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The complete chloroplast genome of *Scrophularia kakudensis* and a comparative analysis of *S. kakudensis* and *S. cephalantha*

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ABSTRACT: The genus *Scrophularia* L. (Scrophulariaceae) comprises 200–270 species worldwide and is a taxonomically challenging lineage, displaying morphological diversity and hybridization. *S. kakudensis* is morphologically similar to the closely related taxa *S. kakudensis* var. *microphylla*, *S. pilosa*, and *S. cephalantha*. Therefore, the purpose of this study was to sequence the chloroplast (cp) genome of *S. kakudensis* using next-generation sequencing and compare it to those of related taxa. The complete cp genome sequence of *Scrophularia kakudensis* was found to be 152,355 bp long, consisting of a pair of inverted repeats of 25,485 bp that separate a large single-copy (LSC) of 83,479 bp from small single-copy regions of 17,909 bp. The cp genome contained 78 protein-coding genes, 30 tRNAs, and four rRNAs. A phylogenetic analysis based on 78 protein-coding genes from six *Scrophularia* species showed *S. kakudensis* and *S. cephalantha* formed with 100% bootstrap values. We compared the complete cp genomes of *S. kakudensis* and *S. cephalantha* and identified seven sequence divergence regions: *matK/rps16*, *rps16/trnQ*, *trnS/trnG*, *rpoB/trnC*, *trnS/trnG*, *rpl32/trnL*, and *ndhD/psaC*. These regions may be useful for determining the phylogenetic relationships among *S. kakudensis*-related species.

Keywords: chloroplast genome, nucleotide diversity, Scrophulariaceae, *Scrophularia kakudensis*

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INTRODUCTION

Scrophularia L. belongs to the family Scrophulariaceae, which comprises approximately 200–270 species (Ortega Olivencia, 2009; Jang et al., 2021). This genus is distributed throughout the Northern Hemisphere and has square stems (sometimes winged stems) and generally opposite leaves. Stiefelhagen (1910) divided this genus into two sections, sect. *Anastomosantes* Stiefelhagen (sect. *Scrophularia*) and sect. *Scorodoniae* (Benth.) Stiefelhagen (sect. *Canina*) which are characterized by perennial subshrubs, petal length, corolla tube shape, and life form. The two series in sect. *Scrophularia* are *Grayanaea* T. Yamaz and *Kakudenses*. T. Yamaz recognized (Yamazaki, 1949).

Seven species of *Scrophularia* (*S. buergeriana*, *S. koraiensis*, *S. kakudensis*, *S. takesimensis*, *S. cephalantha*, *S. kakudensis* var. *microphylla*, and *S. alata*) are distributed throughout Korea (Jang and Oh, 2013). All Korean *Scrophularia* species are included in sect. *Scrophularia*. Two species (*S. alata* and *S. takesimensis*) belong to the series *Grayanae* and five species (*S. buergeriana*, *S. kakudensis*, *S. kakudensis* var. *microphylla*, *S. koraiensis*, and *S. cephalantha*) belong to series *Kakudenses*. However, identifying *S. kakudensis* is difficult due to similar morphological characteristics among *S. kakudensis* var. *microphylla*, *S. cephalantha*, and *S. pilosa*. Jang et al. (2011) suggested that *S. pilosa* should be treated as a synonym for *S. kakudensis*. *Scrophularia cephalantha* and *S. kakudensis* var. *microphylla* are distinct from *S. kakudensis* during the flowering season, with fewer nodes numbers on the stem and the sizes of the leaves, respectively. Recently, the phylogenetic relationships and evolution of several *Scrophularia* species have been studied using chloroplast (cp) genomes (Xu et al., 2018; Jang et al., 2021; Wang et al., 2022). In this study, we sequenced and analyzed the cp genome of *S. kakudensis* and performed a comparative analysis of *S. kakudensis* and *S. cephalantha*.

MATERIALS AND METHODS

We collected young leaves of *S. kakudensis* from Mt. Bohyeonsan (36.16625°N, 128.98097°E). The voucher specimens were deposited at the Daegu National Science Museum. Total DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA). Genomic DNA was sequenced using the Illumina HiSeq X platform (San Diego, CA, USA). We obtained 37,322,320 reads from the 150 bp paired-end sequences. The chloroplast genomes were assembled using GetOrganelle (Jin et al., 2020) and Geseq (Tillich et al., 2017) and Geneious Prime v.2022.1.1 (http://www.geneious.com) were used to annotate the *S. kakudensis* cp genome. Chloroplast genome mapping was performed using OGDRAW v. 1.3.1 (Greiner et al., 2019).

