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PLASTOME ANNOUNCEMENT

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The complete chloroplast genome of *Chloranthus nervosus* Collett ex Hemsl. 1890 (Chloranthaceae)

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ABSTRACT

In this study, we sequenced and assembled the complete chloroplast genome of *Chloranthus nervosus* Collett ex Hemsl. 1890. The total length of the complete chloroplast sequence was found to be 158,002 bp. It consisted of a large single-copy (LSC) region of 87,127 bp, a small single-copy (SSC) region of 18,541 bp, and a pair of inverted repeat (IR) regions, each with a length of 26,167 bp. The overall GC content of the complete chloroplast genome was 38.9%, with the LSC region, SSC region, and IR regions exhibiting GC contents of 37.4%, 34.1%, and 43.1%, respectively. The annotation of the chloroplast genome revealed a total of 131 genes, comprising 86 protein-coding genes, 37 tRNA genes, and eight rRNA genes. Phylogenetic analysis revealed that the seven sampled species of *Chloranthus* were divided into two clades. Within the clade characterized by long filamentous anther connectives, *C. nervosus* showed the closest relation to *C. japonicus*. These findings validated the previous preliminary results on the phylogenetic relationships of the seven species of *Chloranthus* with strong support.

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KEYWORDS
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Introduction

Chloranthus Swartz (Chloranthaceae) plays a crucial role in studying the origin and early evolution of angiosperms (Friis et al. 1986). The genus *Chloranthus* contains two subgenera, i.e. subg. *Tricercandra* and subg. *Chloranthus*, and a total of 11 species (Kong 2000; Liu et al. 2019; He et al. 2022). The two subgenera are distinguished by specific features: subg. *Tricercandra* exhibits long filamentous anther connectives with thecae attached to the base, whereas subg. *Chloranthus* displays short, clavate, or helmet-like connectives with thecae attached to the median or apical parts (Kong 2000). Previous phylogenetic studies on *Chloranthus*, as well as Chloranthaceae, utilized sequences of a limited number of plastid sites and the nuclear ribosome ITS, which consistently supported the monophyly of *Chloranthus* and provided concordant and generally high resolution for intrageneric relationships, except within subg. *Tricercandra* where interspecific relationships had low to moderate support (Kong 2000; Kong and Chen 2000; Kong et al. 2002; Zhang et al. 2011, 2015). Subg. *Tricercandra* consists of four species: *Chloranthus nervosus* Collett ex Hemsl. 1890, *C. japonicus*, *C. augustifolius*, and *C. fortunei*. Among them, *C. nervosus* exhibits polymorphism in flower color, ranging from white or yellow to orange–red (Lu et al. 2020). Therefore, it is necessary to conduct further phylogenetic analysis to validate the relationships within subg. *Tricercandra*, with a particular focus on *C. nervosus*, using larger data that contains more informative sites, such as chloroplast genome data obtained through genome skimming sequencing.

The chloroplast genomes of six *Chloranthus* species have been published and are publicly available from NCBI. These genomes include all species of subg. *Tricercandra* except *C. nervosus*. In this study, we successfully assembled and annotated the complete chloroplast genome of *C. nervosus* (Figure 1) and conducted phylogenetic analysis of *Chloranthus* taxa, particularly in subg. *Tricercandra*. This study will enhance our understanding of the phylogeny of *Chloranthus* and provide preliminary data for future investigations on the taxonomy, phylogeography, and evolution of *Chloranthus*.

