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The complete chloroplast genome of *Lepidagathis incurva* and its phylogenetic relationship

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ABSTRACT: *Lepidagathis incurva* (Acanthaceae) is a tropical and subtropical plant species of ecological significance, yet its genomic resources remain limited, hindering insights into its evolutionary relationships. This study presents the complete chloroplast genome of *L. incurva* to clarify its phylogenetic placement and genomic structure, thereby contributing to the genomic resources of *Lepidagathis*. The assembled genome spans 150,481 bp and exhibits a typical quadripartite structure comprising a large single-copy region (82,200 bp), a small single-copy region (17,347 bp), and two inverted repeat regions (25,467 bp each). A total of 131 genes were identified, including 86 protein-coding genes, 37 transfer RNA genes, and 8 ribosomal RNA genes. Phylogenetic analysis identified *L. incurva* within the subfamily Acanthoideae, forming a well-supported clade with *Barleria prionitis* and *Andrographis paniculata* (bootstrap value = 100%). These findings enrich chloroplast genomic resources for Acanthaceae and provide a foundation for future research on the evolutionary biology and genetic diversity of *Lepidagathis* species.

KEYWORDS: chloroplast genome, phylogenetics, Acanthaceae, evolutionary biology, genomic analysis

INTRODUCTION

Chloroplast (cp) genomes are essential for understanding plant systematics, evolutionary relationships, and genetic diversity [1]. As sites of photosynthesis and other critical metabolic pathways, cp house genomes offer valuable insights into the phylogenetic history and adaptive evolution of plant lineages [2]. Recent advancements in sequencing technologies have enabled the assembly of complete cp genomes, allowing researchers to resolve taxonomic ambiguities and infer phylogenetic relationships with unprecedented precision [3, 4]. These analyses are particularly valuable for identifying taxa within morphologically diverse and ecologically significant plant families [5, 6].

The genus *Lepidagathis* Willd. (Acanthaceae) comprises approximately 150 species distributed across tropical and subtropical regions, demonstrating remarkable ecological adaptability across diverse habitats, from arid landscapes to moist forests [7]. Characterized by diverse floral morphologies and specialized pollination strategies, the genus holds significant ecological and medicinal value [8, 9]. Ethnobotanical studies have documented the medicinal use of *Lepidagathis incurva* for treating blindness and ear infections and as a hemostatic agent [10]. Despite its ecological and medicinal importance, *Lepidagathis* remains largely unexplored at the genomic level, with no complete cp genome reported for the genus to date. Additionally, phylogenetic studies on *Lepidagathis* are scarce, with most classifications relying on morphological characteristics rather than molecular data, complicating taxonomic placement and evolutionary assessments [7, 11].

This study presents the first complete cp genome of *L. incurva*, thereby addressing this knowledge gap. Specifically, we report its complete cp genome sequence and conduct phylogenetic analyses within Acanthaceae. The primary objectives of this study are to (i) characterize the structural features and gene content of the *L. incurva* cp genome and (ii) infer its phylogenetic relationships within *Lepidagathis* and closely related taxa. By integrating genomic and phylogenetic analyses, this study provides a deeper understanding of cp genome evolution and establishes a valuable genetic resource for future research on this genus.

MATERIALS AND METHODS

Plant sampling and DNA isolation

Specimens of *L. incurva* were collected in September 2024 from Tay Ninh, Vietnam (11°24′05.3″ N, 106°14′16.3″ E). Voucher specimens were deposited at the Department of Microbiology – Parasitology under the voucher number FGRC-2024.10.01-Lanchuycong (contact person: Nguyen Hoang Anh Duy; email: nhaduy@ump.edu.vn). Samples were dehydrated with silica gel packets and stored at room temperature to preserve DNA integrity. Genomic DNA was extracted using a DNeasy Plant Mini Kit (Cat. 69104, QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

Sequencing, assembly, and annotation of the cp genome

Genomic DNA was sequenced on the MiSeq platform. The cp genome was *de novo* assembled using NOVOPlasty v4.3.5 to generate contigs [12], which were subsequently imported into Geneious Prime v2025.0.2 for complete genome assembly. Genome annotation was performed using the GeSeq tool, and the accuracy of the annotated genes was validated. Transfer RNA (tRNA) genes were confirmed using tRNAscan-SE [13], whereas protein-coding genes (PCGs) were verified by the BLAST tool [14] (available at https://blast.ncbi.nlm.nih.gov/Blast.cgi). The start and stop codons of PCGs were further curated by aligning them with cp genomes of closely related Acanthaceae taxa, including *Barleria prionitis* [15] (accession number: NC_048478) and *Andrographis paniculata* [16] (accession number: NC_022451). The annotated *L. incurva* cp genome has been deposited in GenBank (accession number: PQ576719). The circular cp genome map was visualized using OGDRAW [17].

