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MITOGENOME ANNOUNCEMENT

Complete mitochondrial genome sequence of *Acer miaotaiense* (Aceraceae)

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ABSTRACT

Acer miaotaiense P. C. Tsoong is a rare and endangered tree endemic to the Qinling Mountains of China and is listed as a national third-class protected plant. In this study, we sequenced the complete mitochondrial genome of *Acer miaotaiense* using the Illumina Novaseq 6000 and Nanopore platforms. The total mitochondrial genome length is 819,227 bp and has 69 genes, including 41 protein-coding, 25 tRNA, and 3 rRNA genes. The genome nucleotide composition was asymmetric, with an overall G + C content of 45.7%. Phylogenetic analysis indicated that *Acer miaotaiense* is closely related to the congeneric *Acer yangbiense*.

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Acer miaotaiense; mitochondrial genome; phylogenetic relationship

Acer miaotaiense, Aceraceae, is a deciduous tree named after its discovery location of Miaotaizi, Liuba County of Shanxi Province, by the Chinese botanist Zhong Buqiu in 1954, and is mainly distributed in the Shanxi and Gansu provinces of the central and western Qinling Mountains in China (IB-CAS 1989). Aceraceae has high utilization value, with its tough wood used in construction, and utensils manufacturing, its seeds for industrial oil, and its fruits, leaves and bark as raw materials of tannin extract (Zhou and Yao 2003). It has thus in recent years become the subject of biological, ecological, and genetic studies. Aceraceae has strict habitat requirements, and its natural populations currently exhibit dynamic characteristics of gradual declination (Meng et al. 2016). These populations' genetic structures are characterized by low levels of genetic diversity associated with strong genetic differentiation, and of which genetic drift and inbreeding have been identified as the main influencing factors (Li et al. 2005). Understanding a species' genetic structure and population genetic differentiation is essential prerequisites for formulating informed protection strategies.

Excellent germplasm resources are the basis for breeding and genetic improvements. Although the complete chloroplast genome of *A. miaotaiense* has been reported (Zhang et al. 2016), the species' genetic information remains incomplete. The highly conserved nature and fast evolutionary rate of mitochondria make them an ideal tool for studying evolution and molecular ecology (Janouskovec et al. 2013). Therefore, in this study, we sequenced the complete *A. miaotaiense* mitochondrial genome, which will be helpful in exploring the species' origin and evolution. This information will promote species molecular systematics and conservation genetics, and provide a reference for conducting theoretical studies.

The plant materials used in this study were collected from the Houzhenzi Ecological Experimental Forest Farm, Zhouzhi County, Xi'an City, Shanxi Province (altitude:1,621m, coordinates:33.856726°N, 107.789374°E), and subsequently submitted to the Acer gene bank (36.77°N, 117.471°E). A specimen was deposited at the herbarium of Shandong Provincial Center of Forest and Grass Germplasm Resources (Biao Han, hanbiao3361@shandong.cn) under barcode number SDF1003756. Total genomic DNA (saved in the DNA library of Shandong Provincial Center of Forest and Grass Germplasm Resources with the code mtq2016cp03) was extracted using a Plant DNA extraction Kit (TIANGEN, Beijing, China) according to the manufacturer's instructions.

We used both Nanopore GridION sequencing (Oxford Nanopore Technology, Oxford Science Park) and Illumina Novaseq 6000 platforms to sequence and construct the library of raw sequence data (Nanopore raw data was 12.9 Gb, N50 is 8,784 bp and Illumina raw data was 10.46 Gb). After filtering, clean data were used to assemble *de novo* the mitochondrial genome using Canu2.1 with default settings (Koren et al. 2017). We used Blastn and tRNA scan-SE 2.0 to annotate the protein coding, rRNA, and tRNA genes (Lowe and Eddy 1997). The annotated mitogenome was deposited in GenBank under the accession number MZ636518. The Blast Web server with 'Algin two or more sequences' option was used to find conserved segments between multiple query sequences, and BMGE (Criscuolo and Gribaldo 2010)