Materials and methods

A sample of *C. nervosus* was collected from Pohong Village (Nabo Town, Baise City, Guangxi, China 23°86′83″ N, 107°15′99″ E) and the voucher specimen (barcode

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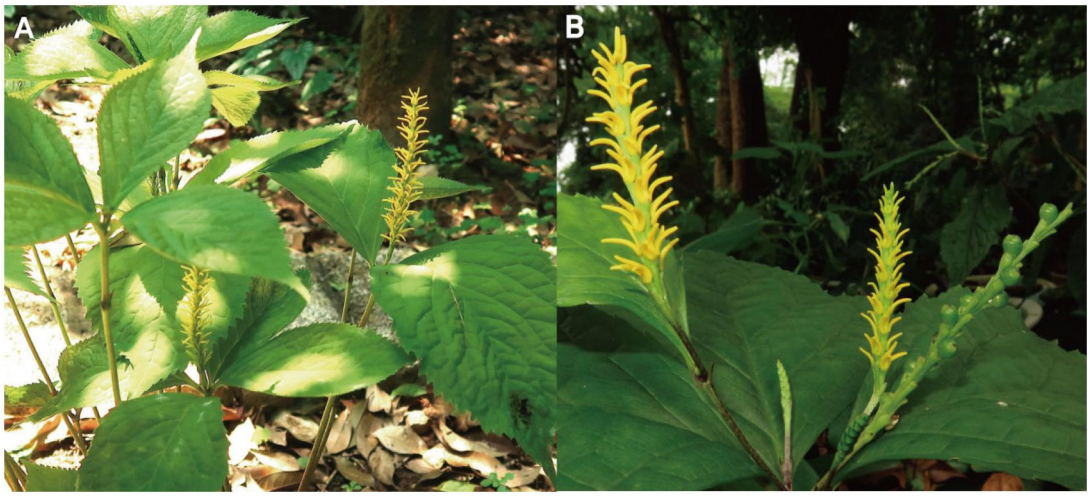

Figure 1. The plant of *C. nervosus*. A whole plant; B inflorescences and fruits. The plant individual has bright yellow long filamentous anther connectives. The photographs were taken by Yong-Bin Lu in Guilin Botanical Garden, Guangxi, China (25°04′36″ N, 110°18′21″ E).

IBK00441921) was deposited at the Herbarium of Guangxi Institute of Botany (http://www.gxib.cn/spIBK/, contact person: Chun-Rui Lin, email: chunruilin@tom.com). The total DNA of *C. nervosus* was extracted by the CTAB method (Doyle and Doyle 1987). Genomic paired-end sequencing (PE150) was performed on an Illumina NovaSeq 6000 at Novogene (Tianjin, China) and approximately 3 G raw reads were generated. After quality filtering using fastp v0.20.1 (Chen et al. 2018), approximately 2.9 G of clean data was obtained. The complete chloroplast genome was *de novo* assembled using GetOrganelle v1.7.5 (Jin et al. 2020) with default parameters. The complete chloroplast genome was annotated using the online tool CPGAVAS2 (Shi et al. 2019) with *C. japonious* (NC_026565) as the reference. The annotation results were manually corrected using Geneious v9.0.2 (Kearse et al. 2012). The circular map of the chloroplast genome was generated using the CPGView program (Liu et al. 2023). The sequence had been submitted to GenBank under the accession number OR198055. To determine the coverage depth of each base, the clean reads were mapped to the assembled chloroplast genome using BWA-MEM v0.7.17 (Li 2013) and SAMtools v1.9 (Danecek et al. 2021). The resulting data were then used to generate a coverage depth map using OriginPro 2020 (OriginLab Corporation, Northampton, MA, USA).

To elucidate the intrageneric phylogenetic relationships, particularly the position of *C. nervosus*, the complete chloroplast genome sequences of 12 species were retrieved from NCBI, including six species in *Chloranthus*, as well as one species each from *Sarcandra*, *Calycanthus*, *Drimys*, *Magnolia*, *Liriodendron*, and *Illicium*. *Illicium oligandrum* was set as the outgroup according to the relationships of angiosperms (Moore et al. 2007). MAFFT v7.490 (Katoh and Standley 2013) was employed to align all sequences, and an in-house R package named alignmentFilter (freely available from: https://github.com/qiangzhang04/alignmentFilter) was used to mask ambiguously aligned segments ($p < .05$) and remove sites with more than 50% gaps. Subsequently, the phylogenetic tree was reconstructed using the maximum-likelihood (ML) method implemented in IQ-TREE v2.0 software (Minh et al. 2020). To determine the best-fit model, we employed the ModelFinder package within IQ-TREE, which identified GTR + F + R3 as the optimal model. We constructed the ML tree based on the GTR + F + R3 model, followed by 5000 ultrafast bootstraps to evaluate the branch support.