The chloroplast genome sequences of six taxa (Table 1), including one outgroup (*Verbascum phoenieum,* MN983301), were included in the phylogenetic analyses. The 78 protein-coding genes shared across taxa were extracted from each chloroplast genome and concatenated. The sequences were aligned using MAFFT (Katoh et al., 2022), and ML analysis was conducted using RAxML (Stamatakis, 2014) with the GTR + GAMMA + I model (rapid bootstrap of 1,000 replications).

The complete cp genomes of *S. kakudensis* and *S. cephalantha* were compared. The nucleotide diversity (Pi) was determined using DnaSP (Rozas et al., 2017). The step size was set to 200 bp, and the window length was set to 600 bp.

RESULTS AND DISCUSSION

The complete cp genome of *S. kakudensis* (NCBI accession number: OR004236) comprised 152,355 bp with a quadripartite structure and two inverted repeat regions (IRs, 25,485 bp) separated by large single-copy (LSC, 83,476 bp) and small single-copy (SSC, 17,909 bp) regions (Fig. 1). The average GC content was 38.0%. It contained 112 genes, including 78 protein-coding, 30 tRNA, and 4 rRNA genes. Six protein-coding genes (*rpl2*, *rpl23*, *ycf2*, *ndhB*, *rps7*, and *rps12*), seven tRNA genes (*trnA-UGC, trnI-CAU, trnI-GAU, trnL-CAA, trnN-GUU, trnR-ACG*, and *trnV-GAC*) and four rRNA genes (4.5S, 5S, 16S, and 23 rRNA) were duplicated in two IR regions. Fifteen genes had one intron, and three genes (*rps12*, *clpP*, and *ycf3*) had two introns.

Comparative genomic analyses of *Scrophularia* revealed that the six cp genomes were highly conserved (Table 1). The total length of *the Scrophularia* species ranged from 152,355 bp (*S. kakudensis*) to 153,175 bp (*S. ningpoensis*). The GC content ranged from 38% to 38.1% (*S. takesimensis*), and the seven *Scrophularia* species contained the same genes (CDS, tRNA, and rRNA).

A phylogenetic analysis was conducted using 78 protein-coding genes from seven *Scrophularia* species and one outgroup (*Verbascum phoeniceum*). The genus *Scrophularia* is a monophyletic group. *S. kakudensis* was closely related to *S. cephalantha* with a 100% bootstrap value, and this clade was a sister to the *S. buergeriana* + *S. ningpoensis* clade (Fig. 2). This result supports the hypothesis that *S. kakudensis* and *S. cephalantha* are included in series *Kakudenses* (Yamazaki, 1949; Jang and Oh, 2013). *Scrophularia cephalantha* is an endemic species (Chung et al., 2023), and this study confirmed that it shows a close relationship between *S. kakudensis* and *S. cephalatha.*

Internal transcribed spacer (ITS) and cpDNA non-coding regions have been widely used to investigate molecular phylogeny at the interspecific level (Taberlet et al., 1991; Baldwin, 1992). In *Scrophularia*, Scheunert and Heubl (2010) tested the nrDNA (ITS) and chloroplast DNA intergenic spacers (*psbA/trnH* and *trnQ/rps16*). However, the relationship among *Scrophularia* species is not well supported. Recently, cp genome sequences have been used as genetic markers for DNA barcoding and phylogenetic relationships, and several studies on the genus *Scrophularia* have analyzed the chloroplast genome (Choi and Park, 2016;Yi and Kim, 2016; Jang et al., 2021; Wang et al., 2022; Guo et al., 2023).

Table 1. Characteristics of the cp genomes in six species of *Scrophularia.*

	Length (bp)				GC contents (%)	Genes			Reference
	Total	LSC	SSC	IR		CDS	tRNA	rRNA	
S. buergeriana	153,631	84,454	17,929	25,624	38	78	30	4	Yi and Kim (2016)
S. takesimensis	152,436	83,542	17,938	25,478	38.1	78	30	4	Choi and Park (2016)
S. henryi	152,868	84,020	17,941	25,454	38	78	30	4	Wang et al. (2022)
S. ningpoensis	153,175	84,257	17,938	25,490	38	78	30	4	Guo et al. (2023)
S. cephalantha	153,016	84,124	17,922	25,485	38	78	30	4	Jang et al. (2021)
S. kakudensis	152,355	83,479	17,909	25,485	38	78	30	4	This study

cp, chloroplast; LSC, large single-copy; SSC, small single-copy; IR, inverted repeat; CDS, coding sequence.

