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# The complete chloroplast genome and phylogenetic analysis of *Lemmaphyllum carnosum* var. *drymoglossoides* (baker) X. P. Wei, 2013

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**ABSTRACT**

*Lemmaphyllum carnosum* var. *drymoglossoides (Baker)* X. P. Wei, 2013 is a valuable medicinal fern in China. Its complete chloroplast genome was determined using Illumina paired-end sequencing. The genome was 157,571 bp in length with 130 genes, including 87 protein-coding genes, eight ribosomal RNA genes, and 35 tRNA genes. It displayed a quadripartite structure consisting of a small single-copy (SSC) of 21,691 bp, a large single-copy (LSC) of 81,106 bp, and two inverted repeats (IRs) of 27,387 bp, respectively. The phylogenetic results indicated that *L. carnosum* var. *drymoglossoides* exhibited the closest relationship with *L. intermedium*, and this study provided new information for the phylogenetic relationship of the Polypodiaceae family.

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*Lemmaphyllum carnosum* var. *drymoglossoides*; chloroplast genome; annotation; phylogenetic analysis

## Introduction

*Lemmaphyllum carnosum* var. *drymoglossoides* (Baker) X. P. Wei, 2013, an epiphytic fern with medicinal value belonging to the Polypodiaceae family (Zhang et al. 2014), was first reported in 1940 with the name *Lepidogrammitis drymoglossoides* (Baker) Ching 1940 (Wei and Zhang 2013) (Figure 1). This species is epiphytic on shaded tree trunks and rocks, 200–1400 m above altitude, and is widely distributed in all provinces south of the Yangtze River basin. The entire plant is used as a traditional herbal medicine in Southwest China and has important applications in ethnomedicine (Chen et al. 2000). It is effective in clearing away heat and toxins, relieving dampness, and eliminating stasis, and it is also used for treating bronchitis, pleurisy, tuberculosis, dysentery, and rheumatoid arthritis (Lin et al. 2000, Yan and Yu 1998). *L. carnosum* var. *drymoglossoides* is easily confused with the *L. carnosum* var. *microphyllum*, and they can only be distinguished by the discrete sori in contrast to the linear coenosori of *L. carnosum* var. *microphyllum* (Lin et al. 2000). Chloroplast genomes have been widely used in species delimitation and phylogeny because of their uniparental inheritance and lower substitution rates than nuclear genomes (Wei et al. 2020, Gu et al. 2022). This study characterizes the first complete chloroplast genome of *L. carnosum* var. *drymoglossoides* using high throughput sequencing technology and reconstructs the phylogenetic relationships utilizing the published chloroplast genome sequences of the Polypodiaceae family.

**Figure 1.** Plant image of *L. carnosum* var. *drymoglossoides*. Sterile leaves oblong or ovate, rounded or obtusely rounded, base cuneate, entire. Fertile leaves ligulate or oblanceolate, narrowly constricted at the base, sometimes the same shape as the sterile leaves, fleshy, leathery when dry. This photograph was taken by Chong-Jian Zhou in Huangshan City, Anhui Province, and was used with the author's permission.

## Materials and methods

*L. carnosum* var. *drymoglossoides* was collected from Fuxi Village (Tangkou Town, Huangshan City, Anhui Province,

