

## The complete chloroplast genome sequences of two ornamental *Epimedium* species (Berberidaceae)

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### ABSTRACT

Both *Epimedium sutchuenense* Franch. 1894 and *E. fargesii* Franch. 1894 are perennial herbs with excellent ornamental values due to their showy flowers. However, little molecular research has been done on these species. Here, the complete chloroplast (cp) genome of *E. sutchuenense* and *E. fargesii* was analyzed and reported. The cp genome sizes of *E. sutchuenense* and *E. fargesii* were 157,263 and 157,133 bp, respectively. Both the two cp genomes contained a pair of inverted repeat regions (IRs) (25,782 and 25,796 bp), separated by the small single-copy (SSC) region (17,106 and 17,071 bp) and a large single-copy (LSC) region (88,593 and 88,470 bp). A total of 113 unique genes were annotated in each of the two cp genomes, including 79 protein-coding genes, 30 tRNA genes, and four rRNA genes. Phylogenetic analysis supported a close relationship between *E. sutchuenense* and *E. wushanense*, while *E. fargesii* had no clear clustering branch.

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## 1. Background

*Epimedium* L., the largest genus of Berberidaceae, is a perennial herb discontinuously distributed in eastern Asia and the Mediterranean regions (Stearn 2002; Ying et al. 2011). The genus contains approximately 68 species/taxa, among which 58 species were distributed in China (Xu et al. 2020). It was noted that 27 Chinese *Epimedium* species were listed in the Red List of China Higher Plants (Qin et al. 2017). Some *Epimedium* species are of outstanding ornamental values (Probst 1998; Ren et al. 2008; Avent 2010). One of such species, *E. sutchuenense* Franch. 1894 is an excellent ornamental groundcover plant because of its rapidly growing rhizomes and attractive foliage and flowers. The species is mainly distributed in Sichuan, Hubei, Shaanxi provinces, and Chongqing city of China (Stearn 2002; Ying et al. 2011). Another species, *E. fargesii* Franch. 1894 is endemic to mountainous areas of Chongqing City, Sichuan, and Hubei Provinces (Stearn 2002; Ying et al. 2011; Xu et al. 2020). Due to its excellent ornamental values, the plant has been over-exploited and endangered (Qin et al. 2017).

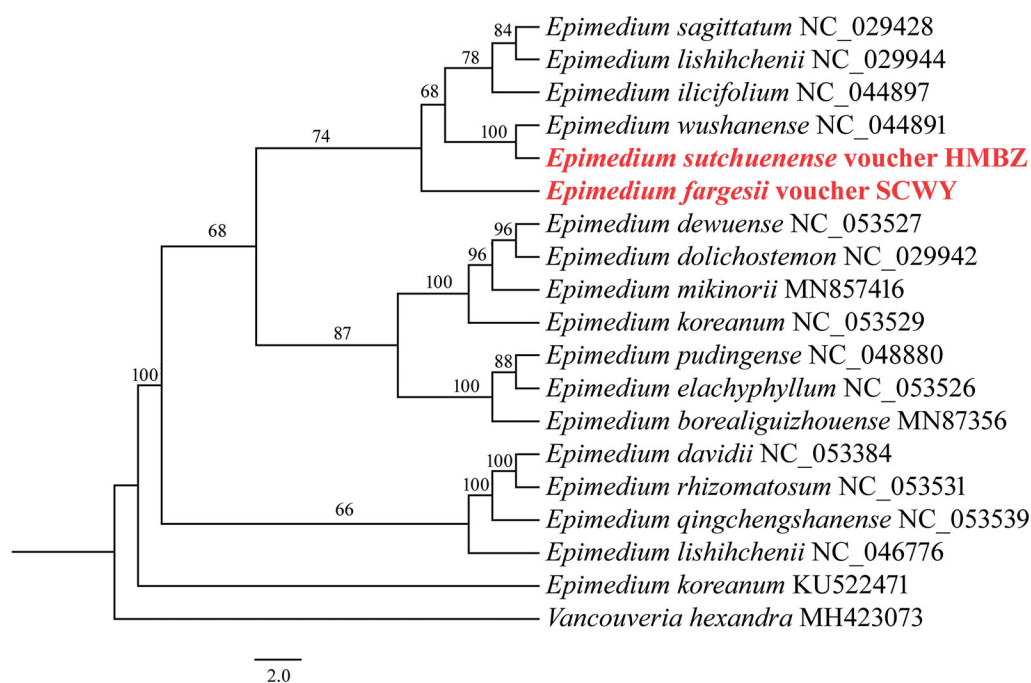
*Epimedium* is one of the most taxonomically difficult representatives of Berberidaceae. Although the complete chloroplast (cp) genomes of same species have been presented recently (Liu et al. 2021a, 2021b), more molecular evidences are indispensable for further investigation of *Epimedium*. In this study, the sampling sites of the current two species were at a distance of about 40 km from those of the two published ones. Given the complex terrains of mountains, the distances would cause the genetic differences within the same species.