Phylogenetic analysis

Phylogenetic relationships within the Acanthaceae family were inferred using the cp genome of *L. incurva* alongside sequences from 22 additional species in Acanthaceae and an outgroup species, *Dolichandrone spathacea* (Bignoniaceae; GenBank accession: NC_054307), as summarized in Table S1. The coding sequences were extracted and aligned using MAFFT v7 with the default parameters [18]. Ambiguous regions and gaps were filtered using Gblocks v0.91b [19]. The optimal evolutionary model GTR+I+G was determined using jModelTest v2.1.10 and subsequently applied to construct a maximum likelihood (ML) phylogenetic tree using IQ-TREE with 1,000 bootstrap replicates [20, 21]. Visualization and editing of the resulting phylogenetic tree were performed using FigTree v1.4.4 (available at http://tree.bio.ed.ac.uk/software/figtree).

RESULTS AND DISCUSSION

Cp genome features of *L. incurva*

The complete cp genome of *L. incurva* was assembled using high-quality sequencing data, yielding 6.18 GB of raw reads. A total of 568,517 reads were mapped to the cp genome, achieving a robust sequencing depth with a minimum coverage of 95×, a maximum of 1,359×, and an average coverage of 558.98× (Fig. S1). This high depth ensured the accuracy and completeness of the assembly.

The *L. incurva* cp genome exhibited a typical quadripartite structure, with a total length of 150,481 bp (Fig. 1). The genome comprises a large single-copy (LSC) region (82,200 bp), a small single-copy (SSC) region (17,347 bp), and two inverted repeat regions (IRs) of 25,467 bp each. The overall GC content was 38.0%, with notable regional variations: 36.0% in the LSC, 32.2% in the SSC, and 43.4% in the IR regions.

A total of 131 genes were identified, including 86 PCGs, 37 tRNAs, and 8 ribosomal RNA genes (rRNAs). Among the 10 intron-containing genes, nine contained one intron (*rps16*, *atpF*, *rpoC1*, *petB*, *petD*, *rpl16*, *rpl2*, *ndhB*, and *ndhA*), and *pafI* contained two (Fig. 2). Notably, the *rps12* gene was trans-spliced, with exon 1 located in the LSC region and exons 2 and 3 located in the IR region (Fig. 3).

Of the 17 genes duplicated in the IR regions, six were PCGs (*ndhB*, *rpl2*, *rpl23*, *rps7*, *rps12*, and *ycf2*), seven were tRNAs (*trnA-UGC*, *trnI-CAU*, *trnI-GAU*, *trnL-CAA*, *trnN-GUU*, *trnR-ACG*, and *trnV-GAC*), and four were rRNAs (*rrn5*, *rrn4.5*, *rrn23*, and *rrn16*).

Comparative genomics and structural variation

The structure and gene content of the *L. incurva* cp genome were consistent with those of other Acanthaceae species, including *Dicliptera tinctoria*, *Echinacanthus longipes*, *Echinacanthus lofouensis*, *Echinacanthus longzhouensis*, and *Echinacanthus attenuatus* [22, 23]. The *L. incurva* cp genome lacked two introns in the *clpP* gene, a feature that distinguished it from closely related taxa. Intron loss in *clpP* was also observed in other plant groups, such as *Cuscuta* species (Convolvulaceae), *Hypericum* species (Hypericaceae) [24, 25]. This pattern was often associated with evolutionary adaptation, particularly in response to selective pressures such as environmental stress and genomic streamlining [26]. Although the functional significance of this intron loss in *L. incurva* remained unclear, its presence suggested potential adaptive evolution within *Lepidagathis*.

