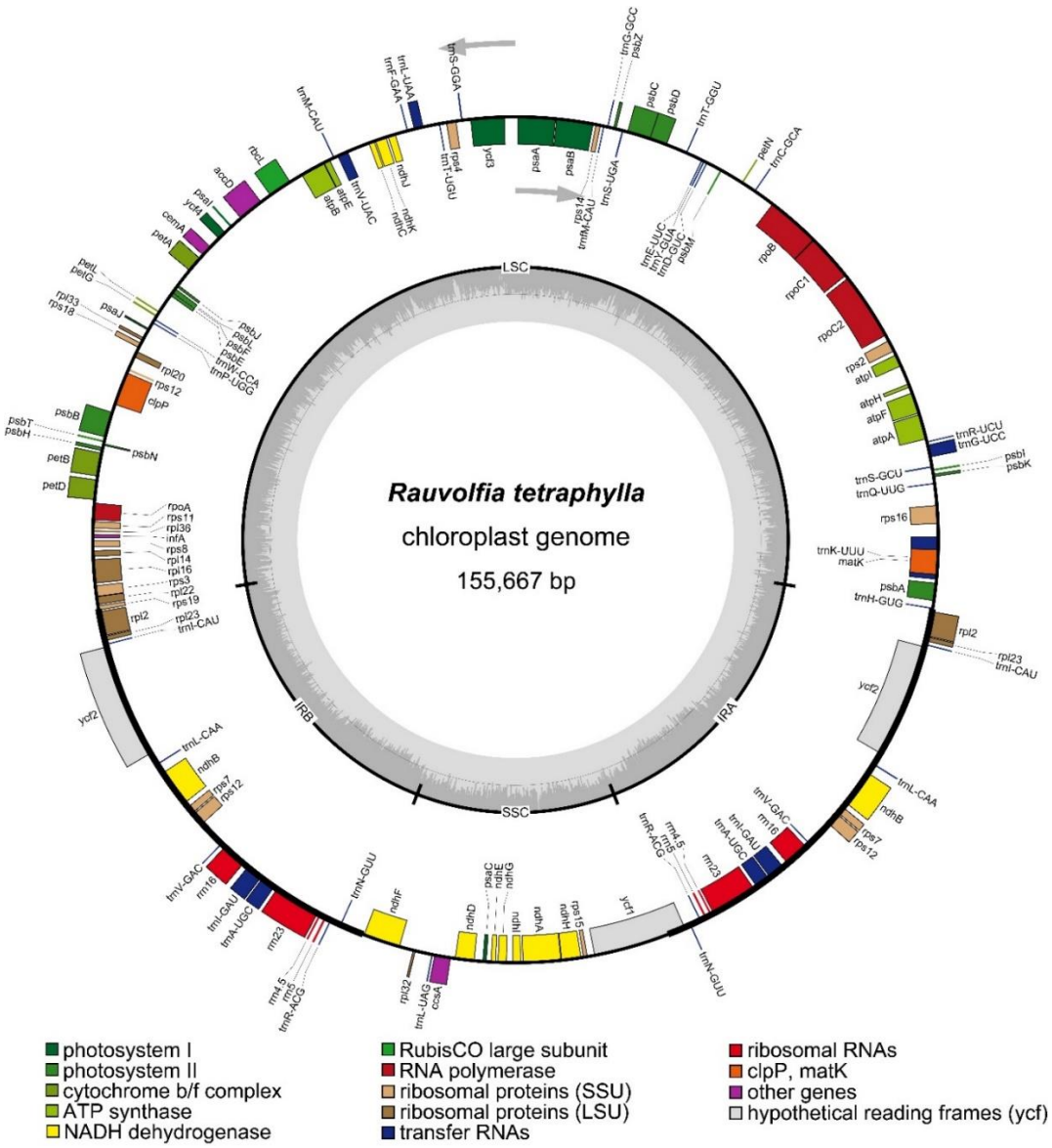

Figure 2 Graphic map of the chloroplast genome of *Rauvolfia tetraphylla* generated by OGDraw. The map contains two main parts. The inner track shows the GC (dark gray) and AT (light gray) contents. The inner circle includes the small single-copy (SSC), inverted repeat regions (IRa and IRb), and large single-copy (LSC) regions. The outer circle shows the location and transcript direction of genes (arrows). Genes are color-coded by function, with the main group shown below the genome map.