Meanwhile, the phylogenetic tree with more species would more accurately explain the evolutionary status of the two species. Here, the complete cp genome of *E. sutchuenense* and *E. fargesii* was sequenced, and the phylogenetic relationship within *Epimedium* was analyzed. The baseline information would be beneficial to the conservation of the horticultural germplasm resources.

## 2. Methods

An individual of *E. sutchuenense* was collected from Shennongjia Forest District, Hubei Province, China (latitude 31.4734, longitude 110.3910); the sample of *E. fargesii* was collected from Chengkou County, Chongqing, China (latitude 31.8391, longitude 108.6277). The specimens of the two species were deposited at the Herbaria of Jiangxi University of Chinese Medicine (JXCM) (<https://www.jxutcm.edu.cn/>, Yanqin Xu, [xuyanqin927@163.com](mailto:xuyanqin927@163.com)) under the voucher numbers Y. Q. Xu and S. Y. Tian 2021006 and Y. Q. Xu and S. X. Liu 2016024, respectively.

Genome DNA was isolated from 0.5 g fresh leaves using the CTAB method (Doyle and Doyle 1987). The purified DNA was used to build a sequencing library with the Illumina NovaSeq 6000 platform. The complete cp genomes of two species were assembled using GetOrganelle v1.71 (Jin et al. 2020). The resultant genomes were annotated by the genome annotator GeSeq (Tillich et al. 2017) with *E. wushanense* (NC\_044891) as the reference, and the results were manually adjusted by Geneious (Kearse et al. 2012). The complete



**Figure 1.** The maximum-likelihood (ML) phylogenetic tree based on 19 complete chloroplast genomes, with *Vancouveria hexandra* as an out-group. Numbers at the right of nodes are bootstrap support values ( $\geq 50$ ).

annotation cp genomes were deposited in GenBank with accession numbers MZ645926 (*E. sutchuenense*) and MZ603801 (*E. fargesii*).

A maximum-likelihood (ML) phylogenetic tree was constructed based on the 19 published complete cp genomes of *Epimedium*, with *Vancouveria hexandra* as an out-group. The ML tree was computed by PhyML v.3.0 (Stéphane et al. 2010) under the best model (F81) evaluated by Jmodeltest (Darriba et al. 2012).

### 3. Results

The lengths of the complete cp genomes of *E. sutchuenense* and *E. fargesii* were 157,263 and 157,133 bp, respectively. Both the two cp genomes exhibited typical quadripartite structures, including two inverted repeat regions (IRs), one large single-copy (LSC) region, and one small single-copy (SSC) region. For *E. sutchuenense*, the lengths of the IR, LSC, and SSC regions were 25,782 bp, 88,593 bp, and 17,106 bp, respectively; for *E. fargesii*, the lengths of the corresponding regions were 25,796 bp, 88,470 bp, and 17,071 bp, respectively. Both the two cp genomes showed overall GC content of 38.78%. The GC contents of *E. sutchuenense* were 37.37, 32.77, and 43.20% in the LSC, SSC, and IR regions, respectively, while these of *E. fargesii* were 37.38, 32.74, and 43.18%, respectively. Each of the two cp genomes was comprised of 113 unique genes, including 79 protein-coding genes, 30 tRNA genes, and four rRNA genes. Among them, 19 genes were duplicated, including seven protein-coding genes, eight tRNA genes, and four rRNA genes.

The phylogenetic analysis showed a close relationship between *E. sutchuenense* and *E. wushanense*, although the two species belong to different series with distinct

morphological traits (Figure 1). The similar phylogenetic pattern was also reported in recent studies within the genus *Epimedium* (Huang et al. 2020; Guo et al. 2021). Contrastingly, *E. fargesii* had no clear cluster with other species of *Epimedium*, which might be associated with the short history of speciation of the species (Zhang et al. 2007; De Smet et al. 2012; Guo et al. 2021).

### Ethical approval

The collecting of all samples in this study followed the Regulations on the Protection of Wild Plants of the People's Republic of China.

### Author contributions

YX and HH contributed the design of the study; ST, QT, and XP performed the sample collection and experiments; ZZ, FL, and XP performed analysis and interpretation of the data. YX, QT, ZZ, and HH were involved in the drafting of the manuscript. YX supervised. YX and HH revised the manuscript critically for intellectual content. All authors were involved in the final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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## Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/>, reference numbers MZ645926 (*E. sutchuenense*) and MZ603801 (*E. fargesii*). The associated BioProject, SRA, and Bio-Sample numbers of *E. sutchuenense* are PRJNA771213, SRR16348881, and SAMN22256841, respectively, and these of *E. fargesii* are PRJNA771209, SRR16350057, and SAMN22256802, respectively.

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