The GC content of the *L. incurva* cp genome (38.0%) was comparable to values reported for other Acanthaceae species, such as *Justicia flava* (38.2%), *Staurogyne concinnula* (38.04%), *Echinacanthus* species (ranging from 38.26% to 38.74%), and 13 Asian Justicia species (ranging from 38.1% to 38.4%) [22, 27, 28]. A higher GC content in the IR regions (43.4%) was attributed to the presence of rRNAs, a common feature of angiosperm cp genomes that was linked to genome stability and functional constraints [22]. Additionally, the distribution of genes across the LSC, SSC, and IR regions followed a conserved pattern observed in angiosperms, supporting the structural stability of the cp genome [29].

Phylogenetic relationships among *L. incurva*

A phylogenetic tree was constructed using the ML method based on protein-coding sequences from 23 species within Acanthaceae, representing three subfamilies: Acanthoideae, Thunbergioideae, and Avicennioideae. The species *D. spathacea* (Bignoniaceae) was included as an outgroup to provide a comparative

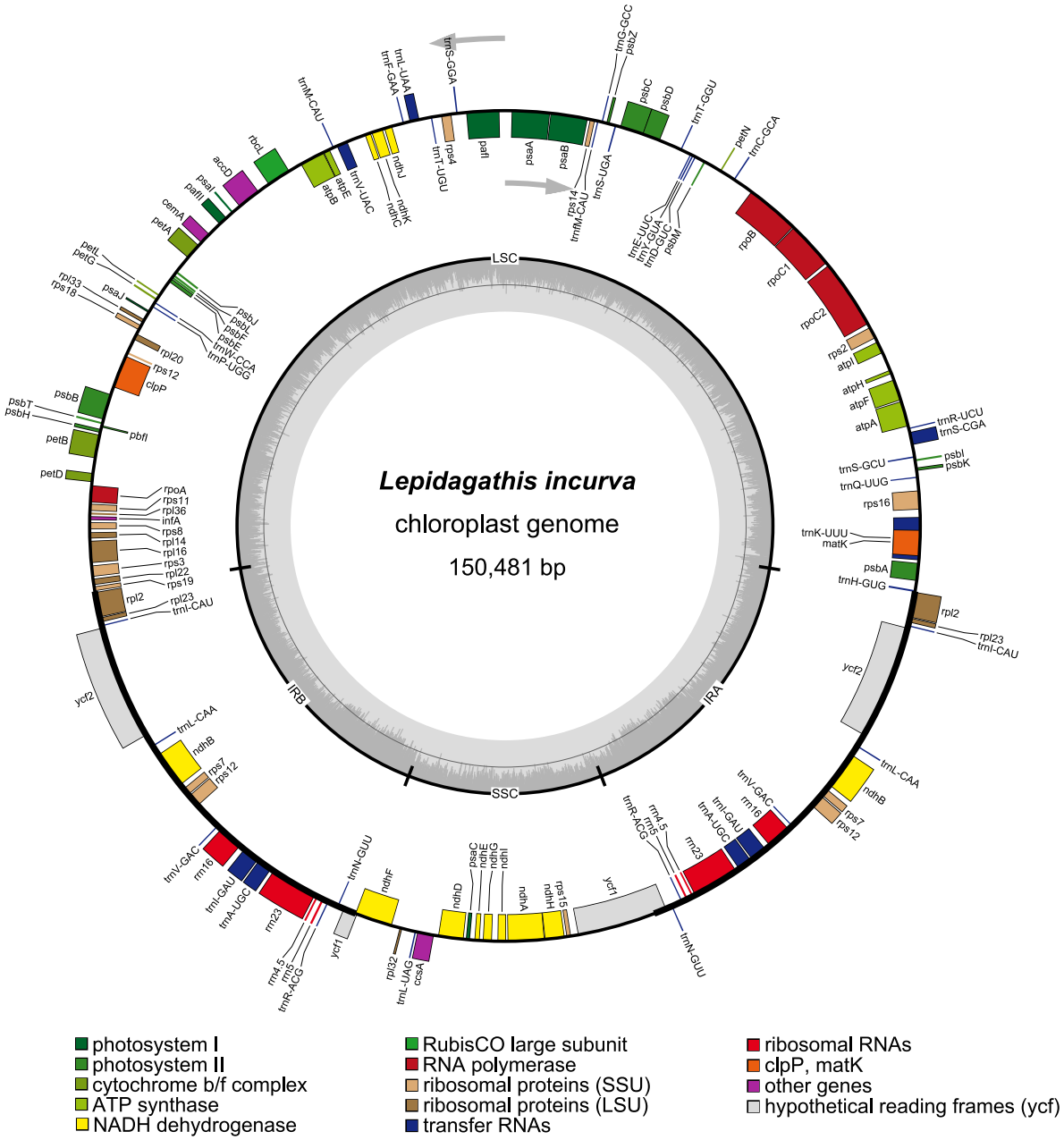

Fig. 1 The cp genome map of *Lepidagathis incurva* illustrates gene locations and orientations. The outer genes are transcribed clockwise, whereas the inner genes are transcribed counterclockwise. Genes are color-coded by functional group, as detailed in the legend. The inner circle represents GC and AT content, with darker gray indicating GC content and lighter gray indicating AT content. Key regions of the genome, including the large single-copy (LSC) region, small single-copy (SSC) region, and inverted repeat regions (IRa and IRb), are highlighted.

reference point for evaluating evolutionary relationships within Acanthaceae. The optimal evolutionary model GTR+I+G, as determined by jModelTest, was applied to ensure accurate tree reconstruction. Phylogenetic analysis placed *L. incurva* within the subfamily Acanthoideae, forming a strongly supported clade with *B. prionitis* and *A. paniculata* (bootstrap value = 100%, in Fig. 4). The inferred tree topology corroborated previous molecular studies, confirming a close evolutionary relationship among these genera [28]. The subfamily Acanthoideae was found to be phylogenetically distinct from Thunbergioideae (*Thunbergia erecta*) and Avicennioideae (*Avicennia marina*), which form separate, well-supported clades. Strong