Results

The chloroplast genome of *C. nervosus* was composed of four regions, with a total length of 158,002 bp. The large single-copy (LSC) region spanned 87,127 bp, the small single-copy (SSC) region was 18,541 bp long, and two inverted repeat (IRa and IRb) regions, each with a length of 26,167 bp (Figure 2). The overall GC content was 38.9%. Specifically, the LSC region had a GC content of 37.4%, the SSC region had a GC content of 34.1%, and the IR regions exhibited a higher GC content of 43.1%. The average coverage depth of *C. nervosus* was 961.5 (Figure S1). This genome contained a total of 131 genes, including 86 protein-coding genes, 37 tRNA genes, and eight rRNA genes. 11 genes contained one intron each, namely: *ndh*A, *rpl*16, *pet*D, *pet*B, *rps*16, *rpo*C1, *atp*F, *rpl*2, *trn*I-GAU, *trn*A-UGC, and *ndh*B. Only the *ycf*3 and *clp*P genes had two introns. Twenty genes were in the IR regions, including eight protein-coding genes (*ycf*1, *rps*12, *rps*7, *ndh*B, *ycf*2, *rpl*23, *rpl*2, and *ndh*F), four rRNA genes (*rrn*16, *rrn*23, *rrn*4.5, and *rrn*5) and eight tRNA genes (*trn*N-GUU, *trn*R-ACG, *trn*A-UGC, *trn*I-GAU, *trn*V-GAC, *trn*L-CAA, *trn*I-CAU, and *trn*H-GUG). There existed 11 cis-splicing genes including *rps*16, *atp*F, *rpo*C1, *ycf*3, *clp*P, *pet*B, *pet*D, *rpl*16, *rpl*2, *ndh*B, and *ndh*A (Figure S2A) and one trans-splicing gene, i.e. *rps*12 (Figure S2B).

The phylogenetic analysis indicated that the seven sampled species of *Chloranthus* were segregated into two clades, corresponding well with the two defined subgenera (Figure 3). *Chloranthus nervosus* was resolved as the sister to *C. japonicus* with maximum bootstrap value (BS = 100%). They in turn formed a strongly supported clade with a lineage consisting of *C. angustifolius* and *C. fortunei* (BS = 100%).

Discussion and conclusion

In this study, we successfully assembled and annotated the chloroplast genome sequence of *C. nervosus* for the first

