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PLASTOME ANNOUNCEMENT

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Characterization of the complete plastid genome and phylogenetic implication of *Micranthes octopetala* (Nakai) Y.I.Kim & Y.D.Kim (Saxifragaceae), endemic to Korea

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ABSTRACT

Micranthes octopetala (Nakai) Y.I.Kim & Y.D. Kim et al. 2015, which belongs to the family Saxifragaceae, is a perennial herb endemic to Korea. *M. octopetala* was originally treated as a synonym of *M. manchuriensis*. However, in 2015, molecular phylogenetic analysis confirmed that *M. octopetala* is an independent species. In this study, the plastid genome of *M. octopetala* was sequenced for the first time, and the taxonomic position of this species was identified. The complete plastid genome of *M. octopetala* has a total length of 149 751 bp (large single copy: 83 083 bp; small single copy: 17 196 bp; inverted repeat: 24 736 bp), containing 130 genes, including 79 CDS, 30 tRNAs, and 4 rRNAs. Moreover, the absence of intron in the *rpl*2 gene, which is a common feature of Saxifragaceae, was confirmed. Phylogenetic analysis based on 79 protein-coding genes from 21 species revealed that *M. octopetala* belongs to the genus *Micranthes,* being a sister to other *Micranthes* species. The plastid genome of *M. octopetala* obtained in this study provides fundamental information for future studies on the genus *Micranthes*.

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Introduction

Micranthes Haw., which belongs to the family Saxifragaceae, is an herbaceous plant genus mainly distributed in the Northern Hemisphere, consisting of approximately 80 species. It previously belonged to the genus *Saxifraga* L. but has been recognized as an independent genus through several molecular phylogenetic studies based on chloroplast DNA genes or internal transcribed spacer (ITS) regions in its genome (Soltis et al. 1996; 2001; Xiang et al. 2012; Deng et al. 2015). Understanding the taxonomic position of *Micranthes* within Saxifragaceae can aid in comparative studies with related genera and contribute to our understanding of the evolutionary history within the family (Deng et al. 2015).

Micranthes octopetala (Nakai) Y.I.Kim & Y.D. Kim et al. 2015, which is endemic to Korea, was treated as an independent species in 2015 by Kim et al. (2015). *M. octopetala* is a perennial plant that typically attains a height of 15–30 cm and grows on the surface of rocks in the shade of deep mountainous areas. Despite controversies regarding the taxonomic position of *M. octopetala*, this has been majorly resolved in a previous study (Kim et al. 2015). *M. octopetala* was originally included in the genus *Saxifraga* (*Saxifraga octopetala* Nakai 1918), but Kim et al. (2015) revealed that *M. octopetala* belongs to the genus *Micranthes* and proposed a new combination-specific name, *Micranthes octopetala*. Although *M. octopetala* was ambiguously treated as *M. manchuriensis*, according to Kim et al. (2015), a phylogenetic tree based on the nrITS region demonstrated that *M. octopetala* and *M. manchuriensis* formed an independent clade. In particular, *M. octopetala* was shown to be phylogenetically close to *M. nelsoniana* and *M. manchuriensis*, with *M. manchuriensis* forming a sister group with *M. nelsoniana* var. *pacifica* and *M. fusca*. In addition, *M. octopetala* is distinguished from *M. manchuriensis* by its distinct morphological features, such as the presence of an underground stolon.

Plastid genomes are often used for phylogenetic analysis because of their high conservation and slow evolutionary rate compared with other genetic materials (Provan et al. 2001; Ravi et al. 2008). However, to date, the plastid genome of *M. octopetala* has remained unknown. To the best of our knowledge, the plastid genome of *M. octopetala* was assembled and presented for the first time in this study, and phylogenetic relationships within the family Saxifragaceae were identified.

