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PLASTOME REPORT

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The complete chloroplast genome sequence and phylogenetic analysis of *Strobilanthes dalzielii* (W.W.Sm.) Benoist 1935 (Acanthaceae)

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ABSTRACT

Strobilanthes dalzielii of Acanthaceae is an herb species with potentially extensive applications for its pharmaceutical and ornamental values. Due to taxonomic complications and limited genetic information, the structural characteristics, and phylogenetic relationships of the *S. dalzielii* chloroplast genome were assembled and characterized here for the first time. The complete chloroplast genome of *S. dalzielii* was 144,580 bp in length. The genome is quadripartite in structure and consists of a large single-copy region (92,137 bp) and a small single-copy region (17,669 bp), which are separated by a pair of inverted repeats (each 17,387 bp). A total of 125 genes were annotated, including 80 protein-coding, 37 transfer RNA, and eight ribosomal RNA genes. The overall GC content was 36.4%. Phylogenetic analysis based on the complete chloroplast genome sequence of 21 taxa within the tribe Ruellieae of Acanthaceae using the maximum likelihood and Bayesian inference methods revealed that *Strobilanthes* diverged after *Ruellia*; *S. dalzielii* is closely related to *S. tonkinensis*. The genomic data obtained from this study will serve as valuable information to the species delimitation and genetic classification of *Strobilanthes*.

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Introduction

Strobilanthes Blume is one of the most diverse genera in Acanthaceae (Ruellieae: Strobilanthinae) and contains about 454 species, which are distributed in the tropical and subtropical regions of Asia, with some species extending to the Pacific region (Hu et al. 2011; Plants of the World Online (POWO), 2023). *Strobilanthes dalzielii* (W.W.Sm) Benoist 1935 is a subshrub or perennial herb that usually grows by streams or wet hill paths with an altitude below 1200 m (Hu et al. 2011; Figure 1). The whole plant has been used as a folk medicine for treatment of venomous snake bites, swelling and pain in throat. Despite it is sourced as folk medicine, studies on *S. dalzielii* are very limited. On the other hand, the morphological diversity, and species richness in *Strobilanthes* has put forward challenges to researchers on its phylogenetic and taxonomic relationships at species level (Moylan et al. 2004). Due to that reason, in this study, we aimed to characterize the complete chloroplast genome of *S. dalzielii* using bioinformatics approaches. To date, only 17 chloroplast genome sequences are publicly available (As of 1 October 2023), which anticipated approximately 4% of the total species present in the genus; the newly assembled chloroplast genome sequence can aid in expanding the genomic information of the species, as well as provide important data to the evolution of the genus. The phylogenetic analysis conducted in this study would reveal the molecular placement of *S. dalzielii* and the relationship among the closely related species at the chloroplast genome level.

Materials and methods

Fresh leaves of *S. dalzielii* were obtained from a natural population located at Mount Jiulian (coordinates: N24°34′39″, E114°26′53″). A voucher specimen (collection number JLSQZJL230601) has been deposited in the Herbarium of Jiulian Mountain National Nature Reserve (contact: Guoliang Xu; email: zxuguoliang@163.com). The species is not categorized as a protected species, obviating the necessity for a permit for specimen collection.

Total genomic DNA extraction was carried out following the cetyltrimethylammonium bromide (CTAB) method as

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Figure 1. *Strobilanthes dalzielii* (A) in its natural habitat, (B) inflorescence. Photos by G. Xu.

described by Doyle and Doyle (1990). A 300-bp genomic library was constructed using the TruSeq DNA Sample Prep Kit (Illumina, USA). Sequencing was conducted using the Illumina Novaseq platform (Illumina, USA). The assembly of the chloroplast genome was executed by feeding the raw NGS data generated by the sequencing machine into the NOVOWrap v.1.20 pipeline (Wu et al. 2021). Gene annotation was performed using GeSEq v2.03 (Tillich et al. 2017), with default parameters, and manual verification was conducted to rectify any potential errors. Gene structures that posed challenges in annotation, including the cis- and trans-splicing genes, were identified using CPGView (Liu et al. 2023), and the annotated chloroplast genome was visualized using OGDraw v1.3.1 (Greiner et al. 2019).

