

## The complete chloroplast genome sequence of *Plectranthus hadiensis* (Lamiaceae) and phylogenetic analysis

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### ABSTRACT

Plants of the genus *Plectranthus* are used for the treatment of digestive problems, skin diseases, and allergies, with a wide variety of uses. Here, the complete chloroplast genome sequence of *Plectranthus hadiensis* (Benth. ex E.Mey) Codd. 1788 was assembled and characterized for the first time. The full length of the chloroplast genome is 152,484 bp, consisting of a small single-copy region of 17,686 bp, a large single-copy region of 83,380 bp, and a pair of inverted repeats of 51,418 bp. The overall GC content is 37.73%. The chloroplast genome contains 131 unique genes, including 87 protein-coding genes, 36 transfer RNA genes, and eight ribosomal RNA genes. Phylogenetic tree construction based on the complete chloroplast genome sequences of 25 species (23 Nepetoideae, two Ajugoideae) of the Lamiaceae family showed that *P. hadiensis* exhibited the closest relationship with *Isodon*.

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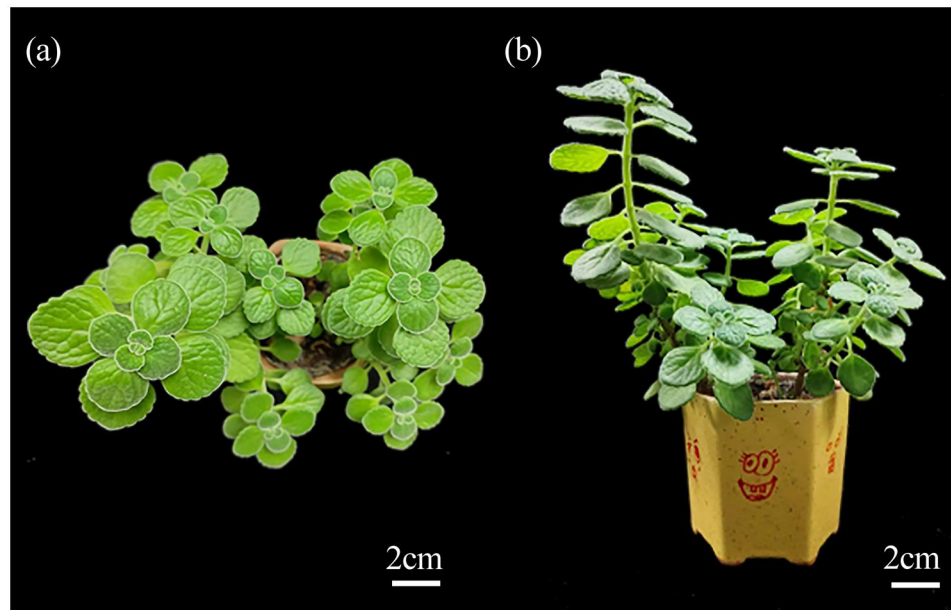
### KEYWORDS

Illumina sequencing;  
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### Introduction

The genus *Plectranthus* (Lamiaceae) has a long history of treating allergies, skin disorders, allergies, and digestive issues


(Lukhoba et al. 2006). *Plectranthus hadiensis* var. *tomentosus*, also known as *Plectranthus tomentosus* (Benth. ex E.Mey.) Codd. 1788, is a semi-succulent subshrub with aromatic



**Figure 1.** The morphological characteristics of *P. hadiensis* used in our research. It is a semi-succulent subshrub with aromatic leaves. Its leaves are opposite, light green, broadly ovate with scalloped margins, and densely covered with short hairs. (a) Top view of the *P. hadiensis* showing the shape of its leaves; (b) front view of the *P. hadiensis* showing the state of the plant (photos were taken by Jiaojiao Hao in Lin'an District, Hangzhou City, Zhejiang Province, China).

