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# Sequencing and characterization of the chloroplast genome of *Aconitum forrestii* Stapf provide insights into phylogenetics in *Aconitum*

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**ABSTRACT**

*Aconitum forrestii* Stapf is an essential traditional Chinese medicine, and is beneficial in dispelling wind, removing dampness, warming, and relieving pain. However, its phylogenetic position of *Aconitum* is not accepted yet. In order to clarify the evolutionary relationship of *A. forrestii*, complete sequencing of chloroplast genome was carried out using Illumina sequencing technology. In total, the chloroplast genome was about 155,869 base pair (bp) in length and carried a typical tetrad structure that included a large single-copy, a small-single copy and two inverted repeat regions. A total of 132 genes were annotated, that included 85 protein -coding genes, 37 transfer RNA genes, eight ribosomal RNA genes, and two pseudogenes. The phylogenetic tree analysis indicated that *Aconitum forrestii* is closely related to *Aconitum episcopale* and *Aconitum delavayi*.

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**KEYWORDS**
*Aconitum forrestii*; phylogenetic analysis; chloroplast genome

*Aconitum* contains various essential medicinal plants, which are widely known in Asian countries such as China, India, and Japan (Liu et al. 2017). Currently, it has been found that there are 200 species of *Aconitum* in China (Liu et al. 2020). Among them, *Aconitum forrestii* Stapf 1910 is a vital medicinal plant in subgenus *Aconitum*, which is mainly distributed in northwest Yunnan (Xu et al. 2013). According to studies, *A. forrestii* is beneficial in dispelling wind, removing dampness, warming, and relieving pain (Liu et al. 2021). The molecular biology of *A. forrestii* however has not been reported yet and the phylogenetic position of *Aconitum* is unknown. Based on the literature survey, chloroplast (cp) genomes are valuable sources of phylogenetic analysis and play a significant role in identifying plants though molecular methods (Wei et al. 2020). The phylogenetic position of four *Aconitum* plant have been confirmed by some scholars (Meng et al. 2018). In this study, the total chloroplast genome of *A. forrestii* was acquired using Illumina Novaseq sequencing and the phylogenetic position in the *Aconitum* was explained.

To conduct the study, fresh leaves of *A. forrestii* were collected from Jade Dragon Snow Mountain of Yuhu village, located in Yunnan province, China (27°22′8″ N, 100°12′2″ E; elevation 3330 m). A specimen was deposited at the College of Pharmacy, Dali University (https://www.dali.edu.cn/, Professor. Cong-long Xia and long7484@126.com) under the voucher number 20200917.

Using a Plant DNA kit (OMEGA, China), the total genomic DNA was extracted. Sequencing of the library was done on the Illumina NovaSeq system (Illumina Inc., San Diego, CA). The raw readings were filtered using Trimmomatic v0.32 (Bolger et al. 2014), and a total of 6.9 GB raw reads were acquired. After the filtration process, NOVOPlasty was used to congregate the chloroplast genome of *A. forrestii* (Dierckxsens et al. 2016). Finally, the annotation of the complete cp genome was done using GeSeq (Tillich et al. 2017).

The complete cp genome of *A. forrestii* had a length about 155,869 bp (GenBank accession number MZ959044), which was composed of a large single-copy (LSC) region of 86,460 bp, a small single-copy (SSC) region of 16,937 bp, and a pair of inverted repeats (IRs) regions of 52,472 bp. Furthermore, the cp genome encoded 132 genes, including 85 protein-coding genes (seven duplications), 37 tRNA genes (seven duplications), eight rRNA genes (four duplications), and two pseudogenes (*rps19*, *ycf1*). The total GC content was found to be 38.07%, and the GC contents in LSC, SSC, and IR regions were 36.2%, 32.6%, and 43.0%, respectively.

For further exploration of the phylogenetic position of *A. forrestii*, 32 complete chloroplast genomes of *Aconitum* were constructed using Maximum Likelihood (ML) phylogenetic tree. In addition, *Berberis bealei* and *Epimedium xichangense* of Berberiaceae served as outgroups. The alignation of the whole cp genome sequences was done using MAFFT v.7 (Katoh and Standley 2013), and the ML bootstrap analysis with 1000 replicates was gathered using RaxML version (Stamatakis 2014). Figure 1 showed that, *A. forrestii*, *A. episcopale*, *A. delavayi*, *A. vilmorinianum* and *A. hemsleyanum* were clustered as a single branch. Meanwhile, *A. forrestii* was found to be a sister to *A. episcopale* and *A. delavayi*, indicating a closed relationship between them. Besides, the ML tree indicated eight species in subgenus *Aconitum*.