To elucidate the molecular placement of *S. dalzielii* within its taxonomic relatives, phylogenetic reconstruction was carried out based on complete chloroplast genome sequence of 21 taxa within the tribe Ruellieae of Acanthaceae. Two closely related species, *Acanthus mollis* (Acantheae; GenBank: OM022238; Ma et al. 2023) and *Barleria strigosa* (Barlerieae; GenBank: ON768805; Kaewdaungdee et al. 2022) were included in the analysis as outgroup. Multiple sequence alignment was performed using MAFFT v7.470 (Katoh and Standley 2013) and phylogenetic analyses were conducted based on the maximum likelihood (ML) through IQ-TREE v1.6.12 (Nguyen et al. 2015) and Bayesian inference (BI) methods using the MrBayes v3.2 (Ronquist et al. 2012) pipeline accessible *via* the CIPRES Science Gateway (Miller et al. 2010). Based on the Bayesian information criterion from the ModelFinder (Kalyaanamoorthy et al. 2017), the optimal DNA substitution model for the chloroplast genome dataset was the transversion model (TVM) with empirical based frequencies (+F) and invariants (+I), discrete Gamma model with default 4 rate categories (+G4) (=TVM + F + I + G4). Branch support was estimated using 1000 replicates according to the ultrafast bootstrapping algorithm (UFboot; Minh et al. 2013). For the BI tree, Markov chain Monte Carlo with 2,000,000 generations was employed, with sampling conducted at intervals of 100 cycles.

Results

The chloroplast genome assembly of *S. dalzielii* has a minimum read mapping depth of 35× and an average read mapping depth of 97.3× (Supplementary Figure 1). The complete chloroplast genome of *S. dalzielii* comes in a typical quadripartite structure and spans a length of 144,580 bp (GenBank: OR713747; Figure 2). The chloroplast genome comprises a large single-copy (LSC) region measuring 92,137 bp and a small single-copy (SSC) region spanning 17,669 bp, which are separated by a pair of inverted repeat (IR) regions, each 17,387 bp in length. In total, 125 genes were annotated, including 80 protein-coding (CDS), 37 transfer RNA (tRNA), and eight ribosomal RNA (rRNA) genes. Among these CDS, nine are cis splicing genes, with two containing two introns, and seven containing a single intron (Supplementary Figure 2 A); the gene structure of the trans-splicing gene *rps*12 was also identified (Supplementary Figure 2B). The overall GC content of the chloroplast genome was 36.4%.

Since both the ML and BI trees exhibited congruent topological structures, only the ML tree was visually presented; the posterior probabilities derived from the BI analysis were integrated into the ML tree (Figure 3). Based on current sampling size, *Ruellia* was first diverged among the four genera in Ruellieae, followed by *Strobilanthes*, as well as *Echinacanthus* and *Hygrophila*. The phylogenetic relationship among members of *Strobilanthes* was well-resolved (ultrafast bootstrap, UFboot ≥95%; posterior probability, PP ≥0.95), except for the branch node indicating the divergence of *Strobilanthes schomburgkii* that showed a weaker ultrafast bootstrap value (UFboot =68%). In the *Strobilanthus* clade, *S. dalzielii* was closely related to *S. tonkinensis*, which they are clustered with other three *Strobilanthes* species namely, *S. biocullata*, *S. pulcherrima*, and *S. sexennis*.

Discussion

The chloroplast genome structure and genome size of *S. dalzielii* were similar to those of other published chloroplast genomes of *Strobilanthes* species, which is quadripartite in structure and has a genome size between 144,012 bp and 144,987 bp (Wang et al. 2021, Wan et al. 2022) However, there are variations in the number of genes among *Strobilanthes* species when compared to *S. dalzielii*, e.g. *S. crispus*, *S. cusia*, and *S. tonkinensis* have more CDS ($n = 84$; Fang et al. 2021, Wu et al. 2021, Wan et al. 2022), while