Materials and methods

Leaves of *M. octopetala* were collected at Mt. Gwangdeoksan, Hwacheon-gun, Gangwon-do province, South Korea (38°06′55.8″N 127°25′52.3″E) (Figure 1) and voucher specimens were deposited in the Korea National Arboretum (http://www.nature.go.kr/kbi/plant/smpl/KBI_2001_030100.do, Hee-Young Gil, E-mail: warmishe@korea.kr) under the voucher

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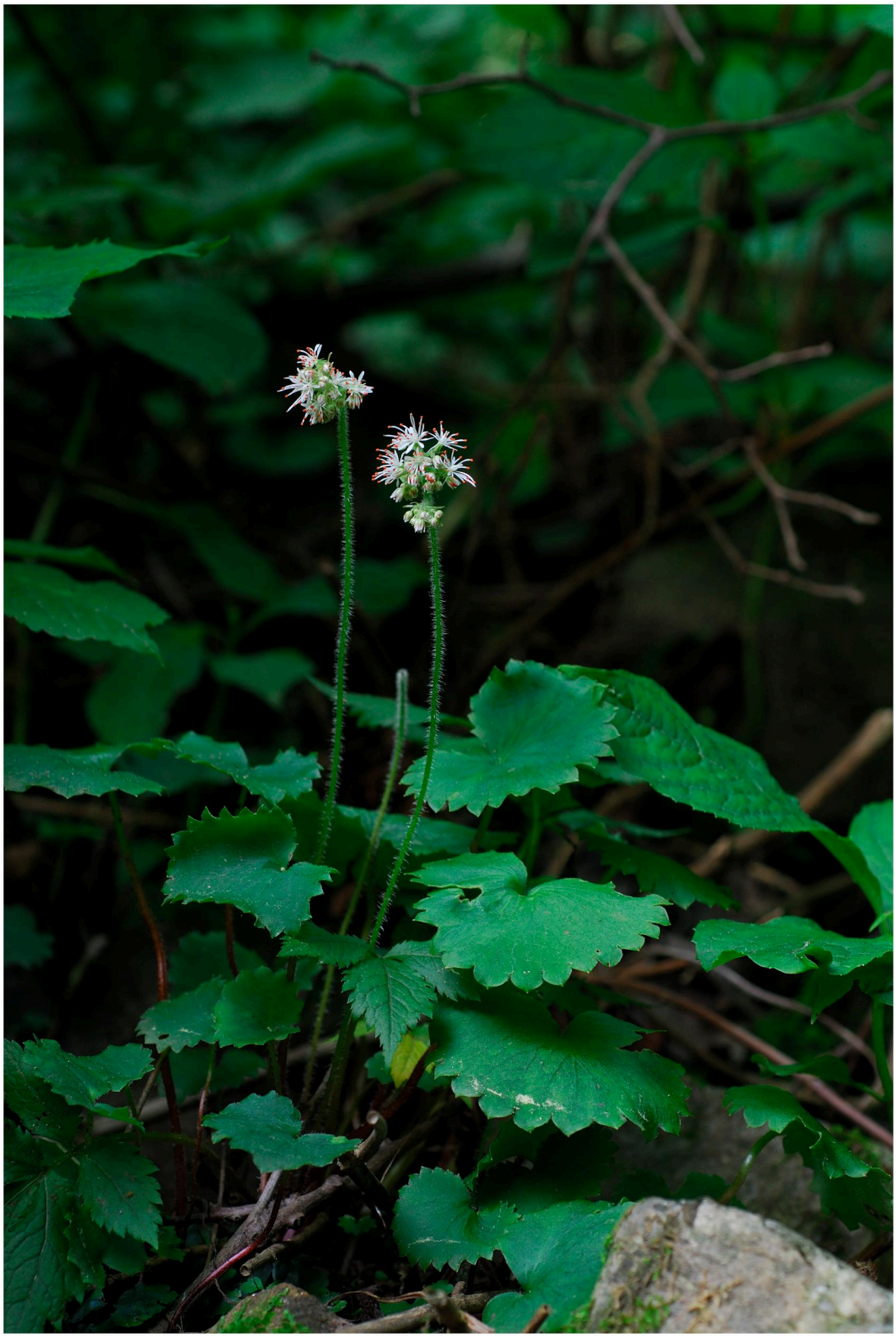