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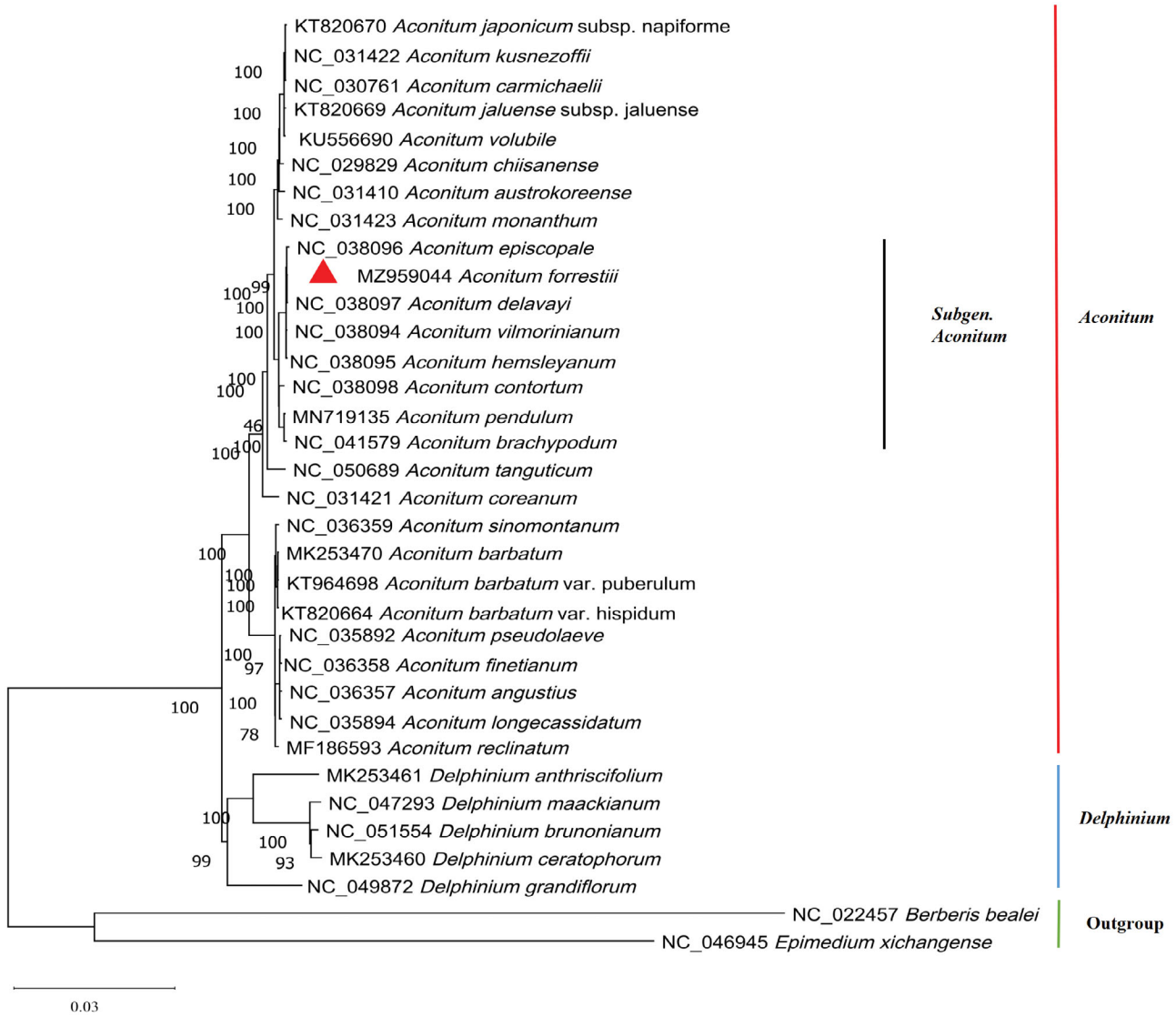


**Figure 1.** Phylogenetic tree inferred from 34 complete chloroplast genome sequences based on Maximuum Likehood. The position of *A. forrestii* is shown in the red triangle and bootstrap values are given for each branch.

To conclude, the study enriched molecular biological research by sequencing the complete cp genome of *Aconitum forrestii* and creating ML trees, which will help understand genetic information and protect the plant's valuable resources.

## Ethical approval

This article is licensed under a Regulations of Yunnan Province on biodiversity protection and approved by Yulong counties (Yunnan Province, China), Dali University (Yunnan province of China).

## Author contributions

Mei-hua Yang performed the experiments, analyzed the data, prepared figures and tables, and authored drafts of the paper; Yun-hui Guan and Ying Wang collected materials and analyzed the data; Cong-long Xia designed the experiment, reviewed and edited the draft; Hai-zhu Zhang and Xu-bing Chen performed the experiments and interpreted the data for the work.

## Disclosure statement

The authors declare no any conflict of interest in the preparation and execution of this manuscript.

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## Data availability statement

The 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. MZ959044. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA762651, SRR15860542, and SAMN21399918 respectively.

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