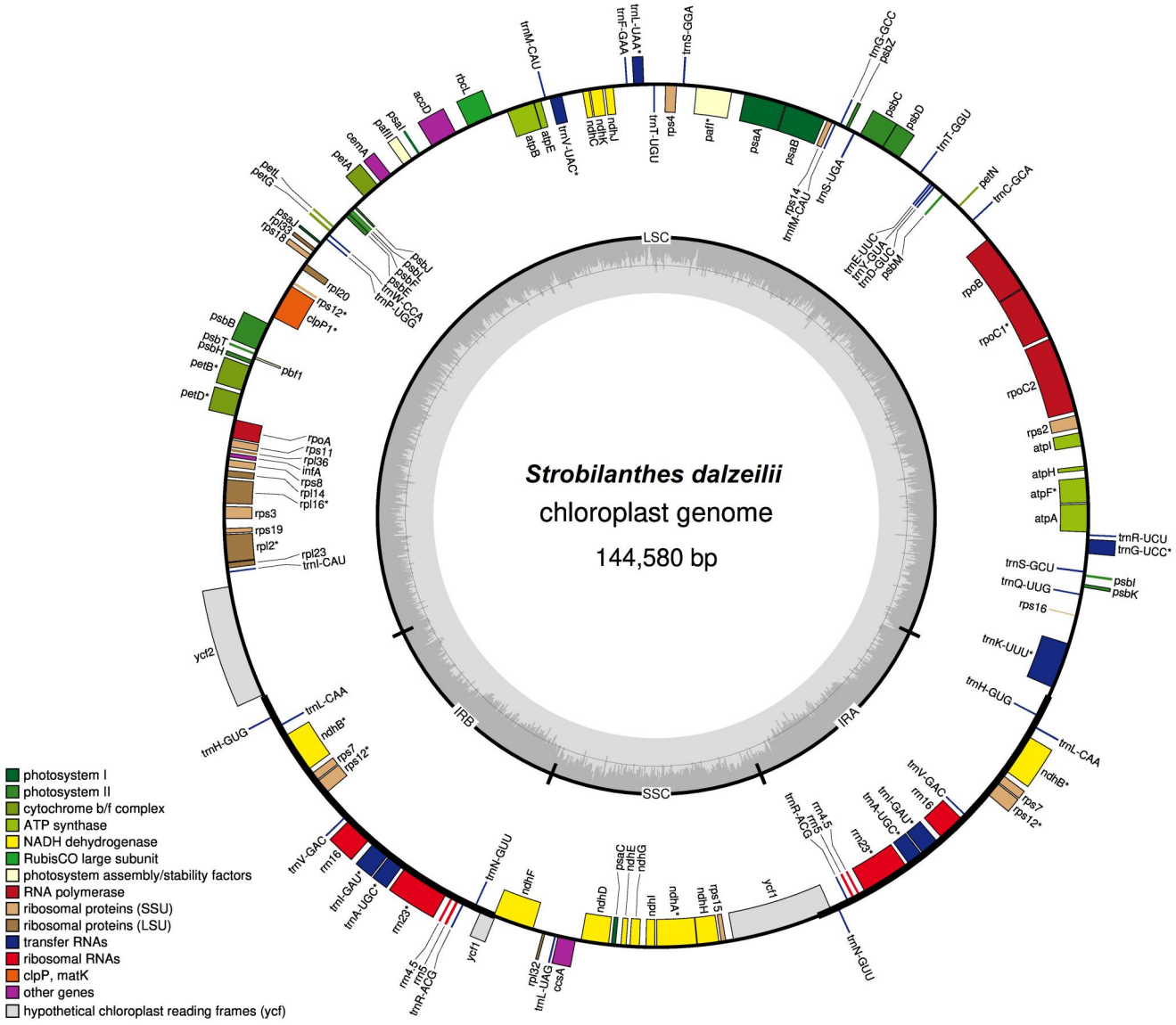

Figure 2. Chloroplast genome map of *Strobilanthes dalzielii*. Genes on inside of map are transcribed in clockwise direction; genes on outside of map are transcribed in counter clockwise direction.

S. biocullata has fewer tRNA genes ($n = 30$; Wang et al. 2021). It is anticipated that the different annotation tools used for characterization could result in inconsistency in the number of genes being annotated (Guyeux et al. 2019).

To date, there was no published molecular data on *S. dalzielii*; hence, the genetic information obtained from this study is likely to be the first and only genetic data available to public. Although genetic studies on *S. dalzielii* is limited, the phylogenetic analysis on *Strobilanthes s.l.* conducted by Moylan et al. (2004) has successfully addressed few concerns that have been debated over the last few centuries. In their study, the combined internal transcribed spacer (ITS) and morphological analysis has revealed conflicts and inconsistency present in past generic classifications; the proposal of infrageneric classification within *Strobilanthes* by Terao (1983) was not accepted. Yet, the molecular placement of most *Strobilanthes* species included in our analysis showed incongruence to that by Moylan et al. (2004). For example, *S. pulceriima* and *S. sexennis* are clustered together when using the complete chloroplast genome dataset but were placed apart when using the combined dataset. However, by assuming that the species identification is accurately conducted, we do not exclude the possibility that the cytonuclear incongruence in the phylogenetic trees is due to presence of hybridization in the genus; although there is no clear evidence of *Strobilanthes* hybrids; the presence of hybrid origin in *Strobilanthes* was somewhat speculated (Wood et al. 2022). Nevertheless, the genomic information generated from this study would serve as useful data for future application in species delimitation and genetic classification of *Strobilanthes* as well as Acanthaceae.

