Characterization of the chloroplast genome of *Meterostachys sikokianus* (Makino) Nakai (Crassulaceae) and its phylogenetic analysis

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**ABSTRACT**

*Meterostachys* is a monotypic genus of Crassulaceae, though its phylogenetic position remains unclear. Here, we report the complete chloroplast (cp) genome sequence of *Meterostachys sikokianus* using the Illumina high-throughput sequencing approach. The cp genome was 149,860 bp in length, containing a large single copy (LSC) of 82,293 bp and a small single copy (SSC) of 16,879 bp, which were separated by a pair of 25,344 bp inverted repeats (IRs). The overall GC content of the *M. sikokianus* cp genome was 37.6%. A total of 113 unique genes were annotated, consisting of 79 protein-coding genes (PCGs), 30 transfer RNAs (tRNAs), and four ribosomal RNAs (rRNAs). Among these genes, eighteen contained one or two introns. A maximum-likelihood (ML) phylogenetic analysis based on 30 accesses of Crassulaceae showed that *M. sikokianus* was most closely related to *Orostachys japonica* and *Orostachys fimbriata*.

The family Crassulaceae consists of species of predominantly succulent appearance, adapted to xerophytic conditions and characterized by a specific type of crassulacean acid metabolism (CAM) (Gontcharova et al. 2006). Among these, *Meterostachys* Nakai is a monotypic genus containing only one species, *Meterostachys sikokianus* (Makino) Nakai. This was initially described as *Cotyledon sikokiana* (Makino 1891). Later, *Meterostachys* was segregated into a new genus because its morphological characteristics differed from those of *Cotyledon* (Hara 1935). However, the phylogenetic relationship and taxonomic position of *M. sikokianus* remain uncertain because allied genera such as *Hylotelephium*, *Orostachys*, and *Sinocrassula* form a polytomy according to all previous phylogenetic studies (Mort et al. 2001; Mayuzumi and Ohba 2004; Gontcharova et al. 2006; Messerschmid et al. 2020). In this study, we report the complete chloroplast (cp) genome sequence of *M. sikokianus* for the first time. This will provide useful genetic information for further studies of the phylogenetic relationships and taxonomic position of *M. sikokianus*.

The plant materials for this study were sampled from Mt. Hwaak (38°00′04″N, 127°31′16″E), Gapyeong-gun, Gyeonggi-do province in South Korea, and a voucher specimen was deposited at the Sangji University Herbarium (voucher no. SJJH000434; KS Cheon, cheonks@sangji.ac.kr). Total DNA was extracted using a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA). Paired-end sequencing for the cp genome of *M. sikokianus* was performed on the MiSeq (Illumina Inc., San Diego, CA, USA) platform. We obtained 3,448,502 raw reads with a length of 301 bp. The assembly and annotation of the mitogenome were accomplished using Geneious prime® v.2021.1.1 (Biomatters Ltd, Auckland, New Zealand). We also compared each gene to the published complete cp genome sequence of Crassulaceae for correct gene annotation. The tRNAs were confirmed using trNAScan-SE (Schattner et al. 2005).

A circular form of the complete cp genome of *M. sikokianus* is a DNA molecule 149,860 bp in length with 37.6 GC content, composed of a large-single-copy (LSC) region of 82,293 bp, a small-single-copy (SSC) region of 16,879 bp, and two inverted-repeat (IR) regions of 25,344 bp. The plastid genome contains a total of 113 unique genes consisting of 79 protein-coding genes, 30 transfer RNA (tRNA) genes, and four ribosomal RNA (rRNA) genes. Of which, eighteen genes are duplicated in IR regions. Additionally, two genes contain two introns (*clpP* and *ycf3*), and sixteen genes contain one intron (*atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpl2*, *rpl16*, *rpoC1*, *rps12*, *rps16*, *trnA-UGC*, *trnG-GCC*, *trnL-GAU*, *trnK-UUU*, *trnL-UAA*, and *trnV-UAC*).

To construct the phylogenetic tree, the complete cp genomes of 29 accesses were selected within the subfamily Sempervivoideae in Crassulaceae. One additional species from the subfamily Kalanchoeae (*Kalanchoe tomentosa*) in Crassulaceae was chosen as an outgroup. The sequences were aligned using MAFFT (Katoh and Standley 2013). A maximum-likelihood (ML) analysis was performed using RAxML v.7.4.2 with 1000 bootstrap replicates and the GTR + I + Γ model (Stamatakis 2006). The ML (maximum-likelihood) tree formed three clades. The first clade consists of *Sedum* alone and formed the most basal part. The second clade is made...
up Phedimus and Rhodiola, with Phedimus forming a sister to Rhodiola. The last clade consists of the remaining genera excluding those mentioned above. Meanwhile, M. sikokianus formed a sister to Orostachys japonica and Orostachys fimbriata, and its phylogenetic position as an independent genus was supported. However, since this study was conducted with only a few taxa, it is judged that additional studies including more diverse taxa are needed.

**Disclosure statement**

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

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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/](https://www.ncbi.nlm.nih.gov/) under the accession no. MZ365442. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA722124, SRR14242008, and SAMN18746007, respectively.

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