

The complete chloroplast genome of *Orthosiphon aristatus* (Blume) Miq. (Lamiaceae)

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ABSTRACT

Orthosiphon aristatus (Blume) Miq. 1858 is a Lamiaceae plant. It is mainly found in southern China. It is an excellent medicinal plant. The complete chloroplast genome of *O. aristatus* is 152,155 bp in length, with an average depth of 287×, and the GC content was 37.86%, a large single-copy (LSC) region of 83,098 bp, a small single-copy (SSC) region of 17,665 bp, and an inverted repeats (IRs) region of 25,696 bp make up the genome's typical tetragonal shape. In addition, the genome consisted of 128 genes, including 85 protein-coding genes, 35 transfer RNA (tRNA), and eight ribosomal RNA (rRNA) genes. A monophyletic group was established by *O. aristatus* and 13 plants from five genera of Lamiaceae, according to the phylogenetic tree. In contrast, an isolated monophyletic group was formed by the alien plant *Cinnamomum aromaticum*. The ML tree bootstrap value was relatively high, and *O. aristatus* was most closely related to *Ocimum tenuiflorum* and *Ocimum basilicum*. This study can help with species identification and phylogenetic analysis within *O. aristatus* and Lamiaceae species.

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1. Introduction

Orthosiphon aristatus (Blume) Miq. 1858 is a Lamiaceae plant. It is primarily found in southern China, where it is frequently used to treat illnesses of the urinary system such as nephritis, cystitis, and urinary calculi (Wen and Wei 2019). It possesses diuretic, uric acid-lowering, antioxidant, and anti-inflammatory effects, according to a recent study (Ameer et al. 2012). Diterpenoids, triterpenoids, phenylpropanoids, and flavonoids are responsible for these pharmacological actions (AbdAziz et al. 2021). There is currently no whole chloroplast (cp) genome information available for *Orthosiphon*. As a result, we sequenced the whole cp genome of *O. aristatus* and examined its phylogenetic connection within the Lamiaceae family. Studying and analyzing *O. aristatus*'s cp genome will help with species identification, clinical safe medication usage, and resource protection for *O. aristatus*. It will also explain *O. aristatus*'s phylogenetic position and genetic link in Lamiaceae plants.

2. Materials and methods

We extracted the genomic DNA of *O. aristatus* from fresh leaves (Figure 1) collected on the campus of Guangxi University of Chinese Medicine (N22°48'14"; E 108°30'4").

The Botanical Herbarium of the Guangxi University of Chinese Medicine houses the gathered specimens (voucher number 202304SCC001, Haicheng Wen, wenhaicheng2015@qq.com). Whole genome DNA is extracted and purified using the CTAB method from *O. aristatus* fresh leaves (Liu et al. 2018); the qualifying samples were utilized to build a library, and the qualified library was sequenced on the Illumina HiSeq 2000 platform (Han et al. 2018; Wen et al. 2023). The segmented contig sequences were spliced using NOVOPlasty (version 2.7.2) software (Dierckxsens et al. 2017) and further assembled into scaffold sequences. GeSeq (version 1.78) software is then used to annotate the sequence, after which it has been submitted to the online NCBI database (accession number: OR387684). The genomic circle map of *O. aristatus* was created using the online CPGView program (<http://www.1kmpg.cn/cpgview>) (Liu et al. 2023). The complete cp gene sequences of 13 Lamiaceae-related plants and one outgroup plant were downloaded from the NCBI database, to explore the phylogenetic relationship of *O. aristatus*. The genome sequences were then compared and corrected using the maximum-likelihood (ML) method and the Tamura-Nei model using MEGA 11 software. The ML tree has been constructed and the bootstrap parameter is set to 1000 repeats to obtain reliable data (Kumar et al. 2018).