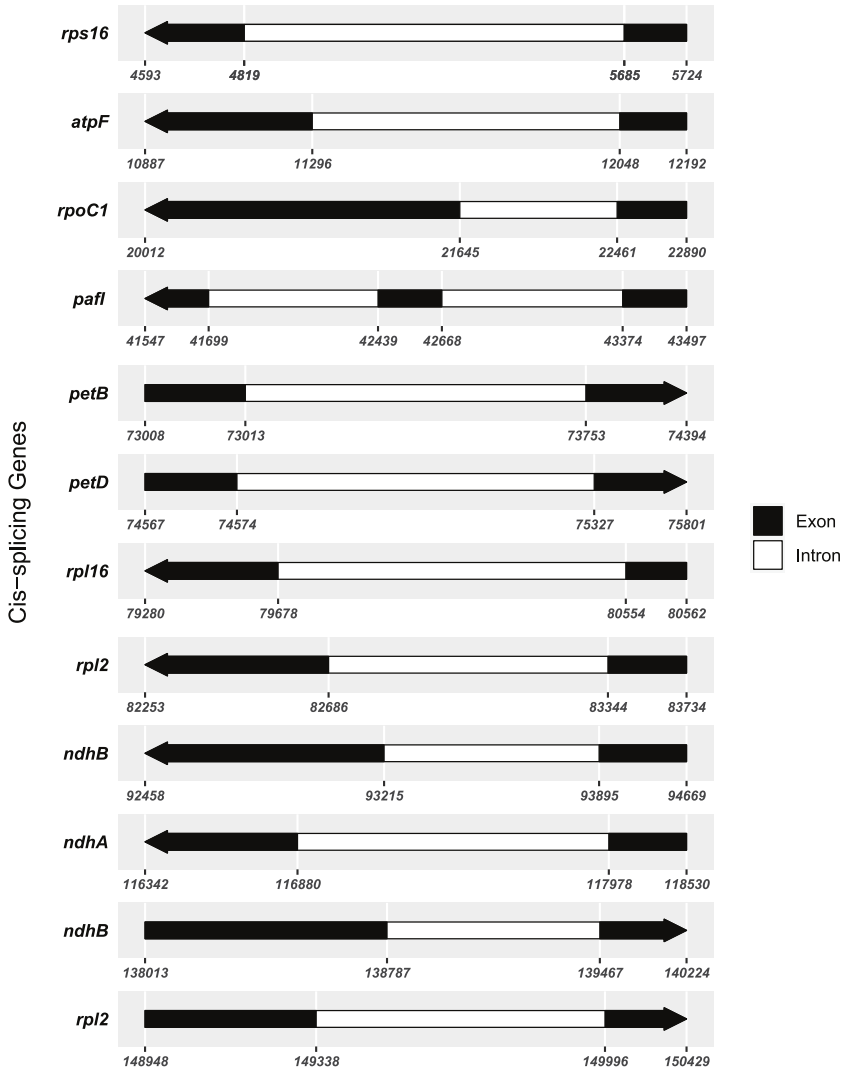

Fig. 2 Cis-splicing gene structures identified in the *Lepidagathis incurva* chloroplast genome.

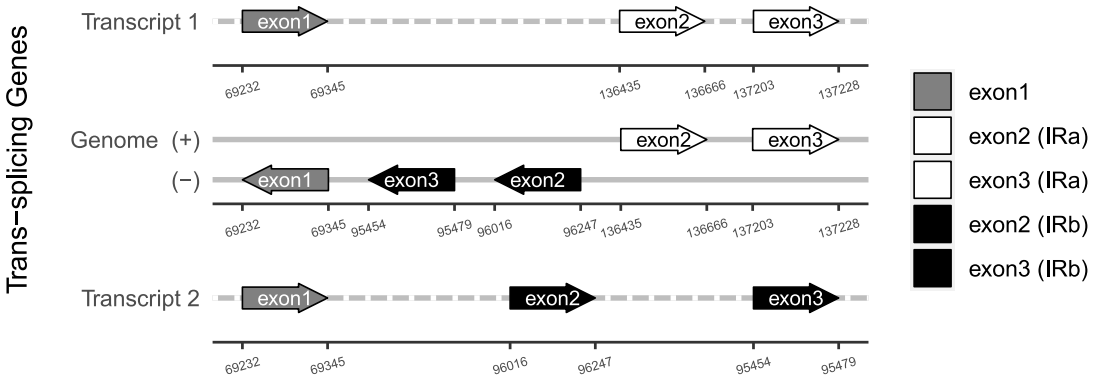

Fig. 3 Trans-splicing gene structures of the *rps12* gene in the *Lepidagathis incurva* chloroplast genome.

bootstrap support across key nodes highlighted the reliability of the phylogenetic placement of *L. incurva*.

These findings contributed to a deeper understanding of Lepidagathis's evolutionary history and underscore the importance of cp genome data in resolving taxonomic uncertainties within Acanthaceae.