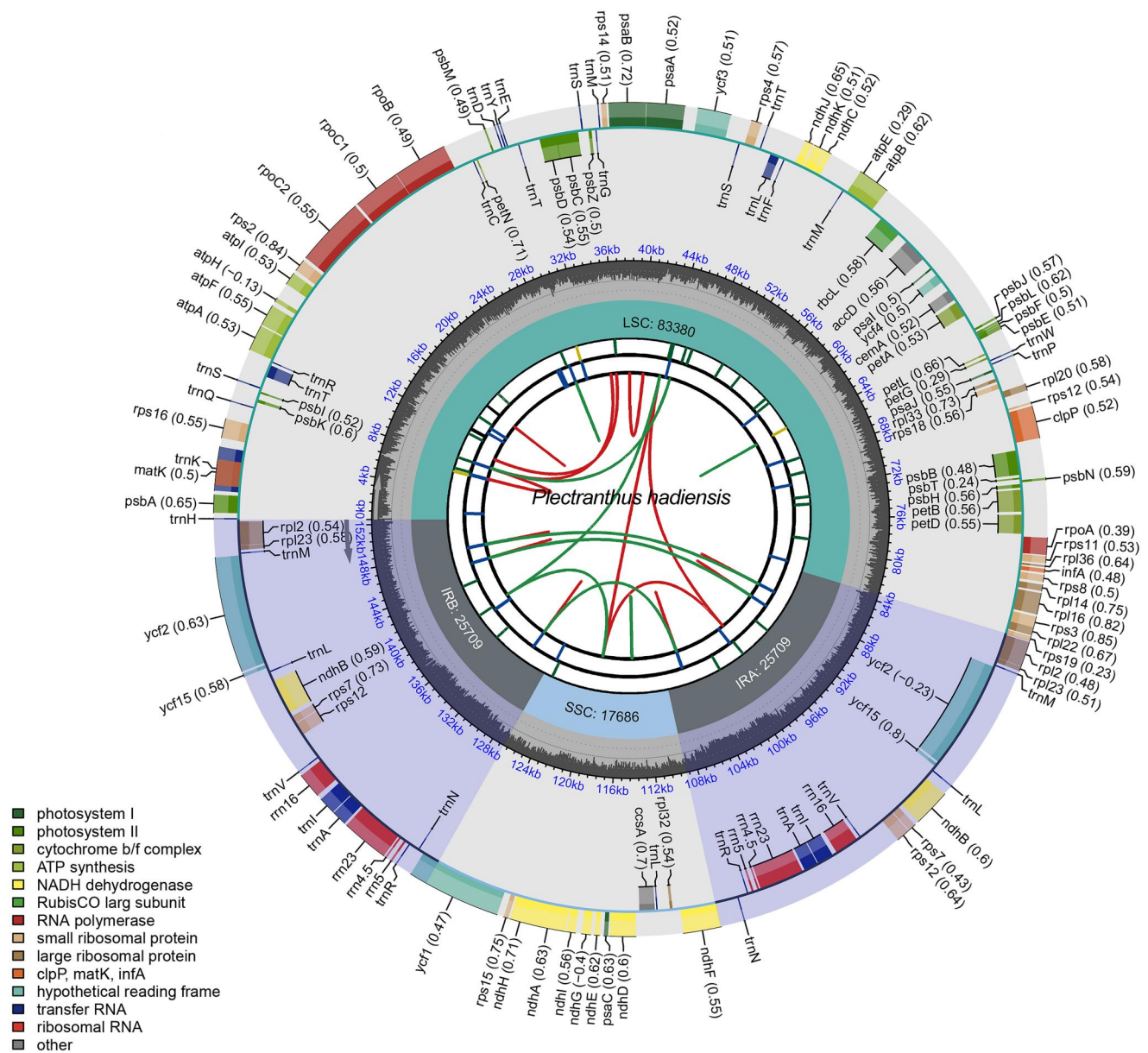
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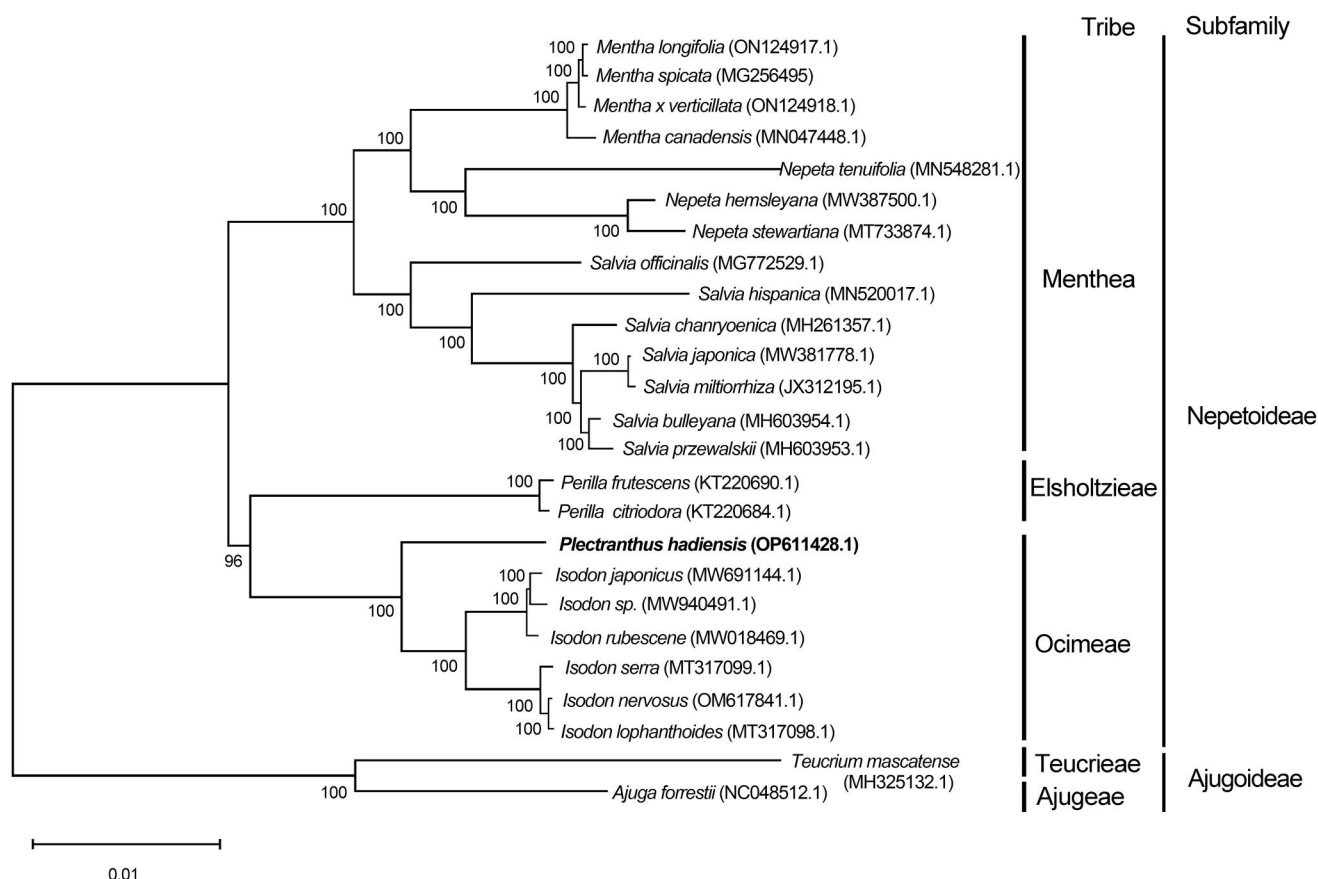
**Figure 2.** Circular map of the complete chloroplast genome of *P. hadiensis* generated by CPGview. The map contains six tracks in default. From the center outward, the first track shows the dispersed repeats. The dispersed repeats consist of direct (D) and Palindromic (P) repeats, connected with red 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. The colors, the type of repeat they represent, and the description of the repeat types are as follows: 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 small single-copy (SSC), inverted repeat (IRA and IRb), and large single-copy (LSC) regions are shown on the fourth track. The GC content along the genome is plotted on the fifth track. The genes are shown on the sixth track. The optional codon usage bias is displayed in the parenthesis after the gene name. Genes are color-coded by their functional classification. The transcription directions for the inner and outer genes are clockwise and anticlockwise, respectively. The functional classification of the genes is shown in the bottom left corner. *Note:* The taxonomy name of *Plectranthus hadiensis* in BioSample and GenBank is *Coleus hadiensis* (the heterotypic synonym of *Plectranthus hadiensis*).