Authors' contributions

SYL, GX: conception and design; ZN, WP, ZC, JYC: analysis and interpretation of data; ZN, WP, ZC, JYC: drafting of the paper; SYL, GX: critical revision of the paper; all the authors approved the final version; and all authors agree to be accountable for all aspects of the work.

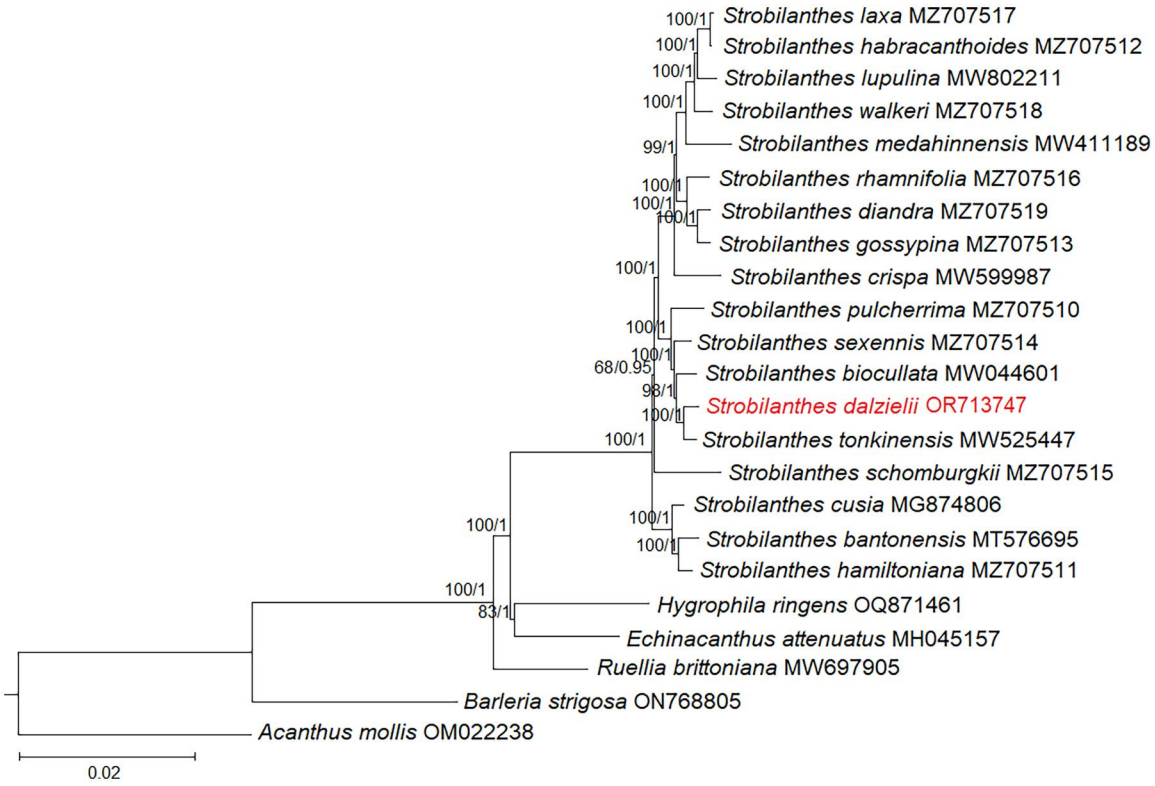

Figure 3. Phylogenetic tree based on the complete chloroplast genome sequence of 21 selected taxa of Ruellieae, while *Acanthus mollis* (GenBank: OM022238; Ma et al. 2023) and *Barleria strigosa* (GenBank: ON768805; Kaewdaungdee et al. 2022) are selected as outgroup. The bootstrap support and posterior probability values are indicated at each branch nodes. GenBank accession number is listed after the species name.

Permission for sample collection

Strobilanthes dalzielii is not a protected plant. The study did not incur any disturbance or damages to its population; thus, permission for sample collection was acknowledged by the nature reserve's administrative office.

Disclosure statement

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

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at http://www.ncbi.nlm.nih.gov under the accession number OR713747. The associated BioProject, SRA, and BioSample numbers are PRJNA772562, SRR26322315, and SAMN37731540, respectively.

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