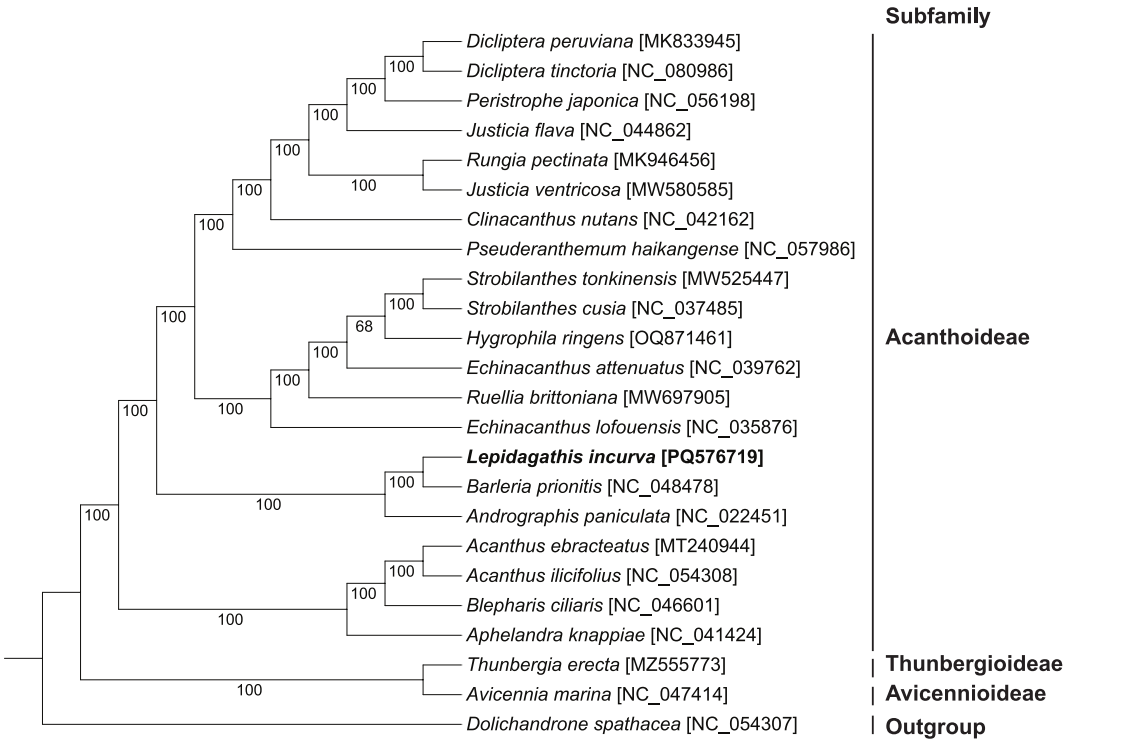

Fig. 4 The phylogenetic tree of *Lepidagathis incurva* and related species within the Acanthaceae family is based on chloroplast protein-coding sequences. The analysis included the following species: *Dicliptera peruviana* [31], *Dicliptera tinctoria* [23], *Peristrophe japonica* (NC_056198), *Justicia flava* [27], *Rungia pectinata* [32], *Justicia ventricosa* [33], *Clinacanthus nutans* [34], *Pseuderanthemum haikangense* [35], *Strobilanthes tonkinensis* [36], *Strobilanthes cusia* [37], *Hygrophila ringens* (OQ871461), *Echinacanthus attenuatus* [22], *Ruellia brittoniana* (MW697905), *Echinacanthus lofouensis* (NC_035876), *Lepidagathis incurva* (PQ576719), *Barleria prionitis* [15], *Andrographis paniculata* [16], *Acanthus ebracteatus* (MT240944), *Acanthus ilicifolius* (NC_054308), *Blepharis ciliaris* [38], *Aphelandra knappiae* [30], *Thunbergia erecta* [39], *Avicennia marina* (NC_047414), and *Dolichandrone spathacea* (NC_054307) [40].

Expanding cp genome sequencing across additional *Lepidagathis* species will further clarify their phylogenetic relationships and evolutionary divergence.

Implications and future directions

This study represented a significant advancement in our understanding of the genomic and evolutionary characteristics of *L. incurva* by providing the first complete cp genome for the genus. Previous phylogenetic analyses of *Lepidagathis* have primarily relied on nuclear and partial cp sequences, which, while informative, provided lower resolution offered by complete cp genomes [7]. Expanding genomic sampling across the genus using full cp genomes will enable a more refined phylogenetic framework, facilitating the identification of cryptic diversity and improving taxonomic classification within Acanthaceae [28]. In addition, this study identified a notable structural variation in the loss of two introns in *clpP*, which may provide insights into the evolution and functional adaptation of cp genomes.