3.2 Gene Content and Genome Organization

A total of 130 genes were annotated, including 85 PCGs, eight rRNAs, and 37 tRNAs. Among the PCGs, nine genes (*rps*16, *atp*F, *rpo*C1, *pet*B, *pet*D, r*pl*16, *rpl*2, *ndh*B, and *ndh*A) contained a single intron, whereas two genes (*paf*I and *clp*P1) contained two introns (Figure 3).

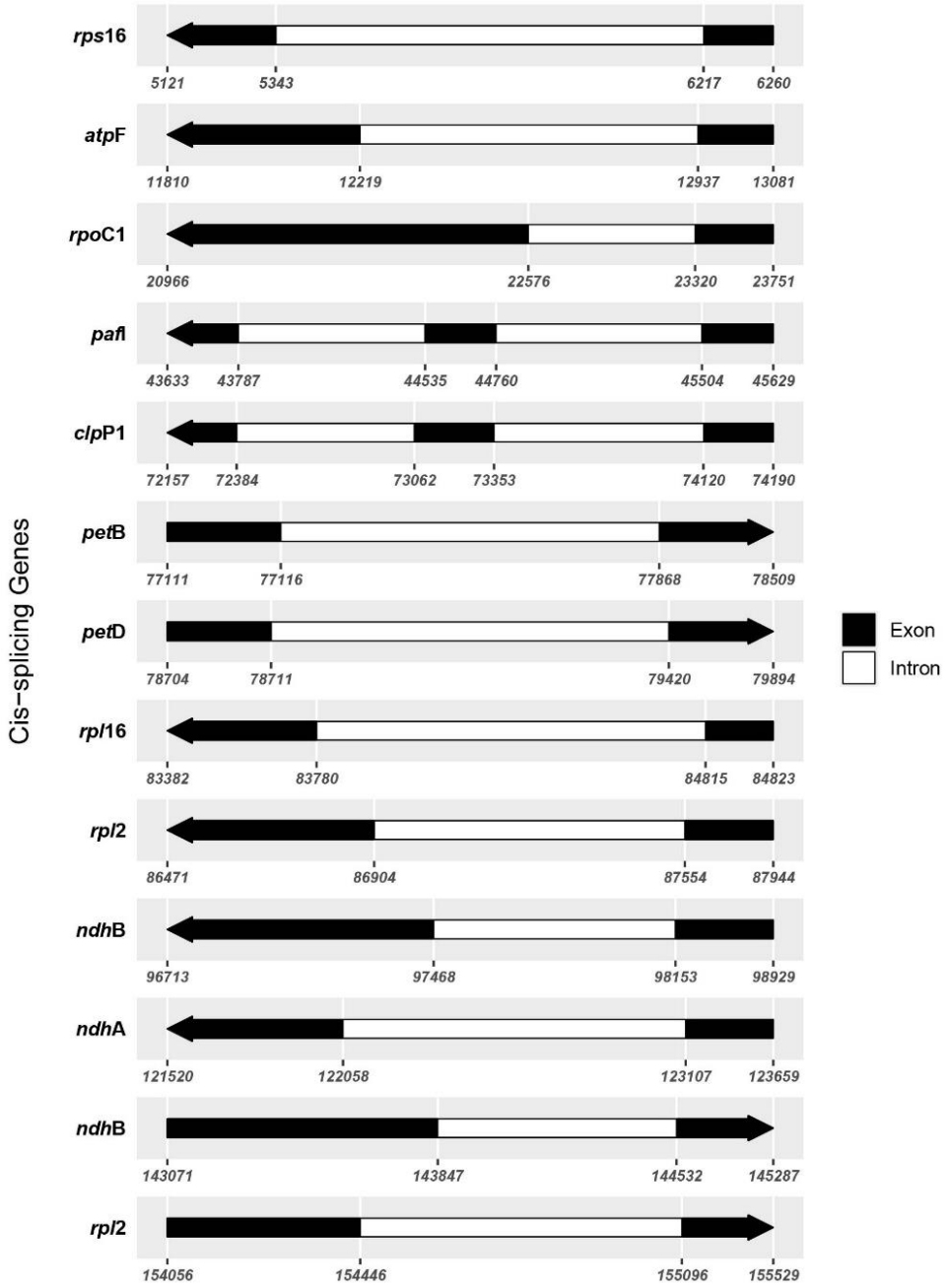

Figure 3 Exon-intron structures of cis-splicing genes in the chloroplast genome of *Rauvolfia tetraphylla*. Black boxes indicate exons and white boxes indicate introns. Arrows show transcriptional orientation, and the numbers beneath each gene represent nucleotide positions in the chloroplast genome. Genes containing a single intron are shown with two exons, whereas *paf*I and *clp*P1 contain two introns and are represented by three exons. The *ndh*B and *rpl*2 genes are shown twice, corresponding to their presence in the inverted repeat regions.

Most genes were located in single-copy regions (LSC and SSC) and therefore occur as single copies, whereas 16 genes are duplicated within the IR regions. These duplicated genes comprised four rRNAs (*rrn*16*, rrn*23*, rrn*4.5*,* and *rrn*5), seven tRNAs (*trn*A-UGC*, trn*I-CAU*, trn*I-GAU*, trn*L-CAA*, trn*N-GUU*, trn*R-ACG*,* and *trn*V-GAC), and six PCGs (*ndh*B*, rpl*2*, rpl*23*, rps*7*, rps*12*,* and *ycf*2). The trans-splicing gene *rps*12 was also identified (Figure 4).