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Figure 1. Reference image of *O. aristatus* taken by Haicheng Wen at the botanical gardens of Guangxi University of Chinese Medicine. (A) Vegetative body. Erect, quadrangular, and pubescent in the back stems. Leaves are opposite; they are ovoid, rhomboid, or ovoid-elliptic, 28.5 cm long and 1.5 cm wide, with a tapered apex, a base that is broadly wedge-shaped or descends to the petiole, a toothed edge or sparse teeth above the base, a tooth tip with small spikes, and pubescent glands on both sides. (B) Blooms. The cymes produce irregular racemes; ovoid-shaped bracts; bell-shaped calyx; pubescent and glandular exteriors; increased following flowering; The corolla is pale purple or white, puberulent on the surface, and has glandular patches on the top lip. It also has a big upper lip that is everted and a three-lobed structure with a broad central lobe, four stamens, somewhat longer than the front pair and extending far beyond the corolla tube; four-lobed ovary, lengthy style, and two-lobed stigma; The disk has a fingerlike shape on the front.

3. Results

O. aristatus has a 152,155 bp long cp genome, with an average depth of $287\times$ (Supplementary Figure 1), a large single-copy (LSC) region of 83,098 bp, a small single-copy (SSC) region of 17,665 bp, and an inverted repeats (IRs) region of 25,696 bp make up the genome's typical tetragonal shape (Figure 2). There is 37.86% of GC content overall, 35.90% of GC content in the LSC region, 31.76% of GC content in the SSC region, and 43.12% of GC content in the IR region. There is little doubt that the GC content in the IR regions is higher than in the two single-copy regions (Table 1). The cp genome *O. aristatus* included 128 genes, including 85 protein-coding genes, 35 transfer RNA (tRNA), and eight ribosomal RNA (rRNA) genes. A monophyletic group was established by *O. aristatus* and 13 plants from five genera of Lamiaceae, according to the phylogenetic tree, while an isolated monophyletic group was formed by the alien plant *Cinnamomum aromaticum* (Figure 3). Supplementary Figures 2 and 3 present the results of cis-splicing for correction and annotation by multi-sequence alignment. Of the 14 genes (*rps16*, *atpF*, *rpoC1*, *rpl2*, *ndhB*, *ndhA*, *trnG-UCC*, *trnL-UAA*, *trnI-GAU*, *trnA-UGC*, *trnK-UUU*, *petB*, *petD*, and *rp116*) all found to contain an intron. two genes (*pafl* and *clpP1*) contained two introns, one small-exon gene (*rps16*), and one trans-splicing gene (*rps12*).

The complete cp genome map of *O. aristatus*. It was generated using CPGView. The circular cp genome map displays 85 protein-coding genes, 35 tRNA genes, and eight rRNA genes. Different categories of genes are labeled with distinct colors. The transcription directions for the inner and outer genes are clockwise and anticlockwise, respectively; genes are color-coded by their functional classification; the functional classification of the genes is shown in the bottom left corner; from the center outward, the first track shows the dispersed repeats. The dispersed repeats consist of direct (D)

and Palindromic (P) repeats, connected with red and green arcs; the second track shows the long tandem repeats as short blue bars; the third track shows the short tandem repeats or microsatellite sequences as short bars with different colors: black: c (complex repeat); green: p1 (repeat unit size = 1); yellow: p2 (repeat unit size = 2); purple: p3 (repeat unit size = 3); blue: p4 (repeat unit size = 4); orange: p5 (repeat unit size = 5); red: p6 (repeat unit size = 6); the SSC, inverted repeat (IRa and IRb), and LSC regions are shown on the fourth track; the GC content along the genome is plotted on the fifth track; the base frequency at each site along the genome will be shown between the fourth and fifth tracks; the genes are shown on the sixth track.

Maximum-likelihood method was used to generate a phylogenetic tree based on 14 representative species. As out-group taxa, *Cinnamomum aromaticum* were used the bootstrap support values are shown at the branches after 1000 bootstrap repeats were computed. The following sequences were used: *Ocimum tenuiflorum* MN687904, *Ocimum basilicum* KY623639, *Orthosiphon aristatus* OR387684, *Isodon japonicus* MW691144 (Wang et al. 2022), *Isodon lophanthoides* MT317098 (Zhang et al. 2020), *Marmoritis complanata* OP186462, *Mentha canadensis* MN047448 (Huaizhu et al. 2019), *Mentha spicata* MG256495 (Wang et al. 2017), *Mentha x piperita* MN736963, *Scutellaria baicalensis* KR233163, *Scutellaria barbata* MW376479, *Clerodendrum japonicum* MW222242 (Long et al. 2021), *Clerodendrum cyrtophyllum* MZ958825, *Clerodendrum colebrookianum* ON149875, and *Cinnamomum aromaticum* MN812496.