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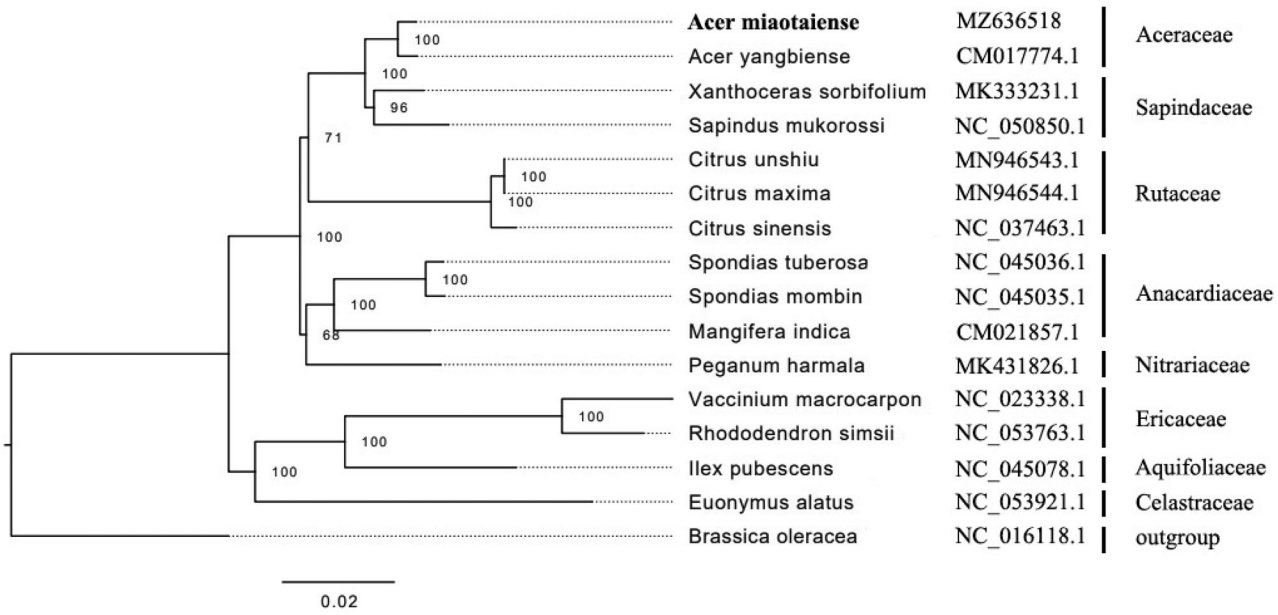

Figure 1. Maximum-likelihood (ML) tree of *A. miaotaiense* and other 14 species based on the conserved segments of mitogenome sequences with *B. oleracea* as outgroup. The accession numbers are showed in the figure, and the numbers behind each node are bootstrap support values.

selected regions for the construction of the evolutionary tree (Bi et al. 2018). The model-finder was then used to chop the model WAG + I + G, and the Maximum Likelihood (ML) phylogenetic tree was constructed by RAxML v8.2.9 software with 1,000 bootstrap replicates (Stamatakis 2014).

The *A. miaotaiense* complete mitochondrial genome was assembled into a single circular-mapping molecule of 819,227 bp with a GC content of 45.7%. The overall A, C, G, and T contents were 27.2, 22.8, 22.9, and 27.1%, respectively. The mitogenome contained 69 genes, including 41 protein-coding genes, 25 tRNA genes, and three rRNA genes. Among the 41 protein-coding genes, seven contained introns—ccmFc and rps14 had a single intron, and nad1, nad2, nad4, nad5, and nad7 had four introns. Five genes (nad1, nad2, nad4, nad5, and nad7) were trans-spliced.

To determine the phylogenetic position of *A. miaotaiense*, phylogenetic analyses were conducted based on the mitogenome sequences of 14 species from GenBank using maximum likelihood (ML) methods, with *Brassica oleracea* as the outgroup. As expected, the phylogenetic results showed that *A. miaotaiense* was closely related to *Acer yangbiense*, and was clustered into a group with *Sapindus mukorossi* and *Xanthoceras sorbifolium*. This analysis also supported Aceraceae and Sapindaceae as sister taxa with a bootstrap probability of 100% (Figure 1), which was consistent with prior results based on morphology and other molecular methods (de Jong 1994; Gadek et al. 1996; Yang and Li 2010).

Ethical approval statement

The authors complied with the international Union for Conservation of Nature (IUCN) policies research involving species at risk of extinction (see Guidelines for appropriate uses of IUCN Red List data), the Convention on Biological Diversity and the Convention on Trade in Endangered Species of Wild Fauna and Flora. The research was approved by the Department of Wild Fauna and Flora Protection of China's National Forestry and Grassland Administration, and the contract number is 2020070316.

Author contributions

Dan Liu (experimental design and thesis writing), Ping Ding (sequencing and conducting experiments), Hai-Li Guo (resource investigation, sampling and thesis writing), Ying Chen (data analysis), Kun Zhao (data analysis), Hai-Ping Yang (sampling and sequencing), Ting Xu (data analysis), Li-Jiang Liu (sampling), Qi Jing (data analysis), Shang-Jun Han (analysis), Bo-Qiang Tong (paper revision), and Wen-Qing Li (experimental design, paper revision); and that all authors agree to be accountable for all aspects of the work.

Disclosure statement

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

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Data availability statement

The genome sequence data are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession no. MZ636518. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA748856, SRR15304752, SRR15304753 and SAMN20353478 respectively.

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