Figure 1. *Micranthes octopetala* in its Native environment at Mt. Gwangdeoksan, Hwacheon-gun, Gangwon-do province, South Korea. The species reference image was taken by Sa-Bum Jang. *M. octopetala* is a perennial herb that grows to 15–30 cm and has 1–4 leaves. Flowers with 8 white petals bloom from June to July. Flowering stems are erect and leafless. The characteristic that can be distinguished from similar species (*M. manchuriensis*) is that it has an underground stolon.

number (Hyh144). Total genomic DNA was extracted from dried leaves using a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) with silica gel. The extracted gDNA was subjected to next-generation sequencing (NGS) using a MiSeq sequencing system (Illumina, Seoul, Korea), and a total of 9 992 786 reads were obtained. To remove poor-quality reads, the raw data were imported and trimmed using Geneious Prime (Kearse et al. 2012) with a 5% error

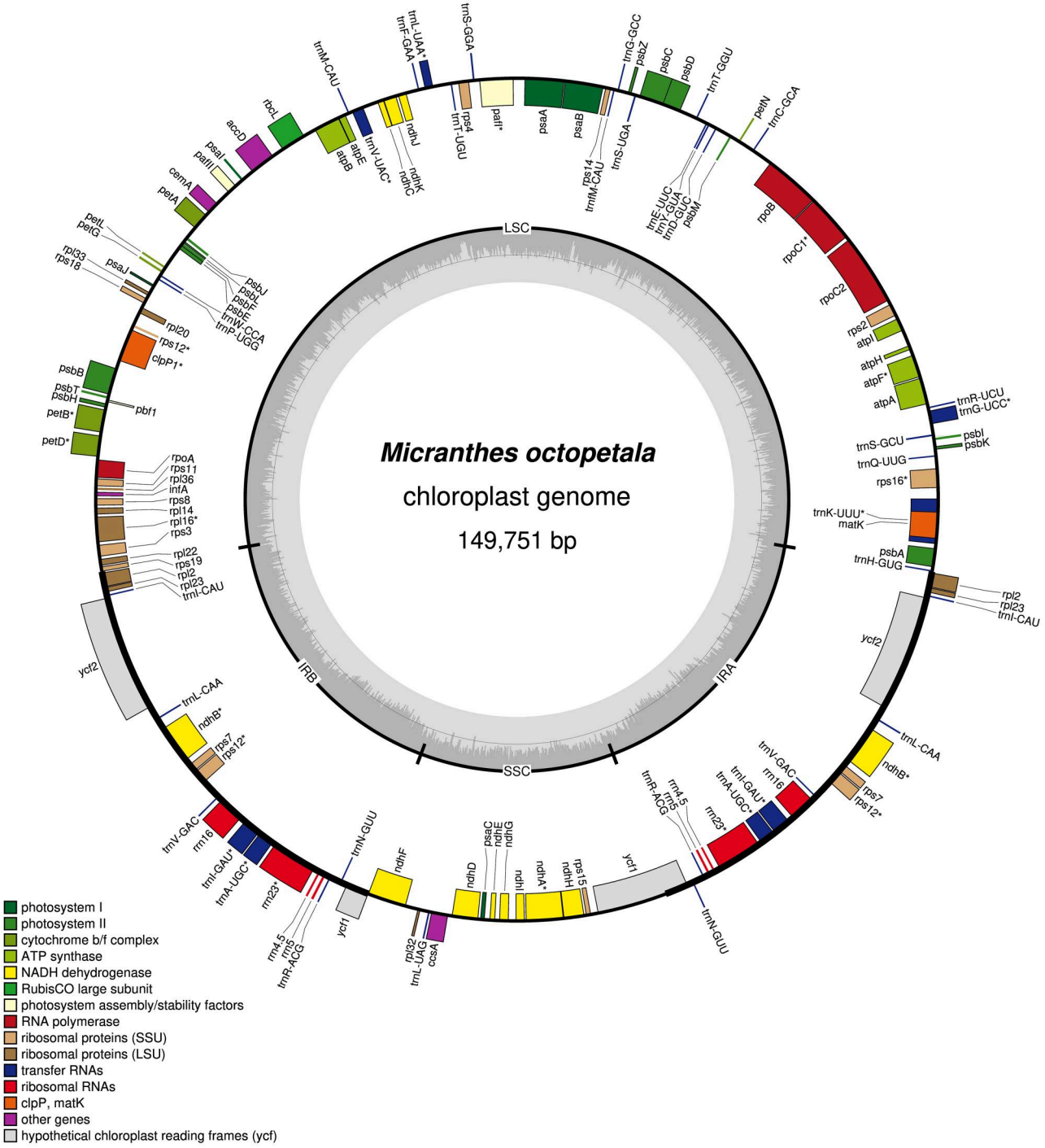

Figure 2. The complete plastid genome of *Micranthes octopetala*. Graphic showing features of its plastome was generated using OGDraw. Colored boxes represent functional groups of plastid-coding genes. The clockwise and counter-clockwise transcribed genes are drawn inside and outside of the circle, respectively. The small gray bar graphs in the inner circle show the GC contents.

probability limitation. Using *M. melanocentra* (GenBank accession = NC_056191.1) as a reference, the "map to reference" option and *De novo* assembly were used so that only raw reads related to the plastid genome were selected and assembled according to the reference (Kearse et al. 2012). The read coverage depth of the assembled genome was 347.21× (Supplementary Figure 1). The gene content and order of the plastome of *M. octopetala* were annotated using GeSeq and scrutinized using Geneious Prime and tRNA scans (Kearse et al. 2012; Tillich et al. 2017; Chan and Lowe 2019). Finally, the OGDraw program was used to visualize the plastid genome of *M. octopetala* (Greiner et al. 2019).

For phylogenetic analysis, 18 species of Saxifragaceae were used, with 3 closely related species of Saxifragaceae (*Ribes glaciale*, GenBank accession number MK887908; *Itea chinensis*, GenBank accession number MH191391; *Penthorum chinense*, GenBank accession number JX436155) being used as outgroups. Using MAFFT, 79 protein-coding genes were extracted from the plastid genome, aligned, and combined to generate molecular data (Katoh and Standley 2013; Zhang

