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Complete chloroplast genome of *Cymbidium ensifolium* (Orchidaceae)

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ABSTRACT

Cymbidium ensifolium is one of the most economical and ornamental significant orchids. It's facing serious genetic resources loss and habitat fragmentation. A conservation strategy is therefore imperative for this endangered orchid. Here, we report on the first complete chloroplast (cp) genome of *C. ensifolium*. Its full-length of 150,257 bp include large single-copy (LSC) region of 85,110 bp, small single-copy (SSC) region of 13,761 bp, and a pair of invert repeats (IR) regions of 25,692 bp. Plastid genome contain 137 genes, 78 protein-coding genes, 38 tRNA genes, and 8 rRNA genes. *Cymbidium ensifolium* was sister to *C. sinense, Cy. Tortisepalum*, and *C. kanran*. The cp genome will help for further research and conservation of *C. ensifolium*.

Cymbidium ensifolium is a terrestrial orchid, distributed in the southern China at latitude 28°N and southeastern Asia and India (Chen 2011). This small perennial plant occurs usually in warm and humid environments with scattered sunlight and well-ventilated and drained conditions (Chen 2011). *Cymbidium ensifolium* is known as ornamental flowering plant, and it is widely used in horticultural gardening (Chen 2011). Unfortunately, the natural populations of *C. ensifolium* have been dramatically decreased and their habitats have been fragmented over the past decades, mainly due to natural habitat loss and over-harvesting (Fay and Chase 2009; Fay 2018). Therefore, *C. ensifolium* is listed in the China Species Red List as an endangered species (Luo et al. 2003; Swarts and Dixon 2009; Seaton et al. 2010). It is urgent to establish an effective conservation strategy for this significant plant. The complete chloroplast genomic data will be useful for population and phylogenetic studies of *C. ensifolium*.

In this study, we assembled the complete cp genome of *C. ensifolium*. Fresh leaf sample of *C. ensifolium* was acquired from Beifeng (119°16′57.90″E, 26°13′12.24″N), Fuzhou City, Fujian Province of China, and voucher specimen deposited at Herbarium of College of Forestry, Fujian Agriculture and Forestry University (specimen code FAFU08014). DNA extraction from fresh leaf tissue, with 350 bp randomly interrupted by the Covaris ultrasonic breaker for library construction. The constructed library was sequenced using PE150 Illumina Hiseq Xten platform, approximately 2GB data were generated. Illumina data were filtered by script in the cluster (default parameter: -L 5, -p 0.5, -N 0.1). Plastid genome were assembled using GetOrganelle pipe-line (https://github.com/Kinggerm/GetOrganelle), it can get the plastid-like reads, and the reads were viewed and edited by Bandage (Wick et al. 2015). Assembled chloroplast genome was annotated by comparing with *C. ensifolium* using GENEIOUS R11.15 (Biomatters Ltd., Auckland, New Zealand) (Kearse et al., 2012). The annotation result was draw using the online tool OGDRAW (http://ogdraw.mpimp-golm.mpg.de/) (Lohse et al., 2013).