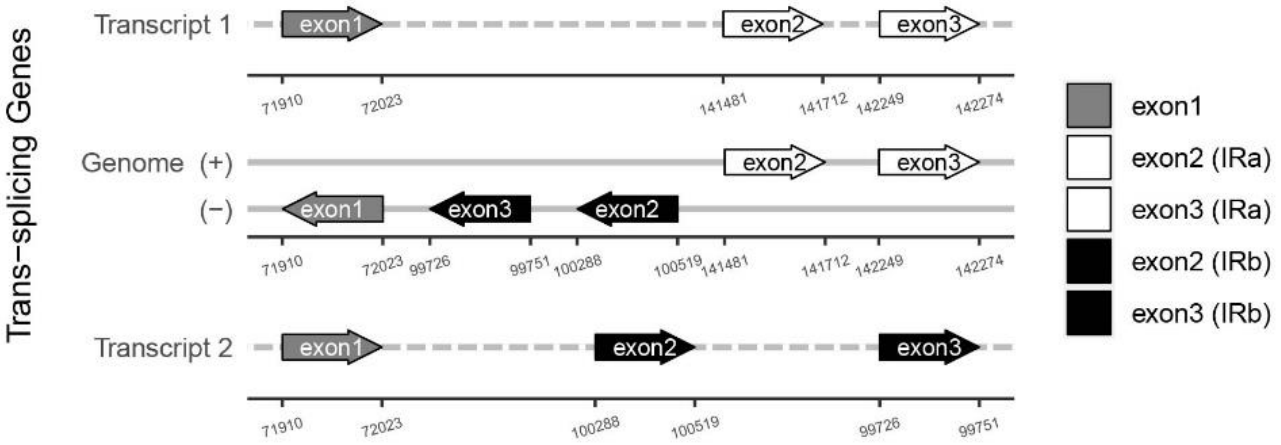

Figure 4 Trans-splicing structure of the *rps*12 gene in the chloroplast genome of *Rauvolfia tetraphylla*. The *rps*12 gene is split into three exons, with exon 1 located in the LSC region and exons 2 and 3 duplicated within the inverted repeat regions (IRa and IRb). Gray, white, and black boxes indicate exons and their locations (exon 1; exons 2 and 3 in IRa; exons 2 and 3 in IRb), as shown in the legend. Arrows denote transcriptional orientation on the plus (+) and minus (-) strands of the chloroplast genome. Dashed lines represent splicing connections, illustrating that exon 1 is trans-spliced with exon 2 and exon 3 to form two mature transcripts (Transcript 1 and Transcript 2). Numbers below the diagrams indicate nucleotide positions in the chloroplast genome.

3.3 Phylogenetic Analysis Based on Chloroplast Genomes

The phylogenetic relationship of *R. tetraphylla* was inferred from 79 concatenated PCGs from the cp genomes of representative Rauvolfioideae species. The resulting tree placed *R. tetraphylla* in a well-supported clade with *R. verticillata* and *R. serpentina* (bootstrap = 100), supporting the monophyly of *Rauvolfia* (Figure 5). Furthermore, *R. tetraphylla* was inferred to be the earliest diverging lineage within the genus. These results provided a robust framework for future investigations of the evolutionary relationships of *R. tetraphylla* within the Rauvolfioideae family.

