**ABSTRACT**

_Forsythia saxatilis_ (Nakai) Nakai is an endemic species in Korea. We present the second complete chloroplast genome sequence of _F. saxatilis_ showing that the chloroplast genome is 156,376 bp in length containing four subregions: a large single-copy (LSC) region of 87,097 bp, a small single-copy (SSC) region of 17,859 bp, and a pair of inverted repeat regions (IRs) of 25,710 bp. The genome contains 131 genes including 88 protein-coding genes, 35 tRNAs, and eight rRNAs. We found 40 base differences in 17 sites between our chloroplast genome and the previously reported chloroplast genome sequences of _F. saxatilis_. All of the differences were found in a region of 832 bp in the LSC region except for one site, which indicated potential sequencing errors in building the previously reported genome because of little substitutions in the homologous region of the other _Forsythia_ species and even of _Abeliophyllum distichum_, a sister to _Forsythia_. The new chloroplast genomes of _F. saxatilis_ and those of _F. x intermedia_ and _F. viridissima_ are identical, which suggests that _F. saxatilis_ is maternally closely related to the other two species.

_Forsythia_ Vahl (Oleaceae) contains 13 species distributed mostly in eastern Asia except for one species in southeastern Europe (Ha et al. 2018). Species of _F. koreana_ (Rehder) Nakai, _F. nakaii_ (Uyeki) T. B. Lee, _F. ovata_ Nakai, and _F. saxatilis_ (Nakai) Nakai have been reported as endemic species in Korea (National Institute of Biological Sciences 2013). In particular, most distribution of _F. saxatilis_ is in the Bukhansan National Park in Korea as an endangered and flagship species of the park (Kim and Chang 2007). Additional fragmented distributions of _F. saxatilis_ are found in limestone area of Gangwon province and Euysung-gun in Gyungbuk province (Han et al. 2011).

The first chloroplast (cp) genome from _F. saxatilis_ has been reported in the phylogenetic study of tribe Forsythieae in Oleaceae (Ha et al. 2018). In this study, we found a new population of _F. saxatilis_ in Opaesan (N20°27’55.14”, W105°17’31.21”) where is near from the Bukhansan National Park and determined the second cp genome with a sample from the new population. It provides comparative genomic evidence that the first cp genome could be affected by potential sequencing errors.

We collected a plant and leaves were preserved in silica gel. A voucher specimen was deposited in the herbarium of the Sungshin University, Korea (S. Kim 2020-001, SWU). DNA extraction was performed using a commercial plant DNA extraction kit (Exgene™; GeneAll, Seoul, Korea), and the extracted DNA was sequenced using the MGISeq-2000 platform (MGI Tech Co. Ltd., Shenzhen, China) with paired-end reads (150 bp in length). The sequencing library was constructed using the MGIEasy DNA library Prep Kit (MGI Tech Co. Ltd., Shenzhen, China) with an insert size of 500 bp.

The sequencing generated a total of 20,477,062 raw reads. After short-read quality filtering, the reads were mapped against the previously reported cp genome from _F. saxatilis_ (MF407179) using Geneious version 9.1.8 (Kease et al. 2012). We also performed _de novo_ assembly of the mapped reads using Geneious, which produced the same sequence as the mapped result. Comparing our cp genome with the previously reported one from _F. saxatilis_, we found 40 base differences in 17 sites. The sites included two short repeating unit differences (TTATAAA and ATGCTA), two three-concatenated base changes, two homopolymer indels, three homopolymer size differences, and eight single-nucleotide substitutions between the two cp genomes. Remarkably, all of the differences except for one site were found in the intergenic region between _aptF_ and _atpH_ and parts of the two genes, which was a relatively short region (832 bp) of the large single-copy (LSC). Furthermore, there were not so many substitutions in the region of the other _Forsythia_ species and even of _Abeliophyllum distichum_, a sister to _Forsythia_ in Oleaceae (Ha et al. 2018). Therefore, we believe that this region in the previously reported cp genome from _F. saxatilis_ is likely to be affected by potential sequencing errors.

The assembled cp genome (MT887608) was annotated using Geneious version 9.1.8 (Kease et al. 2012) based upon the annotation of _F. mandschurica_ (NC_048504.1), one of the
reference cp genomes in Forsythia. The size of the cp genome of F. saxatilis was 156,376 bp, consisting of a LSC region of 87,097 bp and a small single-copy (SSC) region of 17,859 bp separated by each of the two identical inverted repeat regions (IRs) of 25,710 bp. The cp genome encoded 88 protein-coding genes, 35 tRNAs, and eight rRNAs.

A phylogenetic analysis was performed using (1) the new cp genome of F. saxatilis, (2) 15 cp genomes of Forsythia species, and (3) the cp genome of Abeliophyllum distichum Nakai (NC_031445) (Figure 1). These 17 sequences were aligned by the MAFFT program (Katoh and Standley 2013) and a maximum-likelihood analysis was performed to create a phylogenetic tree using IQ-TREE (Nguyen et al. 2015) with the best model (K3Pu + F + I) including 1000 replicates of bootstrapping.

The tree showed that our cp genome was clustered with those of F. koreana (MF407176), F. × intermedia (= F. viridissima × F. suspense var. fortunei) (NC_036982), and F. viridissima (MF407182) (Figure 1). The previous cp genome of F. saxatilis (MF407179) was more distantly related to them than our cp genome. The new cp genome sequence of F. saxatilis was exactly the same as those of F. × intermedia and F. viridissima, and differed only in two bases from that of F. koreana. Autapomorphic characters found in the previous cp genome of F. saxatilis probably due to potential sequencing errors resulted in the rather longer external branch leading to the previous cp genome of F. saxatilis.

We report the second complete chloroplast genome in F. saxatilis. This allowed comparison with the previously reported cp genome in the species, suggesting the possibility of sequencing errors. The base substitution rate and phylogenetic relationship among species in Forsythia provided by this study elucidate the evolutionary history such as a hybridization origin.

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 NCBI at https://www.ncbi.nlm.nih.gov/ (reference number: MT887608) or available from the corresponding author.

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