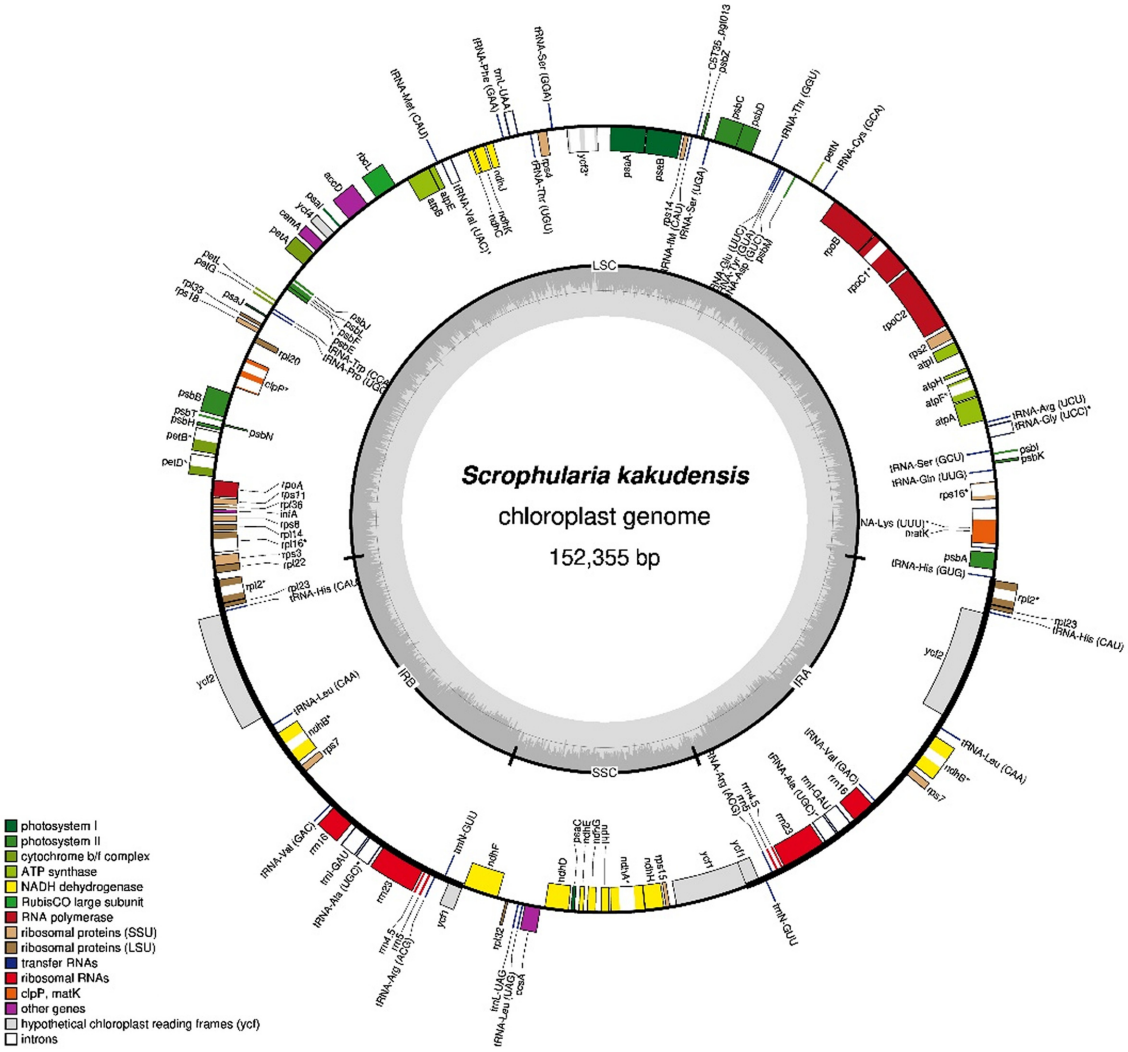

Fig. 1. A circular map and annotation of the chloroplast genome of *Scrophularia kakudensis*. The genes are transcribed clockwise on the inside and counterclockwise on the outside. The darker gray in the inner circle corresponds to the GC content.