leaves. The plant is native to South Africa and Asia, including South Korea (Ji et al. 2019). The natural products of *P. hadiensis* contain phenolics and essential oils, including terpenoids (Abdel-Mogib et al. 2002). Recent studies demonstrated that *P. hadiensis* possesses a wide range of biological characteristics, including cytotoxic, antioxidant, antimicrobial, and anti-inflammatory activities (Marques et al. 2002; Mothana et al. 2010; Kong et al. 2013; Sun et al. 2022). In the present study, the whole chloroplast genome of *P. hadiensis* was characterized and assembled for the first time to reveal the genetic

taxonomy at the molecular level and provide a foundation for the evolutionary analysis of *P. hadiensis* within the Lamiaceae family.

## Materials and methods

Fresh *P. hadiensis* leaves were collected from the garden of Zhejiang Agriculture and Forestry University (30°15'23.71"N, 119°43'41.76"E), Zhejiang Province, China (Figure 1). A specimen was deposited at the Shanghai Chenshan Herbarium,



**Figure 3.** Maximum-likelihood phylogenetic tree based on 25 chloroplast genomes of Lamiaceae. *Teucrium mascatense* and *Ajuga forrestii* are outgroups. The position of *P. hadiensis* is marked in bold. Bootstrap values are shown on each node ( $N = 1000$ ). Scale bar = 0.01. The following sequences were used: *Mentha longifolia* ON124917.1 (Zubair Filimban et al. 2022), *Mentha spicata* MG256495 (Wang et al. 2017), *Mentha x verticillata* ON124918.1 (Zubair Filimban et al. 2022), *Mentha canadensis* MN047448.1 (Li et al. 2020), *Nepeta tenuifolia* MN548281.1 (Wang et al. 2021), *Nepeta hemsleyana* MW387500.1 (Bautista et al. 2022), *Nepeta stewartiana* MT733874.1, *Salvia officinalis* MG772529.1 (Du et al. 2022), *Salvia hispanica* MN520017.1 (Zhao et al. 2020), *Salvia chanryoenica* MH261357.1 (Ha et al. 2018), *Salvia japonica* MW381778.1 (Liang et al. 2019), *Salvia miltiorrhiza* JX312195.1 (Gao et al. 2020), *Salvia bulleyana* MH603954.1 (Liang et al. 2019), *Salvia przewalskii* MH603953.1 (Liang et al. 2019), *Perilla frutescens* KT220690.1 (Lang et al. 2020), *Perilla citriodora* KT220684.1 (Mo et al. 2017), *Plectranthus hadiensis* OP611428.1, *Isodon japonicus* MW691144.1 (Wang et al. 2022), *Isodon sp.* MW940491.1, *Isodon rubescens* MW018469.1 (Yue et al. 2021), *Isodon serra* MT317099.1 (Zhang et al. 2023), *Isodon nervosus* OM617841.1, *Isodon lophanthoides* MT317098.1 (Zhang, Ma, et al. 2020; Zhang, Xia, et al. 2020), *Teucrium mascatense* MH325132.1 (Khan et al. 2019), and *Ajuga forrestii* NC048512.1 (Tao et al. 2019). The genome length is shown in Supplementary Table S2.