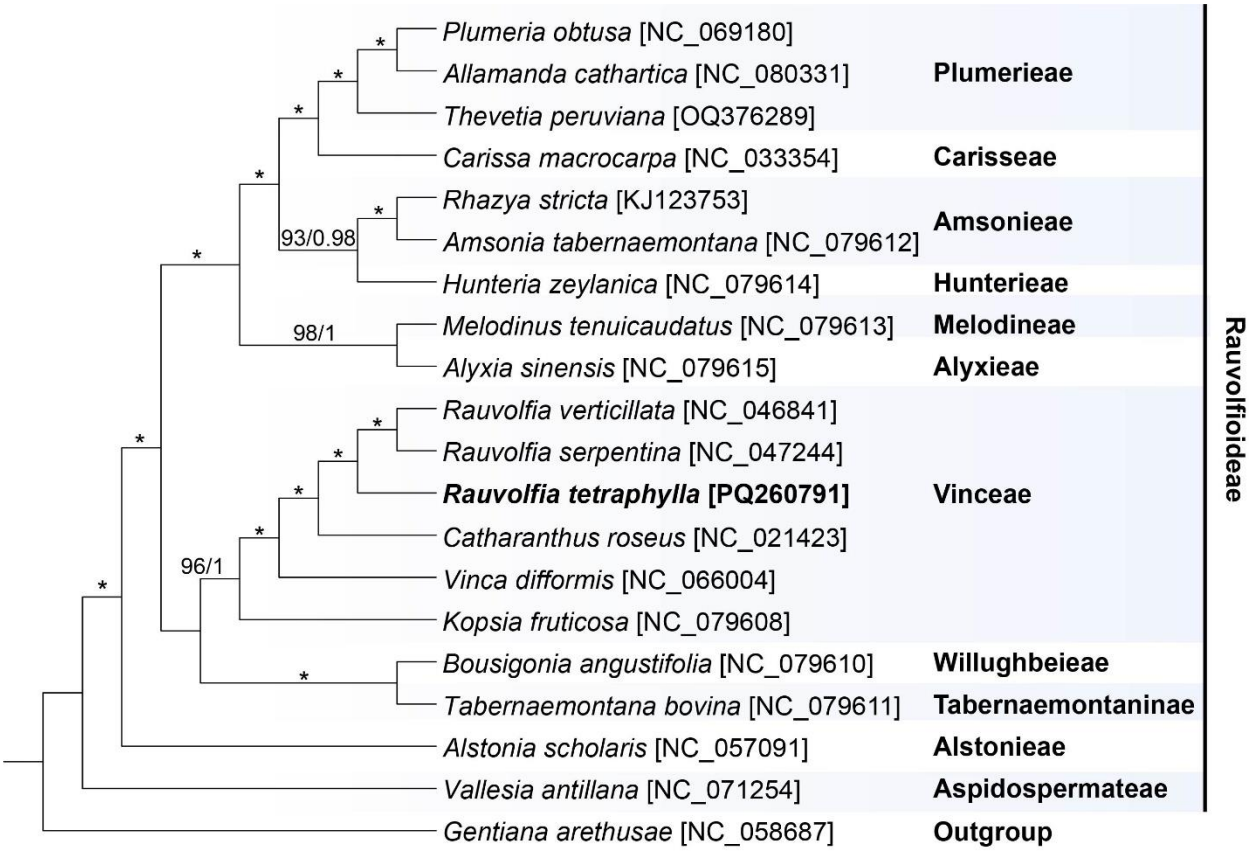

Figure 5 Phylogenetic cladogram of Rauvolfioideae inferred from chloroplast protein-coding genes. Support values at each node are presented as bootstrap support (BS) followed by Bayesian posterior probability (PP). An asterisk (*) indicates maximal support (BS = 100, PP = 1). Each taxon is labeled with its scientific name and corresponding GenBank accession number. Tribe names are shown on the right side of the tree. The species are used in phylogenetic analysis, including: *Gentiana arethusae* (NC_058687, outgroup, [14]), *Vallesia antillana* (NC_071254, [15]), *Alstonia scholaris* (NC_057091, [16]), *Tabernaemontana bovina* (NC_079611, unpublished), *Bousigonia angustifolia* (NC_079610, unpublished), *Kopsia fruticosa* (NC_079608, unpublished), *Vinca difformis* (NC_066004), *Catharanthus roseus* (NC_021423, [17]), *Rauvolfia tetraphylla* (PQ260791, this study), *Rauvolfia serpentina* (NC_047244, unpublished), *Rauvolfia verticillata* (NC_046841, [18]), *Alyxia sinensis* (NC_079615), *Melodinus tenuicaudatus* (NC_079613, unpublished), *Hunteria zeylanica* (NC_079614, unpublished), *Amsonia tabernaemontana* (NC_079612, unpublished), *Rhazya stricta* (KJ123753, [19]), *Thevetia peruviana* (OQ376289, [20]), *Allamanda cathartica* (NC_080331, unpublished), *Plumeria obtusa* (NC_069180, [21]), and *Carissa macrocarpa* (NC_033354, [22]).

4. Discussion and Conclusions

The family Apocynaceae is a large and diverse angiosperm group, comprising approximately 5,000 species in 378 genera [23]. Within this family, *Rauvolfia* is a pantropical genus of shrubs and small trees widely recognized for its medicinal importance. Species of *Rauvolfia* produce a broad range of bioactive monoterpene indole alkaloids, including reserpine from *R. serpentina*, a

compound with well-documented antihypertensive activity [24]. Despite this pharmacological value, genomic resources for the genus have remained limited, with only three complete cp genomes publicly available as of March 2026, including that of *R. tetraphylla*. Notably, the first reported cp genome of the genus, from *R. verticillata* in 2019, indicated a close phylogenetic relationship between *Rauvolfia* and *Catharanthus* [18]. In this study, the cp genome of *R. tetraphylla* represents an important addition to the genomic resources of the genus. It provides a valuable foundation for future comparative genomic and evolutionary studies.