4. Discussion and conclusions

The genomic structure of *O. aristatus* consists of two IRs, an SSC region, and an LSC region similar to homologous species *Isodon japonicus* (Wang et al. 2022). The ML tree bootstrap

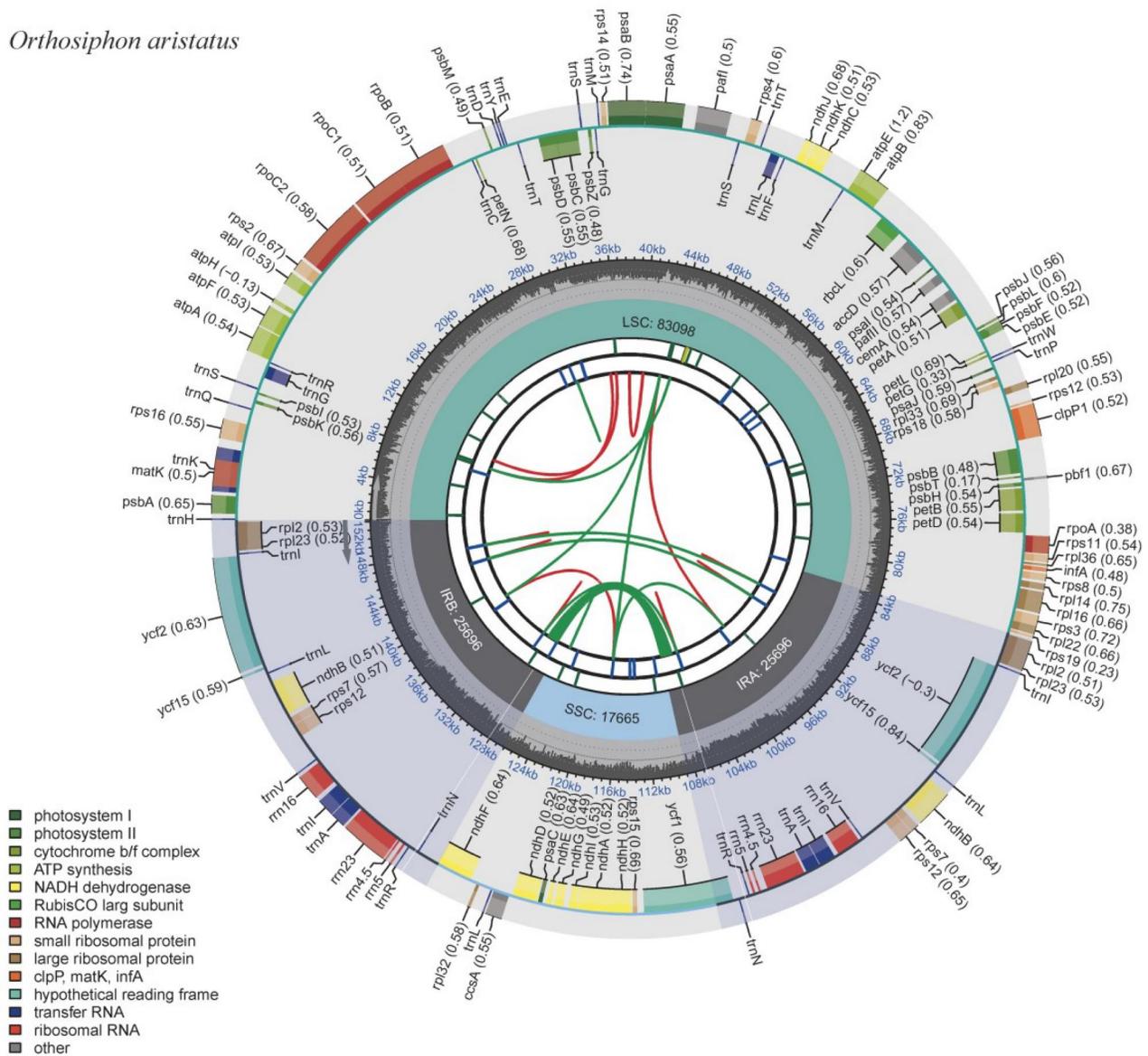
Orthosiphon aristatus

Figure 2. Chloroplast genome map of *O. aristatus*.