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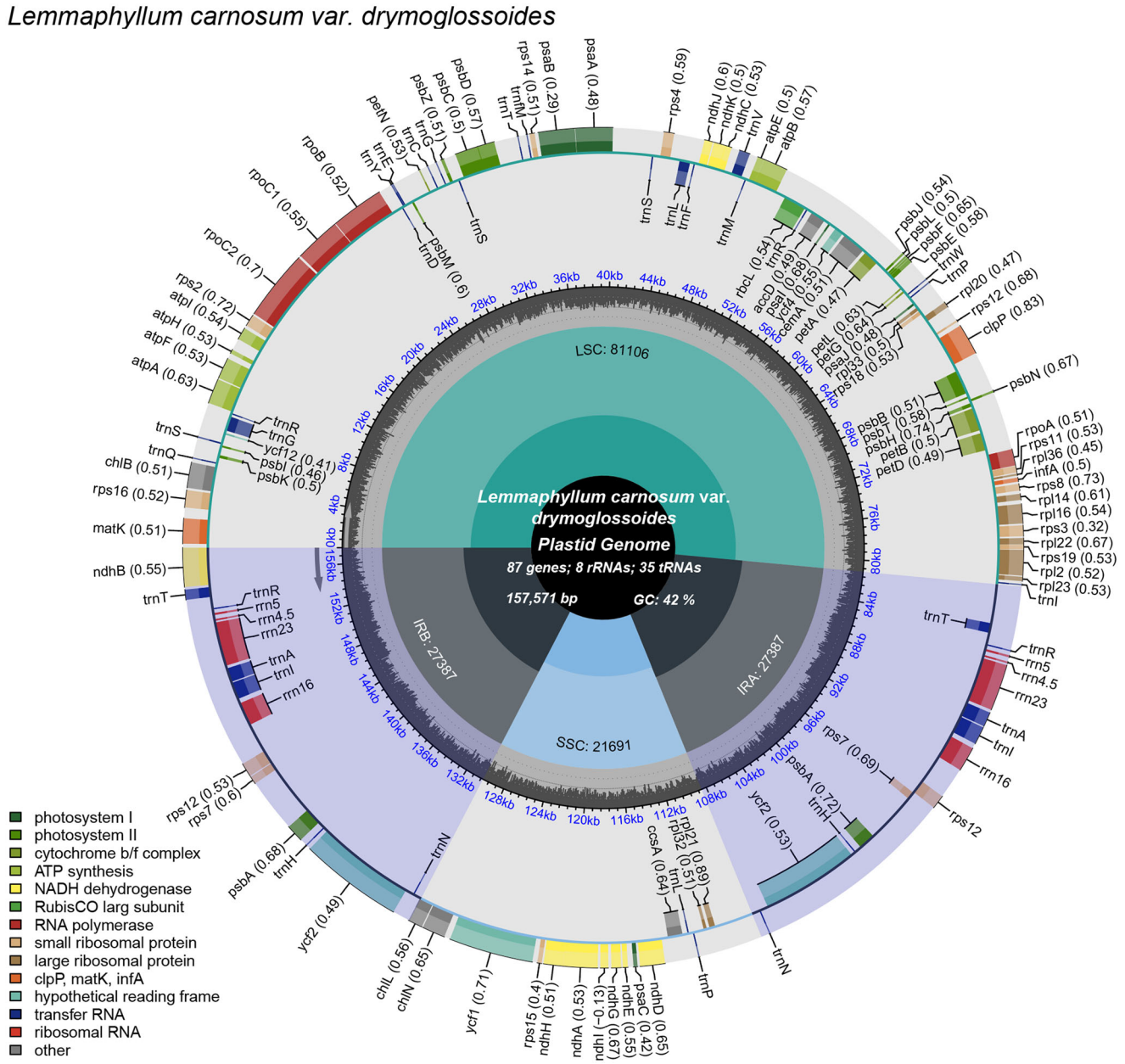


**Figure 2.** Chloroplast genome map of *L. carnosum* var. *drymoglossoides*. Genes drawn outside the outer circle are transcribed counterclockwise, and genes drawn inside the outer circle are transcribed clockwise. Genes belonging to diferent functional groups are color-coded. The different colored legends in the bottom left corner indicate genes with different functions. The dark grey inner circle indicates the GC content of the chloroplast genome and the presence of nodes in the LSC, SSC, IR regions.

China N30°07′, E118°12′) and deposited at Chengde Medical University (Jinxin Liu, Liujx_23@163.com) with the voucher number HPAA0005. The total genomic DNA was isolated from fresh, healthy fronds using modified CTAB methods. The DNA quality was estimated using a Qubit 4.0 Fluorometer (Thermo Fisher Scientific Inc., USA), followed by shearing to prepare a PCR-free library of 350 bp. High-throughput sequencing was performed using the Illumina NovaSeq 6000 system, generating a total of 2.2 G of pair-end raw data. Trimmomatic v0.38 (Bolger et al. 2014) was used to clean the sequencing adapters and low-quality reads. The clean data was then *de novo* assembled with GetOrganelle v1.7.3.5 (Jin et al. 2020). The depth of coverage was calculated by mapping the reads onto the chloroplast genome sequence with bowtie2 v2.3.4.3 to determine the correctness of the assembly (Langmead and Salzberg 2012). The assembled chloroplast genome was annotated using the GeSeq (Tillich et al. 2017) and CPGAVAS2 online webserver (www.herbal genomics.org/cpgavas2) (Shi et al. 2019), and corrected *via* a blasting search against *Lemmaphyllum intermedium* and *L. carnosum* var. *microphyllum* (GenBank: NC_053788, MN623356) as references. Chloroplast Genome Viewer (CPGView) was used to draw the circular map of chloroplast genome (Liu et al. 2023).