In addition to phylogenetic studies, this newly sequenced cp genome served as a valuable resource for evolutionary and ecological research. Future studies could evaluate whether *Lepidagathis* species exhibited cp genome modifications linked to habitat specialization, as observed in other Acanthaceae members [22]. Additionally, given the medicinal significance of *L. incurva*, integrating the cp genome data with metabolomic and transcriptomic analyses could reveal the genetic pathways involved in the biosynthesis of bioactive compounds [10].

From a conservation perspective, cp genome data provide informative markers for population genetics and phylogeography. By analyzing intraspecific cp genome variation, future work can assess the genetic diversity, population structure, and evolutionary history, which are critical for the conservation and sustainable utilization of *Lepidagathis* species [30]. Ultimately, expanding cp genome sequencing across *Lepidagathis* and integrating nuclear genomic data will provide a comprehensive phylogenomic framework, enhancing our understanding of species evolution and diversification within Acanthaceae.

CONCLUSION

This study provided the first complete cp genome of *L. incurva*, thereby generating a crucial genomic resource for the genus and Acanthaceae. The findings

improved phylogenetic resolution, identified structural variations, and established a foundation for future comparative genomic and evolutionary studies. By expanding cp genome sequencing across *Lepidagathis*, integrating nuclear data, and exploring functional adaptations, future research could further elucidate the evolutionary dynamics and taxonomic complexities of this ecologically and medicinally significant genus.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2026.089.