Shanghai, China, under the voucher number CSH0065563 (Contact: Binjie Ge, Email: [gebinjie@csnbgsh.cn](mailto:gebinjie@csnbgsh.cn)). The genomic DNA was extracted by using the cetyltrimethyl ammonium bromide (CTAB) method with minor modification (Montero-Pau et al. 2018), and then the DNA concentration, quality, and integrity were determined by using a Qubit Fluorometer (Invitrogen, Carlsbad, CA) and a NanoDrop Spectrophotometer (Thermo Scientific, Waltham, MA). The constructed libraries were sequenced using the Illumina NovaSeq platform by following the standard Illumina protocols (Illumina, San Diego, CA). The library was sequenced into a paired-end sequencing mode under PE400 library. FastQC was used to rapidly assess the quality of sequencing data (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>). Raw sequencing data were pre-processed using AdapterRemoval (version 2.1.7) to remove adapter sequences (Lindgreen 2012). Then, SOAPEC (v2.0) software was used to correct all the reads based on the K-mer frequency to obtain high-quality data (Luo et al. 2012). The high-quality reads were assembled with Fast-Plast to obtain the chloroplast genome (<https://github.com/mrmckain/Fast-Plast>).

The chloroplast genome of *P. hadiensis* was annotated using Geseq (<https://chlorobox.mpimp-golm.mpg.de/geseq>),

and the Genome Map was drawn with CPGview (<http://www.1kmpg.cn/cpgview>). Finally, the complete chloroplast genome sequences and annotations of *P. hadiensis* were submitted to GenBank under the accession number OP611428.

## Results

The chloroplast genome of *P. hadiensis* is a typical circular structure, 152,484 bp in length, consisting of inverted repeat regions (IRa and IRb) of 51,418 bp, a large single-copy (LSC) region of 83,380 bp and a small single-copy (SSC) region of 17,686 bp (Figure 2). The genome encodes a total of 131 unique genes, including 87 protein-coding genes, 36 transfer RNA genes, and eight ribosomal RNA genes. The total GC content was 37.73%, and the GC contents in LSC, SSC, IRa, and IRb regions were 35.75%, 31.4%, 43.14%, and 43.15%, respectively. As the evidence for correct assembly of the genome, the coverage depth figure is provided in Supplemental Figure S1. The *cis*-splicing genes and the *trans*-splicing gene rps12 are shown in Supplemental Figure S2, and the genes containing intron are listed in Supplemental Table S1.



To evaluate the phylogenetic position of *P. hadiensis*, chloroplast genomes of 25 species (23 Nepetoideae, two Ajugoideae) belonging to the family of Lamiaceae were downloaded from GenBank (Supplemental Table S2). Sequence alignment was performed using Mafft version 7 software (Kato and Standley 2013), and the maximum-likelihood tree was constructed via MEGA11 software under the Tamura-Nei model with 1000 bootstrap replicates (Tamura et al. 2011; Kumar et al. 2018). The phylogenetic tree revealed that *P. hadiensis* was clustered in subfamily Nepetoideae belonging to the tribe Ocimeae, and plants in *Plectranthus* had a close relationship with *Isodon* (Figure 3).

## Discussion and conclusions

As the center of photosynthesis and energy transduction, chloroplasts play a crucial role in the growth and yield of plants (Yu et al. 2014). In this study, the complete plastome of *P. hadiensis* was sequenced, assembled, and annotated for the first time. The genomic structure of *P. hadiensis* consists of a pair of IRs, an SSC region, and an LSC region similar to that in the majority of other angiosperms (Liu et al. 2022). The phylogenetic results indicated that *P. hadiensis* exhibited the closest relationship with *Isodon* in the subfamily Nepetoideae. In conclusion, the study provides a molecular basis in revealing the phylogenetic relationships and providing genetic information for the advancement of systematics research among Lamiaceae species, the exploitation of plants, and resource protection.

## Author contributions

Zhujun Zhu and Youjian Yu designed the experiment; Jiaojiao Hao and Yanchi Lu analyzed the data and wrote the manuscript; Menghuan Dang, Rui Xia, and Liai Xu were involved in the interpretation of the data. All authors read and approved the final manuscript before submission.

## Ethical approval

*P. hadiensis* is not an endangered or a protected species, and the sample collection site is neither privately owned nor protected for which no specific permissions were required.

## Disclosure statement

The authors reportedly had no conflict of interest.

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## Data availability statement

The data of this study are openly accessible in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/> under the accession number OP611428.

The associated BioProject, SRA, and BioSample numbers are PRJNA875654, SRR21387184, and SAMN30629428, respectively.

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