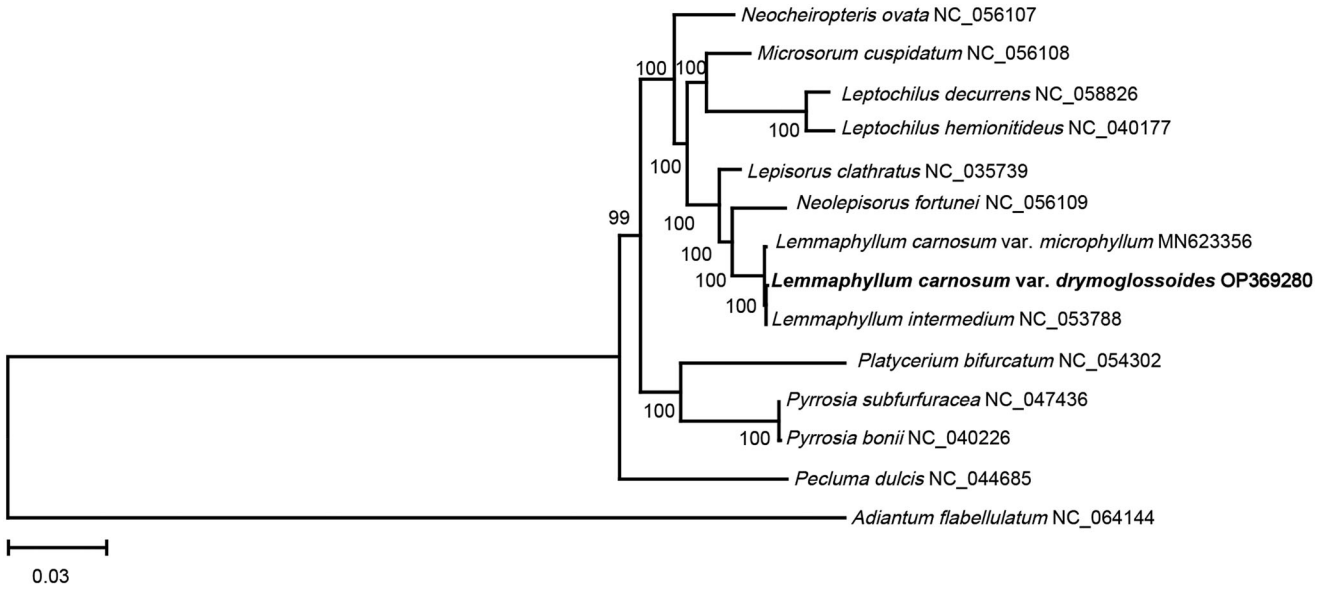


**Figure 3.** The phylogenetic position for *L. carnosum* var. *drymoglossoides* according to the ML phylogenetic tree constructed based on 14 chloroplast genomes. The following sequences were used: *Lemmaphyllum carnosum* var. *drymoglossoides* OP369280, *Adiantum flabellulatum* NC_064144, *Lemmaphyllum carnosum var. microphyllum* MN623356 (Liu et al. 2020), *Lepisorus clathratus* NC_035739 (Wei et al. 2017), *Leptochilus decurrens* NC_058826 (Su et al. 2019), *Leptochilus hemionitideus* NC_040177 (Min et al. 2018), *Lemmaphyllum intermedium* NC_053788 (Wang et al. 2021), *Microsorum cuspidatum* NC_056108, *Neolepisorus fortunei* NC_056109, *Neocheiropteris ovata* NC_056107 (Liu et al. 2021), *Pecluma dulcis* NC_044685 (Samuli and Cárdenas 2019), *Platycerium bifurcatum* NC_054302, *Pyrrosia bonii* NC_040226 (Cai et al. 2018), *Pyrrosia subfurfuracea* NC_047436 (Min et al. 2019). The sequences used for the tree structure are coding sequences. The bootstrap support values are shown on the nodes.