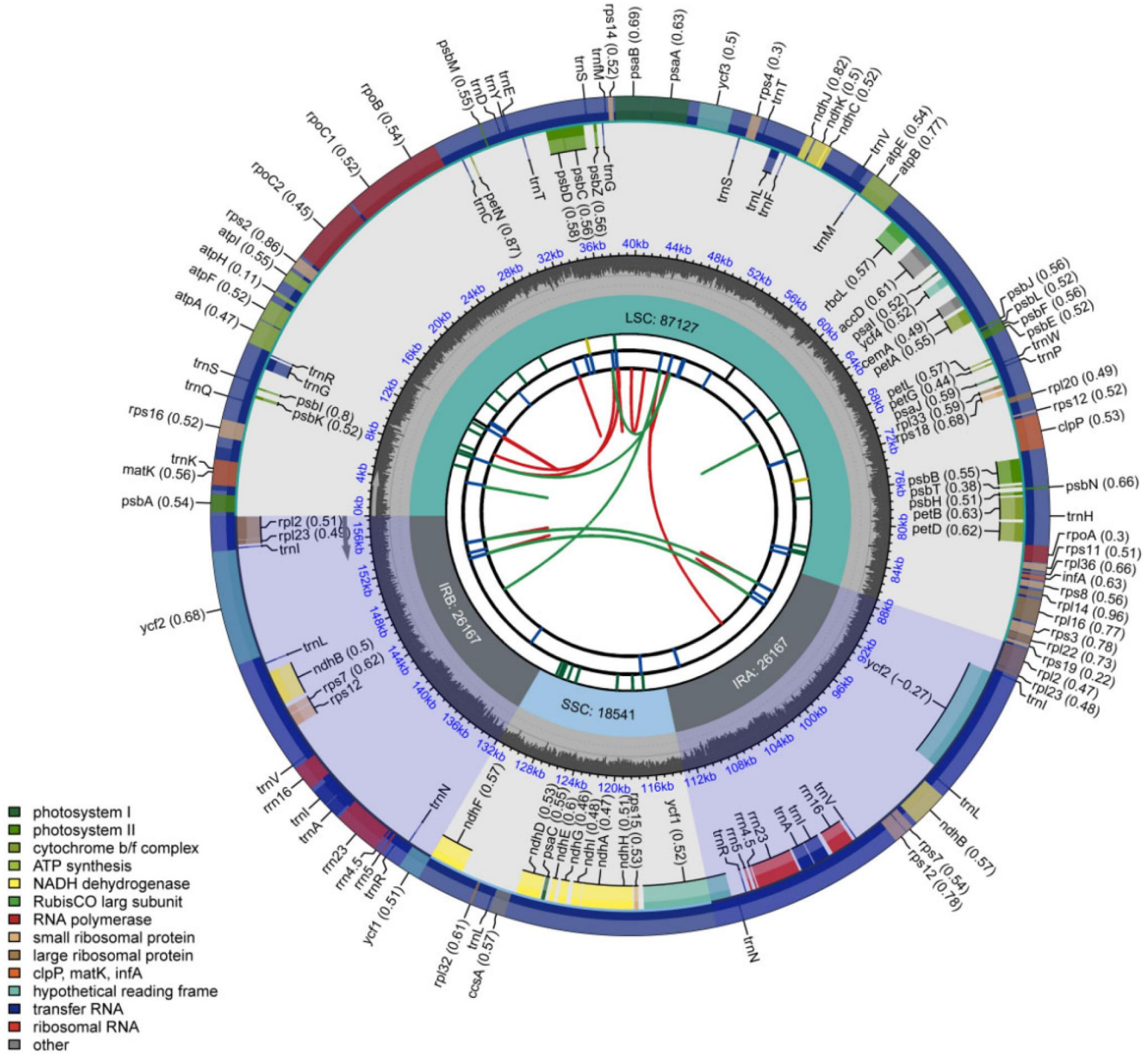

Figure 2. Circular chloroplast genome map of *C. nervosus*. The map is made up of six circles. Starting from the center, the first circle represents the distribution of repeats. The second circle denotes the tandem repeats with short bars. Short bars in the third circle indicate microsatellite sequences. The positions of the LSC, SSC, IRa, and IRb regions are indicated on the fourth circle. The fifth circle shows the GC content. Genes with different functions are color-coded on the sixth circle, and the optional codon usage bias is shown in parentheses after the gene name.

time. The findings from our phylogenetic analyses revealed a clear division of the seven sampled species of *Chloranthus* into two distinct clades that align with the two defined subgenera. Notably, *C. nervosus* was found to share the closest relationship with *C. japonicus* with strong support. The results obtained were consistent with those of previous studies. However, unlike the results of previous studies, the interspecific relationships in subg. *Tricercandra* in our results had a high resolution, which may be due to that the complete chloroplast genome sequences used in this study contain more informative sites, whereas only a few fragments used in the previous studies had much fewer informative sites (Kong 2000; Kong et al. 2002; Zhang et al. 2011, 2015). These results not only corroborated the preliminary relationships and further clarified unresolved interspecific relationships presented in earlier studies but also provided a robust foundation for future studies on the phylogeny and evolution of *Chloranthus*.