Table 1. Structure and composition of *O. aristatus* chloroplast genome.

Region	GC (%)	Length (bp)
Chloroplast genome	37.86	152,155
LSC	35.90	83,098
SSC	31.76	17,665
IRA	43.12	25,696
IRB	43.12	25,696

value was quite high, and *O. aristatus* was most closely related to *Ocimum tenuiflorum* and *Ocimum basilicum*, indicating that they might have shared an ancestor. Their revealed genetic information will be beneficial to further refine detailed and in-depth investigations into this species of evolutionary history.

This study is the first to analyze the cp genome of *O. aristatus* and employs phylogenetic trees to examine the connections between similar species. The phylogenetic tree suggests a close relationship between *O. aristatus*, *O.*

tenuiflorum, and *O. basilicum*, and this work provides new data on the phylogeny, species identification, species resource conservation, and genetic investigations of *O. aristatus*.

Author contributions

HCW and WW designed the experiment and obtained the funding. YJS and BL performed laboratory work (sample collection, DNA extraction, library construction, and sequencing). YJS and BL performed bioinformatics analyses. HCW and WW wrote and revised the manuscript, and all authors reviewed it.

Ethics statement

In the present study, *O. aristatus* plants were collected from the campus of Guangxi University of Chinese Medicine, and permission for the collection of samples was obtained from the Nanning Wild Animal and Plant Protection Station. We complied with the regulations of the Guangxi Zhuang Autonomous Region on the protection of wild plants.

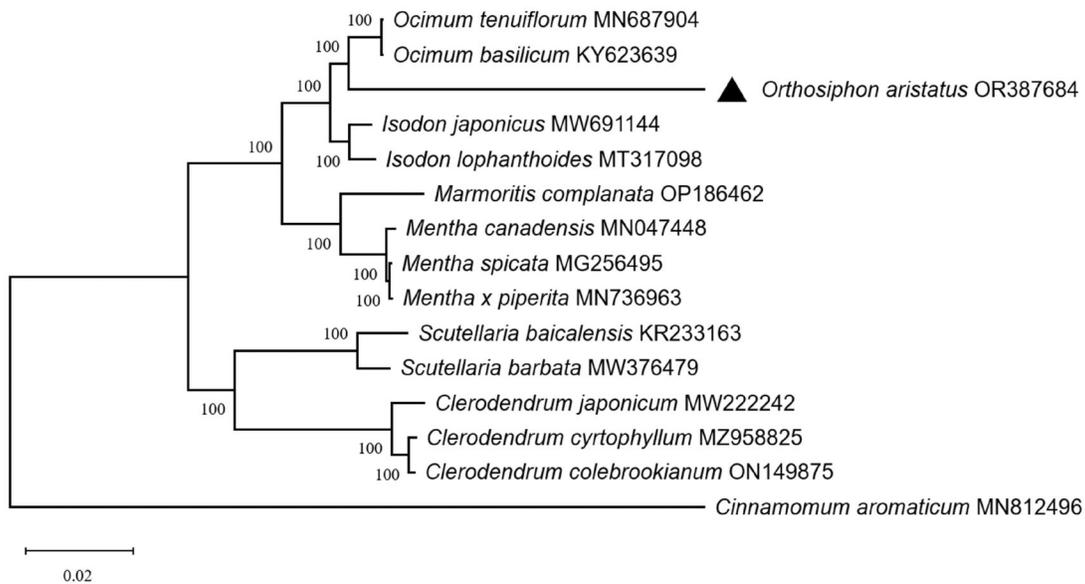


Figure 3. Maximum-likelihood phylogenetic tree composed of the complete gene sequences of 14 related species obtained based on MEGA X analysis.

Disclosure statement

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

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Data availability statement

Genomic sequence data supporting the findings of this study are publicly available at the NCBI GenBank at <https://www.ncbi.nlm.nih.gov> under accession number OR387684. The associated BioProject, Bio-Sample, and SRA numbers are PRJNA1000189, SAMN36761994, and SRR25457748, respectively.

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