## Result

The chloroplast genome of *L. carnosum* var. *drymoglossoides* is 157,571 bp in length, with an average depth of 513.14 X (Supplementary Figure 1). The depth of some ares are about 80X because the low-complexity regions affect the efficiency of reads mapping, such as 'AATACCCCCCCCCC'. The chloroplast genome of *L. carnosum* var. *drymoglossoides* was circular with a quadripartite structure consisting of a small single-copy (SSC) of 21,691 bp, a large single-copy (LSC) of 81,106 bp, and two inverted repeats (IRs) of 27,387 bp, respectively (Figure 2). Furthermore, the *L. carnosum* var. *drymoglossoides* chloroplast genome encoded 130 genes, including 87 protein-coding genes, eight rRNA genes, and 35 tRNA genes. Gene structure analysis was done for *rps16*, *atpF*, *rpoC1*, *clpP, petB, petD, rpl16, rpl2, ndhA, ndhB* difficult to annotate genes (Supplementary Figure 2). The GC content of the entire genome was 41.8%, displaying LSC, SSC, and IR GC levels of 40.5%, 37.9%, and 45.3%, respectively. One intron occurred in 16 genes: *rps16, trnG-UCC, atpF, rpoC1, trnL-UAA, trnV-UAC, clpP, petB, petD, rpl16, ndhB, trnT-UGU, trnA-UGC, trnI-GAU, ndhA, trnI-GAU,* and *rpl2*. Only the *clpP* gene displayed two intors. Fifteen genes were duplicated in the IR regions, namely *ndhB, trnT-UGU, trnR-ACG, rrn5S, rrn4.5S, rrn23S, trnA-UGC, trnI-GAU, rrn16s, rps12, rps7, psbA, trnH-GUG, ycf2,* and *trnN-GUU*.

To conduct the phylogenetic analyses, the complete chloroplast genome sequences of 13 ferns were retrieved from the National Center for Biotechnology Information (NCBI). The alignment of 80 protein-coding genes was first created using the muscle v5 (Edgar 2022). and then concatenated to a super alignment with a length of 67900 bp. A maximum likelihood (ML) tree was constructed with RAxML v8.2.12 (Stamatakis 2014) using *Adiantum flabellulatum* as an outgroup. Furthermore, 14 chloroplast genomes of 14 species, including 13 species in the Polypodiaceae family, and one species (*Adiantum flabellulatum*) in the Adiantaceae family in the Cornaceae family, were used for phylogenetic analysis. The results indicated that *L. carnosum* var. *drymoglossoides* exhibited the closest relationship with *L. intermedium* with a high bootstrap value of 100 (Figure 3). This study provides new evidence for the phylogenetic study of the genus *Lemmaphyllum*.

## Discussion and conclusion

In this study, the chloroplast genome sequence of *L. carnosum* var. *drymoglossoides* was assembled for the first time and the structure of this species was annotated. The phylogenetic results indicated that *L. carnosum* var. *drymoglossoides* exhibited the closest relationship with *L. intermedium*, and this study provided new information for the phylogenetic relationship of the Polypodiaceae family.

## Ethical approval

The material involved in the article does not involve ethical conflicts. This species is neither endangered on the cites catalogue nor collected from a natural reserve, so it did not need specific permissions or licenses. All collection and sequencing work was strictly executed under local legislation and related laboratory regulations to protect wild resources.

## Author contributions

JXL and LCS conceived and designed the experiments; HYZ and XYL performed the experiments; ZLZ and YT analyzed the data and modified the article; ZLZ and HYZ wrote the paper. All authors agree to be accountable for all aspects of the work.

## Disclosure statement

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

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## Data availability statement

The genom esequence data that support the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession no. OP369280. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA682118, SRR23238769, and SAMN32925244, respectively.

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