**The complete chloroplast genome sequence of Anemarrhena asphodeloides Bunge**

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**ABSTRACT**

*Anemarrhena asphodeloides* represents the only species in monotypic genus *Anemarrhena*. Its rhizome is called Zhi Mu and has been used in traditional medicine in China for more than 2000 years. We sequenced the complete chloroplast (CP) genome of this plant. The CP genome of *A. asphodeloides* is 157,734 bp in length, containing a pair of 53,313 bp inverted repeat regions (IRs) separated by one large and one small single copy region (LSC and SSC) of 85,851 and 18,570 bp, respectively. The overall AT content of the CP genome is 62.15%. The phylogenetic analysis strongly supported that *A. asphodeloides* comprises a monophyly, and it is affiliated to Subfam. Agavoideae (Asparagaceae). The results will be useful for conservation, phylogenetics, and identification of this species.

*Anemarrhena asphodeloides* Bunge is one of the important medicinal plants, which has been used for over 2000 years in China. It has the effects of clearing away heat and reducing fire, nourishing yin, and moistening dryness (Han and Park 1997; Chinese Pharmacopoeia Commission 2015). Presently, the wild *A. asphodeloides* has become quite rare in China as a result of over-exploitation and the deterioration of natural conditions (Sun et al. 2008). *A. asphodeloides* is the only species in genus *Anemarrhena*, a taxonomically controversial group, whose phylogenetic position has frequently changed during the past 40 years (Delectis Florae Reipublicae Popularis Sinecae 1980; APG 2016). In this study, we sequenced the complete chloroplast genome of *A. asphodeloides*, in order to give a phylogenomic insight to systematic position of the genus *Anemarrhena* and provide genetic information for conservation of this species.

Fresh leaves of *A. asphodeloides* were collected from Pingshan County, Hebei Province in China (Latitude: 38°13′0.09″N, Longitude: 114°6′13.69″E). The voucher specimen (Chao Zhi 147311) was identified by Prof. Zhi Chao and deposited in the herbarium of the School of Traditional Chinese Medicine, Southern Medical University. The genomic DNA was extracted from 100 mg of fresh leaves with the modified CTAB method (Yang et al. 2014). Sequencing was performed on an Illumina HiSeq 4000 in high output mode with 2 × 125 bp paired-end reads at Beijing Genomics Institute (BGI, Shenzhen, China). The CP genome of *A. asphodeloides* was assembled by SOAPendv02.04 (Luo et al. 2012) with *Agave attenuata* as a reference (Accession No. NC032696). The Dual Organellar Genome Annotator (DOGMA) software (Wyman et al. 2004) was used for genome annotation. The annotated sequence had been deposited in GenBank (Accession No. MH669277).

The complete CP genome of *A. asphodeloides* was a double-stranded circular DNA of 157,734 bp in length. Its quadripartite structure was composed of two inverted repeated regions (IRa and IRb) of 53,313 bp, separated by a large single-copy (LSC) region of 85,851 bp and a small single-copy (SSC) region of 18,570 bp. The complete CP genome contains a total of 112 genes, including 88 protein-coding genes, 20 tRNA genes, and 4 rRNA genes. Eighteen genes were duplicated in the IR regions. Fifteen genes contain one intron, while three genes have two introns. The over-all A-T content of the chloroplast genome is 62.15%.

A phylogenetic analysis was performed based on 19 complete CP genome, to reveal the phylogenetic position of genus *Anemarrhena*. All the CP genome sequences were aligned with MAFFT (Kato and Standley 2013). The maximum likelihood (ML) tree was inferred in MEGA 6.0 based on GTR + R + I model using 1000 bootstrap replicates (Tamura et al. 2013). The reconstructed phylogenetic tree showed that genus *Anemarrhena* was closely related to genus *Agave* and was affiliated to Subfam. Agavoideae (Asparagaceae), which is consistent with the APG IV. (Angiosperm Phylogeny Group 2016) (Figure 1).

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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Figure 1. Maximum-likelihood (ML) tree based on the chloroplast genome of 19 taxa, including Anemarrhena asphodeloides and two outgroup taxa (Stemona mairei and S. japonica). The bootstrap support values are indicated at the nodes.