In this study, the cp genome of *Rauvolfia tetraphylla* was 155,667 bp in length, with an overall GC content of 37.8%, and contained 85 PCGs, 37 tRNA genes, and 8 rRNA genes (Table 1). Comparative whole-plastome analysis using MAUVE showed that the cp genome of *R. tetraphylla* was highly conserved, particularly in comparison with the two congeneric species, *R. serpentina* and *R. verticillata* (Figure S2). Among these plastomes, sequence similarity was high, and gene order was largely collinear, indicating strong structural stability within *Rauvolfia*. Nevertheless, *R. tetraphylla* exhibited an intermediate plastome size, being larger than *R. serpentina* (155,102 bp) but smaller than *R. verticillata* (155,856 bp). Its GC content was also very similar to that of the other two species (37.9-38.0%). In terms of gene content, the plastome of *R. tetraphylla* was identical to that of *R. verticillata* but contained two more genes than that of *R. serpentina*. In detail, we found that *rps*19 and *trn*H are absent from the cp genome of *R. serpentina* (Figure S3 and Figure S4).

Table 1 Chloroplast genome features of three *Rauvolfia* species.

Species	***Rauvolfia serpentina***	***Rauvolfia tetraphylla***	***Rauvolfia verticillata***
Accession no.	NC_047244	PQ26079	NC_046841
Total length	155,102	155,667	155,856
%GC	38.0	37.8	37.9
LSC	85,367	86,333	86,084
IR	25,742	25,740	25,736
SSC	18,251	17,854	18,300
Total genes	128	130	130
(PCGs/tRNAs/rRNAs)	(84, 36, 8)	(85, 37, 8)	(85, 37, 8)

Comparison of the IR boundary structures among the three *Rauvolfia* plastomes showed that *R. tetraphylla* most closely resembled *R. verticillata*, particularly in the organization of the IR/SC junctions, with only minor positional differences in *rps*19, *ndh*F, *ycf*1, and *trn*H near the boundary regions (Figure S5). In contrast, the absence of *rps*19 and *trn*H in *R. serpentina* appeared to have contributed to shifts in the JLB and JLA boundaries. Given that the lengths of the LSC, SSC, and IR regions were highly similar across all three species, these boundary differences were more plausibly explained by gene loss than by IR expansion or contraction.

Given the limited availability of cp genome data for *Rauvolfia* in public databases, the present comparison provided only a preliminary view of plastome variation within the genus. Our results showed that the plastome of *R. tetraphylla* was highly similar to that of *R. verticillata* in overall structure, gene content, and gene order. In contrast, *R. serpentina* displayed more pronounced differences, particularly the apparent absence of *rps*19 and *trn*H, which may have indicated a distinct plastome configuration within the genus. However, it remained unclear whether this

pattern reflected a lineage-specific evolutionary feature or individual-level variation in the sampled accession. Broader taxon sampling and more extensive intraspecific sampling would therefore be necessary to clarify the extent and evolutionary significance of cp genome variation in *Rauvolfia*. Such data would provide a more robust basis for understanding plastome evolution and structural diversity within the genus.

The phylogenetic relationships among the major lineages of Apocynaceae have been broadly resolved. In 2018, Fishbein et al. used plastid data from more than 1,000 species across the family to clarify relationships within Apocynaceae. However, that study focused primarily on higher taxonomic levels, such as tribes and subfamilies, and did not include any *Rauvolfia* representatives [25]. At the genus level, Simões et al. (2007) used a combination of molecular markers and morphological evidence to establish an initial phylogenetic framework for *Rauvolfia*, although only two species were sampled [26]. Consistent with that earlier work, our plastome-based phylogeny recovered *Rauvolfia* as a monophyletic lineage with strong bootstrap support (BS = 100, PP = 1), further supporting the genus's monophyly [26]. However, a previous study of tribe Vinceae has also suggested that many infrageneric classifications within *Rauvolfia* were not monophyletic; specifically, most traditional sections, series, and subseries were recovered as paraphyletic, whereas only a few historical groupings were supported as monophyletic [27]. Therefore, these findings highlight the need for broader taxon sampling (only three out of 87 recognized species in this study), which would not only improve our understanding of evolutionary relationships in *Rauvolfia* but also provide a stronger basis for reassessing its infrageneric classification [28, 29].

