

PLASTOME ANNOUNCEMENT



## Complete chloroplast genome sequence of a subtropical tree, *Actinodaphne cupularis* (Lauraceae)

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### ABSTRACT

*Actinodaphne cupularis* is a multi-purpose tree that grows in central or southwest China and is one of the primary source plants for making *Hawk tea*. In this study, we firstly assembled and characterized the complete chloroplast (cp) genome of *A. cupularis* using Illumina pair-end sequencing. The results revealed that the total length of the cp genome was 152,748 bp with 39% of the guanine-cytosine content, including a pair of 20,066 bp reverse repeat regions, a large single-copy region with 93,788 bp, and a small single-copy region with 18,828 bp. One hundred twenty-four genes were identified, including eight rRNA genes, 36 tRNA genes, and 80 protein-coding genes. In addition, the maximum likelihood phylogenetic tree was constructed between the cp genome of *A. cupularis* and its related species. The results suggested that *A. cupularis* was more closely related to *Actinodaphne obovate* and then formed a sister clade with the *Machilus* genus. This study not only analyzed the cp genome characteristic information of *A. cupularis* but also provided a specific basis and foundation for future research.

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*Actinodaphne cupularis* (Hemsley) Gamble (Journ. Linn. Soc. Bot. 26: 380. 1891) is an indeciduous shrub or small tree naturally distributed in the forests of southwestern and southern China, including mountain slopes, streamsides, thickets, and dense forests with 300–1300 m (Huang and Henk 2008). The flowering period is from October to November, and the fruiting period is from August to September. As one of the raw materials for processing *Hawk tea*, *A. cupularis* has high value in making beverages (Tan et al. 2016; Feng et al. 2019). However, the primary research on genetics and molecular biology of *A. cupularis* is limited, which leads to the slow progress of germplasm resources protection and comprehensive utilization, and restricts industrial development. Here, we report the complete cp genome sequence of *A. cupularis* uploaded to NCBI (GenBank No: OL979482).

The materials used in this experiment were collected from the natural habitats of Dazhou city (107°50'E, 31°21'N), Sichuan province, China. No protected or endangered species were involved in this study, and no specific permissions were required for this sample. Fresh leaves were immediately dried in silica gel after collection sent for sequencing. The voucher specimen was deposited at the Herbarium of Forestry College, Guizhou University (specimen code YA202108AC03, Xingyong Cui, [cuixy0520@163.com](mailto:cuixy0520@163.com)). The genomic DNA of *A. cupularis* was extracted by the CTAB method (Doyle and Doyle 1987), and the quality was detected by 1% agarose gel electrophoresis and ultraviolet spectrophotometer. Based on passing the quality verification, the DNA was randomly

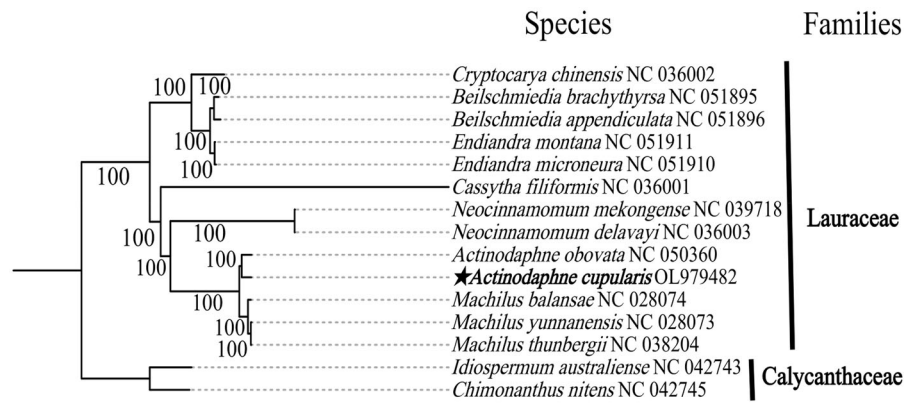
sheared by the Covaris M220 focused-ultrasonicator, in which 350 bp fragments were used for library construction, and then paired-end 150 bp sequencing was performed using Illumina HiSeq 4000 platform. The raw reads obtained after sequencing were first filtered using the NGS QC toolkit\_v2.3.3 (Patel and Jain 2012) to remove the low-quality sequences at the joints and both ends to obtain high-quality sequences to be analyzed. The chloroplast genome of *A. cupularis* was assembled by inputting the script of GetOrganelle (Jin et al. 2020), and then clean reads were viewed by Bandage (Wick et al. 2015). The online annotation tool CPGAVAS2 (Shi et al. 2019) annotated the cp genome and compared it with the cp sequence of *Lindera erythrocarpa* as a reference. The tRNAscan-SE was used to verify the tRNA genes of *A. cupularis* (Schattner et al. 2005). MAFFT v7.271 (Katoh and Standley 2013) software was used for multiple alignments of sequences, and Maximum Likelihood (ML) was used to analyze the phylogenetic relationship after manual inspection and adjustment. The ML phylogenetic tree was generated by IQ-TREE v1.6.12 (Minh et al. 2020). GTR + F + I + G4, chosen based on Bayesian Information Criterion, was selected as the best-fit model according to the ModelFinder method.

In this study, the entire length of the cp genome sequence of *A. cupularis* was 152,748 bp and presented a typical circular shape. The structure consisted of a large single-copy region (LSC) of 93,788 bp, a small single-copy region (SSC) of 18,828 bp, and a pair of reverse repeat regions (IR) of 20,066 bp. The cp genome's total guanine-cytosine (GC)

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**Figure 1.** Maximum-likelihood phylogenetic tree of *A. cupularis* based on complete cp genomes of 14 previously reported species (all the sequences were downloaded from NCBI GenBank; Numbers on the nodes are bootstrap values from 1000 replicates).

content was 39%. According to the annotation results, the cp genome of *A. cupularis* contains 124 genes, including 80 protein-coding genes, 36 tRNA genes, and eight rRNA genes. The phylogenetic analysis based on the cp genomes of 13 species in Lauraceae and two from Calycanthaceae suggested that *A. cupularis* was more closely related to *Actinodaphne obovata* and then formed a sister clade with the *Machilus* genus (Figure 1). The results of this study are helpful for the further study of the phylogeny of the *Actinodaphne* genus and the utilization of tea-like species resources.

### Author contributions

BX conceived the project and designed the study. XB and JP performed the sampling and experiments. XB performed the data analysis and wrote the manuscript. BX edited the manuscript. All authors read and approved the final manuscript.

### Disclosure statement

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

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### Data availability statement

The data that support the findings of the study are openly available in GeneBank at <https://www.ncbi.nlm.nih.gov/>. The complete chloroplast genome has been deposited in GeneBank with accession number

OL979482. The associated Bio-Sample, SRA, and BioProject numbers are SAMN25010770, SRS11758195, and PRJNA797553, respectively.

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