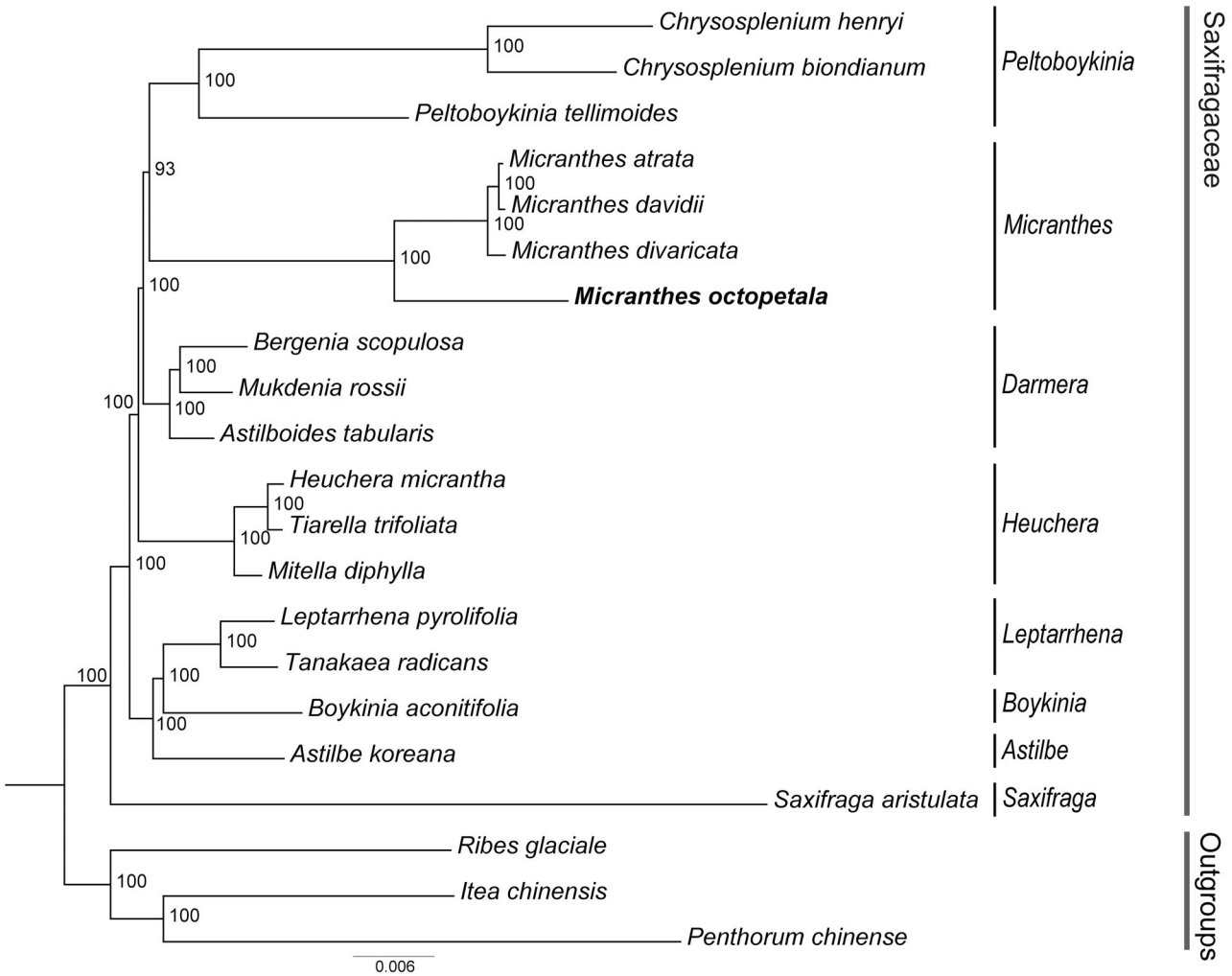

Figure 3. The ML tree of 18 species of Saxifragaceae based on concatenated protein-coding genes. *M. octopetala* is marked in bold. Bootstrap support values shown at the branches. The following sequences were used: *Chrysosplenium henryi* OK336532, *Chrysosplenium biondianum* OK336542, *Peltoboykinia tellimoides* MZ779205 (Yang et al. 2022), *Micranthes atrata* ON720899 (Yuan et al. 2023), *Micranthes davidii* ON720901 (Yuan et al. 2023), *Micranthes divaricata* ON720902 (Yuan et al. 2023), *bergenia scopulosa* KY412195 (Bai et al. 2018), *mukdenia rossii* MG470844 (Liu et al. 2018), *astilboides tabularis* MT316511 (Ha et al. 2020), *heuchera micrantha* OL489769, *tiarella trifoliata* MH708572, *mitella diphylla* MH708564, *leptarrhena pyrolifolia* MN496070 (Folk et al. 2020), *tanakaea radicans* MW300581 (Su et al. 2021), *boykinia aconitifolia* MN496058 (Folk et al. 2020), *astilbe koreana* MK990830, *Saxifraga aristulata* ON720851 (Yuan et al. 2023), *Ribes glaciale* MK887908, *Itea chinensis* MH191391 (Dong et al. 2018), and *Penthorum chinense* JX436155 (Dong et al. 2013).

et al. 2020). Maximum likelihood (ML) analysis was performed based on the concatenated molecular data using the IQ-tree software of the PhyloSuite program (Nguyen et al. 2015; Zhang et al. 2020). According to ModelFinder in PhyloSuite, the best-fit model was GTR + F + I + G4, and an ML tree with 5000 bootstrap replications was constructed (Zhang et al. 2020).