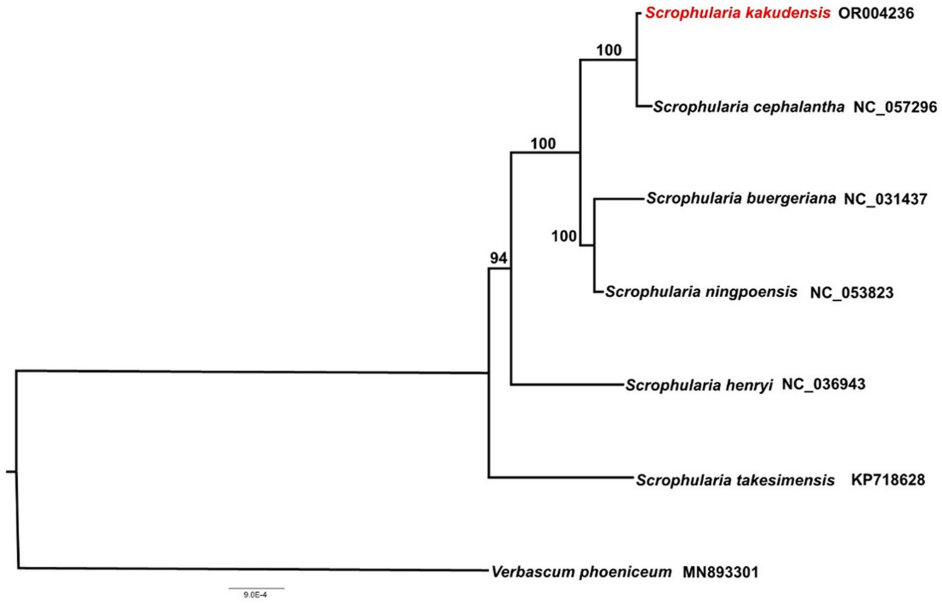

Fig. 2. A maximum-likelihood tree of *Scrophularia kakudensis* and five other *Scrophularia* species based on 78 chloroplast protein-coding genes.

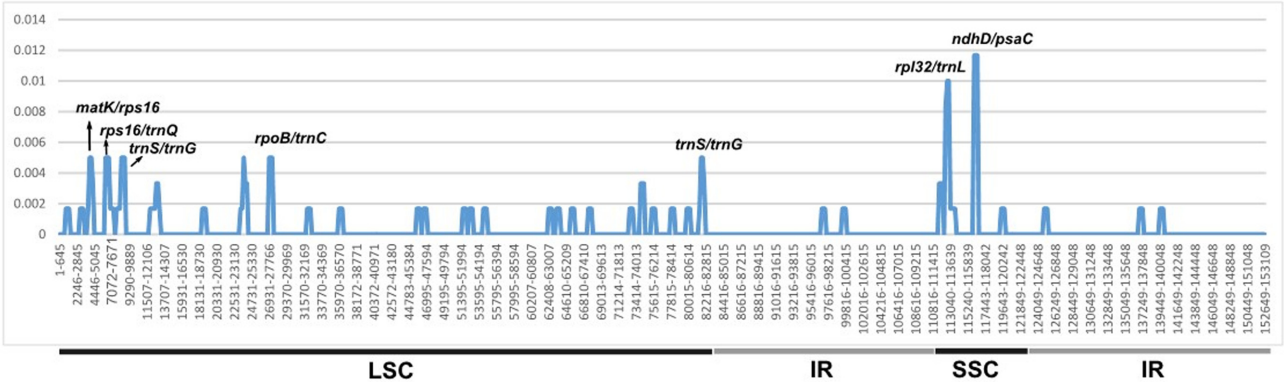

Fig. 3. Comparison of the nucleotide variability (Pi) values between *Scrophularia kakudensis* and *S. cephalantha*. LSC, large single-copy; IR, inverted repeat; SSC, small single-copy.

The nucleotide diversities between *S. kakudensis* and *S. cephalantha* were compared. Nucleotide diversity (Pi) ranged from 0–0.01167. Most of the variable regions were located between the *ndhD/psaC* regions (Pi = 0.01167). This result suggests that seven regions (*matK/rps16*, *rps16/trnQ*, *trnS/trnG*, *rpoB/trnC*, *trnS/trnG*, *rpl32/trnL*, and *ndhD/psaC*) were highly informative markers to identify species (Fig. 3).

Xu et al. (2018) suggested that nine markers (*trnH/psbA*, *rps15*, *rps18/rpl20*, *rpl32/trnL*, *trnS/trnG*, *ycf15/trnL*, *rps4/trnT*, *ndhF/rpl32*, and *rps16/trnQ*) could be used as DNA barcodes to distinguish *Scrophularia*. The results from this study indicate that, compared to the nine markers presented in the previous study, the *rpl32/trnL* and *ndhD/psaC* regions are more useful for studying *S. kakudensis* and its relatives. This study reports the complete chloroplast genome of *S. kakudensis* and provides useful information on the phylogenetic relationships within *Scrophularia* species.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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