Ethical approval

The *C. nervosus* specimens used in this study are not endangered, protected, or privately owned. Therefore, no permission was necessary for collecting plant materials in this study. We confirm that the research adheres to ethical guidelines and local legislation.

Author contributions

Shu-Ting Yao: analyzed data and drafted the manuscript. Yong-Bin Lu: collected materials, conducted species identification, and facilitated the

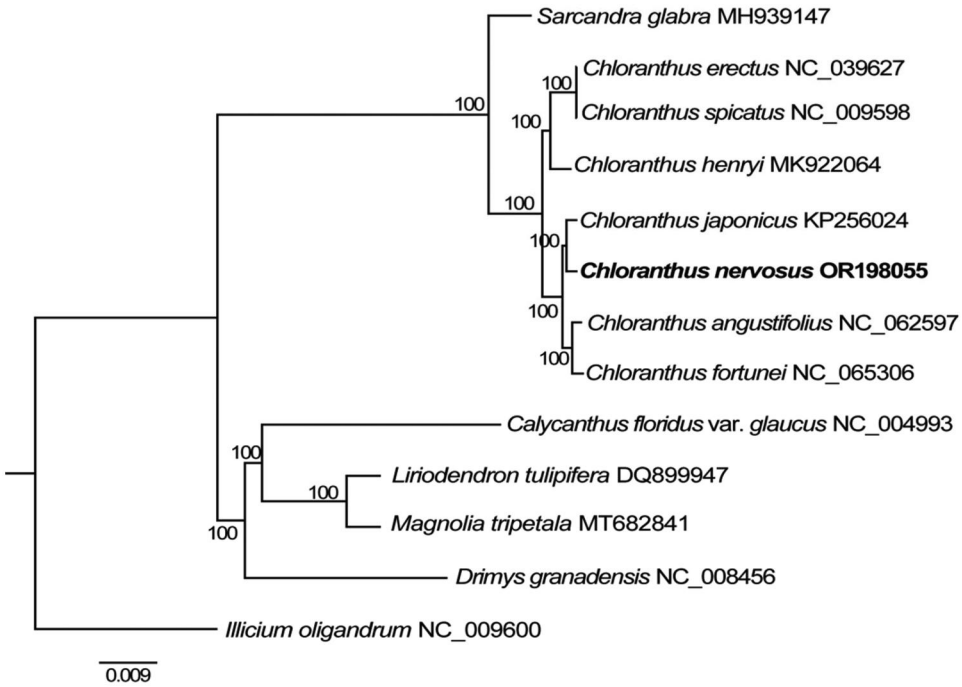

Figure 3. The maximum-likelihood (ML) tree was constructed based on the eight chloroplast genome sequences. The bootstrap support values are shown above branches. The following sequences were used: *Chloranthus erectus* NC_039627 (Zeng et al. 2018), *C. spicatus* NC_009598 (Hansen et al. 2007), *C. henryi* MK922064 (Liu et al. 2019), *C. japonicus* KP256024 (Sun et al. 2016), *C. nervosus* OR198055 (this study), *C. fortunei* NC_065306 (Kang et al. 2022), *Sarcandra glabra* MH939147 (Wang et al. 2020). *Calycanthus floridus* var. *glaucus* NC_004993 (Goremykin et al. 2003), *Liriodendron tulipifera* DQ899947, *Magnolia tripetala* MT682841, *Drimys granadensis* NC_008456 (Cai et al. 2006), and *Illicium oligandrum* NC_009600 (Hansen et al. 2007).

sample preparation for sequencing. Zhan-Jiang Zhang and Cui Li: provided financial program support and helped to revise the manuscript. Peng-Fei Wang and Xin-Mei Qin: designed the study, provided guidance for the analysis, and revised the manuscript. All authors have reviewed and approved the final manuscript for publication.

Disclosure statement

No potential conflicts of interest are reported by the authors.

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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 (https://www.ncbi.nlm.nih.gov/) under accession no. OR198055. The associated BioProject, SRA, and BioSample numbers are PRJNA990663, SRR25131669, and SAMN36271954, respectively.

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