The present study improves our understanding of the cp genome features and phylogenetic placement of *R. tetraphylla*. A broader sampling of cp genomes across *Rauvolfia* will be important for gaining deeper insight into plastome evolution within the genus, refining infrageneric relationships, and providing a stronger foundation for future studies on the evolution, utilization, and conservation of this medicinally important group.

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Author Contributions

T-TTH: Conceptualization, methodology, investigation, formal analysis, writing - original draft, writing - review and editing. TNN: Conceptualization, methodology, writing - review and editing, correspondence. A-DHN: Software, data curation, writing, review, and editing. MTQ: Conceptualization, resources, supervision, writing, review and editing, correspondence. All authors have read and approved the final version of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

Data Availability Statement

The sequence of the complete chloroplast genome of *Rauvolfia tetraphylla* is available in GenBank (https://www.ncbi.nlm.nih.gov/) under accession number PQ260791.

AI-Assisted Technologies Statement

Artificial intelligence (AI) tools were used solely for language editing and grammar correction during the preparation of this manuscript. Specifically, TrinkaAI (https://www.trinka.ai/) was used to improve the clarity and readability of the English text. All scientific content, data analysis, interpretation, and conclusions were developed entirely by the authors. The authors reviewed and edited all AI-assisted content and took full responsibility for the accuracy and integrity of the manuscript.

Additional Materials

The following additional materials have been uploaded to the page of this paper.

1. Figure S1: Sequencing depth across the complete chloroplast genome of *Rauvolfia tetraphylla*. The x-axis represents the genomic regions, including the large single-copy (LSC), inverted repeat B (IRb), small single-copy (SSC), and inverted repeat A (IRa) regions, while the y-axis indicates sequencing depth (×). Coverage was generally high across the genome, with an average depth of 1,104.6×, a maximum depth of 3,504×, and a minimum depth of 295×.
2. Figure S2: Whole-plastome sequence similarity comparison of *Rauvolfia tetraphylla* with related Apocynaceae species. The upper red plot on each track represents nucleotide sequence identity across the chloroplast genomes (genomic coordinates indicated along the top). The lower gene map summarizes annotated features along the reference plastome, with coding sequences (white), tRNA genes (green), and rRNA genes (red).
3. Figure S3: Comparison of the *rps*3-*rpl*2 region among representative Apocynaceae chloroplast genomes. Gene organization in *rps*3-*rpl*2 is shown for *Rauvolfia tetraphylla*, *R. serpentina*, and *R. verticillata*. The red-boxed area highlights the *rps19* variable genes, illustrating differences in presence/absence and local structure across genomes. Grey/white blocks on the reference line represent aligned sequence segments and interruptions (gaps/indels) across taxa within this region.
4. Figure S4: Structural comparison of the *trn*H-*psb*A-*trn*K region among three *Rauvolfia* species. The gene arrangement of the chloroplast region containing the *trn*H-GUG, *psbA*, and *trn*K-UUU segments is compared across *Rauvolfia tetraphylla*, *R. serpentina*, and *R. verticillata*. The red boxed area highlights the variable *trn*H-GUG genes, illustrating differences in presence/absence and local structure among genomes. Grey/white blocks on the reference line represent aligned sequence segments and interruptions (gaps/indels) across taxa within this region.
5. Figure S5: Comparison of inverted repeat boundaries in the chloroplast of *Rauvolfia tetraphylla* and related taxa. Schematic maps show the positions of the four junctions between plastome regions-LSC/IRb (JLB), IRb/SSC (JSB), SSC/IRa (JSA), and IRa/LSC (JLA). Colored blocks represent the large single-copy (LSC), small single-copy (SSC), and the two

inverted repeats (IRb and IRa). Genes located at or near each junction are indicated, and the numbers denote the distances (bp) from genes to the corresponding borders or the lengths of gene fragments spanning junctions.

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