Results

We confirmed that the total length of the plastid genome of *M. octopetala* was 149 751 bp, with 37.8% GC content (Figure 2). It exhibited the typical quadripartite structure of angiosperms, with the lengths of the large single-copy (LSC), small single-copy (SSC), and inverted repeat (IR) regions being 83 083, 17 196, and 24 736 bp, respectively. We also found that the GC content of the IR region (43.3%) was higher than that of the LSC (35.7%) and SSC regions (31.7%). We identified a total of 130 genes in *M. octopetala* plastid genes, including repetitive genes in the IR region, consisting of 79 CDS, 30 tRNAs, and 4 rRNAs (Supplementary Table 1). We detected that 14 genes (*atp*F, *ndh*A, *ndh*B, *pet*B, *pet*D, *rpl*16, *rpo*C1, *rps*16, *trn*A-UGC, *trn*G-UCC, *trn*I-GAU, *trn*K-UUU, *trn*L-UAA, and *trn*V-UAC) contained 1 intron, whereas 2 coding genes (*clp*P1 and *paf*I) had 2 introns (Supplementary Figure 2). In addition, we found that *rps*12 is a trans-splicing gene located in the IRs and LSC regions (Supplementary Figure 3). We further confirmed that the intron of *rpl*2 was deleted.

The ML tree with GTR + F + I + G4 model constructed based on the 79 concatenated protein-coding genes showed that Saxifragaceae is a monophyletic family, with most nodes exhibiting a high support value (Figure 3), as described in previous studies (Soltis et al. 2001; Deng et al. 2015). We determined that *M. octopetala* belongs to the genus *Micranthes* and was positioned as a sister to other *Micranthes* species. We confirmed that the group closest to the *Micranthes* group was the *Peltoboykinia* group (*Chrysosplenium*, *Peltoboykinia*).

Discussion and conclusions

In this study, the plastome of *M. octopetala*, a Korean endemic species, was assembled and its phylogenetic relationships were confirmed. The plastid genome length of *M. octopetala* was determined to be 149 751 bp, containing a total of 130 genes. The absence of introns in the *rpl*2 gene of *M. octopetala*, which is a characteristic commonly observed in Saxifragaceae plants, was demonstrated (Downie et al. 1991; Dong et al. 2013; Liu et al. 2020). Gene losses occur frequently during the process of plant evolution and provide evidence for elucidating the evolutionary history of plants (Jansen et al. 2007; Han et al. 2022). In the case of *M. octopetala*, the loss of the *rpl*2 intron can be attributed to homologous recombination and gene conversion mechanisms, which represent the most plausible causal factors (Gu et al. 2016; Ding et al. 2019; Han et al. 2022). Phylogenetic analysis based on the plastid genome was performed to infer phylogenetic relationships between *M. octopetala* and related taxa. Similar to the results of a previous study, *M. octopetala* was identified to belong to the genus *Micranthes* and was thus located as a sister group to other *Micranthes* species (Kim et al. 2015). In this study, we obtained the plastid genome of *M. octopetala*, which will aid future phylogenetic and evolutionary studies of the genus *Micranthes*.

Ethical approval

The materials used in this study did not involve ethical conflicts. Therefore, no specific permission or license was required.

Authors' contributions

Tae-Hee analyzed the data and drafted the manuscript. Young-Ho collected plant materials and performed the experiments. Sang-Chul analyzed the data and revised the manuscript. Hyuk-Jin conceived of and designed the original study. All authors agreed to the submitted version of the manuscript.

Disclosure statement

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

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Data availability statement

The genome sequence data supporting the findings of this study are available in the GenBank of NCBI at https://www.ncbi.nlm.nih.gov/ under accession no. OQ835312. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA956675, SRR24198730, and SAMN34227787, respectively.

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