The complete plastid genome sequence of *C. ensifolium* (GenBank accession MK841484) was 150,257 bp in length, with a large single-copy (LSC) region of 85,110 bp, a small single-copy (SSC) region of 13,761 bp, and a pair of inverted repeats (IR) regions of 25,692 bp. A total of 137 gene species were annotated, including 78 protein-coding (PCG), 38 transfer RNA (tRNA), and 8 ribosomal RNA (rRNA) gene species. The complete genome GC content was 37.10%. In order to reveal the phylogenetic position of *C. ensifolium*, a phylogenetic analysis was performed based on 10 complete cp genomes of *Cymbidium* (*C. aloifolium, C. tracyanum, C. tortisepalum, C. sinense, C. mannii, C. macrorhizon, C. lancifolium, C. kanran, C. goeringii, C. faberi*) and two taxa (*Eulophia zollingeri, Oncidium sphacelatum*) as outgroups, all of them were downloaded from NCBI GenBank. The sequences were aligned using MAFFT v7.388 (Katoh and Standley 2013), and phylogenetic tree was constructed using RAxML (Stamatakis 2014) (Figure 1). The relationship between *Cymbidium, Oncidium*, and *Eulophia* was strongly supported (bootstrap = 100%). The maximum likelihood phylogenetic analysis shows that *C. ensifolium*s was closely related to *C. sinense, C. tortisepalum* and *C. kanran*.
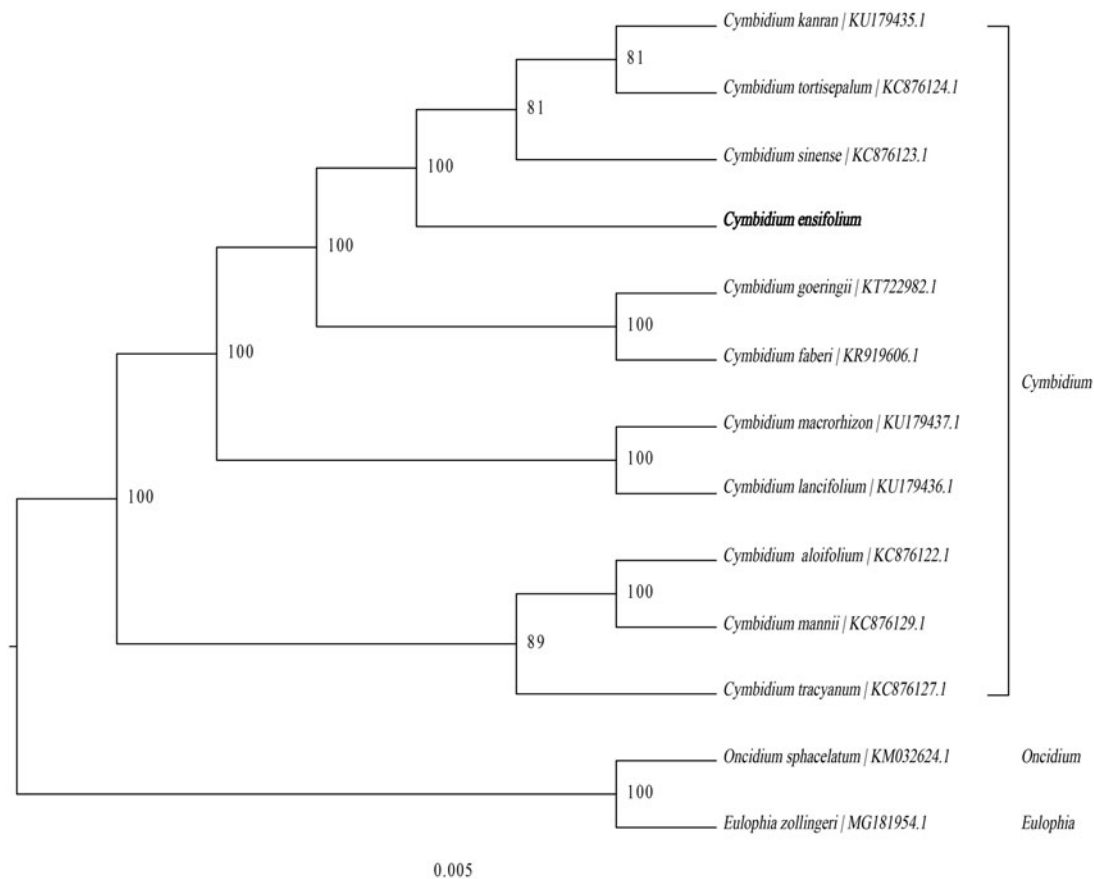
Figure 1. Maximum-likelihood (ML) tree based on 11 complete cp genome in *Cymbidium*, with *Oncidium sphacelatum* and *Eulophia zollingeri* as out group, bootstrap support value near the branch.

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