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Appendix A. Supplementary data

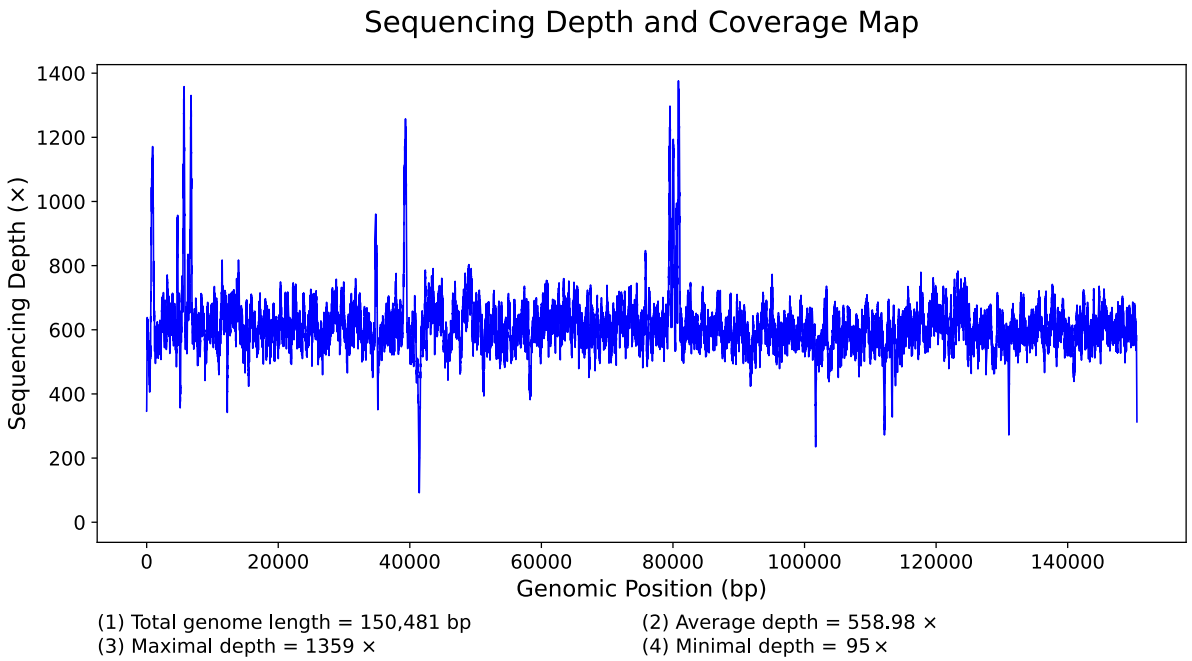

Fig. S1 Sequencing depth and coverage map of the *Lepidagathis incurva* chloroplast genome.

Table S1 GenBank accession numbers of reference sequences used for phylogenetic analysis.

No.	Name of the species	GenBank accession number
1	*Dicliptera peruviana*	MK833945
2	*Dicliptera tinctoria*	NC_080986
3	*Peristrophe japonica*	NC_056198
4	*Justicia flava*	NC_044862
5	*Rungia pectinata*	MK946456
6	*Justicia ventricosa*	MW580585
7	*Clinacanthus nutans*	NC_042162
8	*Pseuderanthemum haikangense*	NC_057986
9	*Strobilanthes tonkinensis*	MW525447
10	*Strobilanthes cusia*	NC_037485
11	*Hygrophila ringens*	OQ871461
12	*Echinacanthus attenuatus*	NC_039762
13	*Ruellia brittoniana*	MW697905
14	*Echinacanthus lofouensis*	NC_035876
15	*Lepidagathis incurva*	PQ576719
16	*Barleria prionitis*	NC_048478
17	*Andrographis paniculata*	NC_022451
18	*Acanthus ebracteatus*	MT240944
19	*Acanthus ilicifolius*	NC_054308
20	*Blepharis ciliaris*	NC_046601
21	*Aphelandra knappiae*	NC_041424
22	*Thunbergia erecta*	MZ555773
23	*Avicennia marina*	NC_047414
24	*Dolichandrone spathacea*	NC_054307