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PLASTOME REPORT

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Comprehensive analysis of the chloroplast genome and phylogenetic relationships of *Sasa quelpaertensis* Nakai

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ABSTRACT

Jeju-Joritdae (*Sasa quelpaertensis* Nakai) is a broad-leaved bamboo grass endemic to Mount Halla, Jeju Island, South Korea. In this study, we report the complete chloroplast genome sequence of *S. quelpaertensis*. Its chloroplast genome is 139,730 bp in size and consists of a large single-copy (LSC, 83,351 bp) region, one small single-copy (SSC, 12,788 bp) region, and two inverted repeats (IRs, 21,796 bp each). The chloroplast genome of *S. quelpaertensis* encodes 131 genes, including 86 protein-coding, 37 tRNA, and 8 rRNA genes. The overall GC content of the *S. quelpaertensis* chloroplast genome is 38.86%. Phylogenetic analysis using the chloroplast genome sequence showed that *S. quelpaertensis* is closely related to *Sasa veitchii* and *Sasella kogasensis*. These findings provide valuable genomic resources for future studies of the *Sasa* genus in South Korea and other countries encompassing its distribution area.

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Jeju-Joritdae; bamboo grass; *Sasa palmata*; Poaceae; plastome genome

Introduction

Sasa quelpaertensis Nakai (1933), a dwarf-bordered bamboo from the Poaceae family, is an endemic species confined to Jeju Island, South Korea (Nakai 1933). *Sasa quelpaertensis* is a rhizomatous, perennial bamboo grass that forms dense clumps and reaches heights up to 1 m. The culms are erect, thick, and solid, with an average diameter of 1.5 cm. Its leaves are oblong-lanceolate in shape and measure 7–20 cm long and 2.5–5.0 cm wide. They are dark green with a cream-colored, bleached margin (Kim 2008). The leaf sheaths are densely pubescent with stiff hairs, giving them a rough texture. *Sasa quelpaertensis* has a panicle inflorescence with spikelets that emerge from the leaf axils, initially covered with brownish-red bracts. The spikelets contain several flowers, and the lemma of the lower floret is pubescent.

S. quelpaertensis plays a critical ecological role in the Jeju Island forest ecosystem and is used for a variety of purposes, including ornamental gardening, herbal tea making, and traditional medicine (Kim 2008; Lee et al. 2019). Recent research has demonstrated that *S. quelpaertensis* possesses diverse health-promoting effects, including anti-obesity, anti-depressant, anti-viral, anti-inflammatory, and anticancer effects (Kang et al. 2012, 2013; Kim et al. 2014; Kang and Lee 2015; Kwon et al. 2020). This plant is gaining attention as a forage option for livestock, which could help to reduce the negative impact on habitat diversity (Lee et al. 2010, 2023).

Despite its ecological and economic importance, the taxonomic status of *S. quelpaertensis* remains controversial, with some authors treating it as a synonym of *Sasa palmata* (Chang et al. 2014). Conversely, *S. quelpaertensis* can be differentiated from *S. palmata* based on the morphological features of its aerial parts (Kim 2008). Comprehensive taxonomic investigations using molecular phylogenetic approaches are needed to clarify the taxonomic status of *S. quelpaertensis*.

Chloroplasts are plant organelles that play a vital role in photosynthesis, amino acid and lipid synthesis, and metabolite storage. Chloroplast genomes are widely used in phylogenetic research to investigate the evolutionary history of organisms due to their high degree of conservation and slow evolutionary rate (Yang et al. 2022). Several studies have reported the complete sequence and structural features of chloroplast genomes from temperate woody bamboo species (tribe Arundinarieae, Poaceae), including *Ampelocalamus actinotrichus* (Zhang, Chen, et al. 2019), *Dendrocalamopsis variostrata* (Lin et al. 2019), *Gelidocalamus xunwuensis* (Zhang, Guo, et al. 2019), *Acidosasa gigantea* (Zheng, Yang, et al. 2020), *Chimonobambusa hejiangensis* (Liu et al. 2021), *Indosasa hispida* (Tu et al. 2022), and *Phyllostachys edulis* (Jing et al. 2023). Moreover, a comprehensive phylogenetic analysis of 40 chloroplast genomes from Arundinarieae unveiled 12 distinct lineages within the tribe (Ma et al. 2017). Further complete plastome analysis in 62 species of *Frgesis*, the largest genus of Arundinarieae, revealed the genetic

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variability and relationships within the taxonomically challenging genus in Arundinarieae (Lv et al. 2023).

To date, the complete sequence and genomic features of *S. quelpaertensis* chloroplast genomes remain uncharacterized, and its evolutionary history is not fully understood. There are no entire or chloroplast genome sequences for any species of the genus *Sasa* reported in the NCBI and CpGDB databases, except for *Sasa veitchii* (Singh et al. 2020). This study aims to characterize the complete chloroplast genome sequence of *S. quelpaertensis* to elucidate its genetic diversity and evolutionary history.

Materials and methods

Fresh leaf samples were collected from the Koreyri population site in Jochun-eup, Jeju City, South Korea (126.6374057 E, 33.4251433 N) (Figure 1). A voucher specimen (ID number: 10034277) was deposited at the National Institute of Forest Science, South Korea (http://www.nifos.go.kr, Eun-Young Yim, curie580@korea.kr). Chloroplast isolation and DNA extraction were performed as described previously, with a minor modification (Takamatsu et al. 2018). The library was prepared using the TruSeq Nano DNA Library Preparation Kit (Illumina, San Diego, CA, USA) and sequenced on an Illumina Novaseq 6000 platform (Macrogen, South Korea). The chloroplast genome was *de novo* assembled using NOVOPlasty v4.2.1 (Dierckxsens et al. 2017). The read coverage plot was generated using BAM Coverage Plotter (Pierre 2015). The assembled chloroplast genome was annotated using the Plastid Genome Annotator (PGA) (Qu et al. 2019), and the annotated map was drawn with OGdraw v1.3.1 (Greiner et al. 2019), incorporating the annotation results. *Cis-* and *trans-*splicing transcripts were identified and visualized using cpgview (http://www.1kmpg.cn/cpgview) (Liu, Ni, et al. 2023).

Figure 1. Photograph of a collected sample of *S. quelpaertensis* Nakai. The photograph was taken at the sampling site near Koreyri, Jochun-eup, Jeju City, South Korea (126.640822 E, 33.417596 N). Scale bar = 1 cm.

Pairwise sequence alignment of the chloroplast DNA sequences from *Sasa palmata* against the chloroplast genome of *S. quelpaertensis* was performed using the BLASTN program (Altschul et al. 1997). A total of 30 chloroplast open reading frames (ORFs) and intergenic DNA sequences from *S. palmata* were downloaded from GenBank and used as queries in the BLASTN program. PhyloSuite v1.23 (Zhang et al. 2020) was used to perform a phylogenetic analysis of chloroplast genomes from 26 species of the *Arundinarieae* tribe downloaded from GenBank, with *Bambusa emeiensis* as an outgroup. Protein-coding genes shared by all genomes were identified, followed by the alignment of each gene using MAFFT v7.505 (Rozewicki et al. 2019). Subsequently, Gblocks 0.91b (Talavera and Castresana 2007) was used to mask sequences, and the end-to-end connections of the genes for each species were used as input in the phylogenetic analysis. Maximum-likelihood phylogenies were constructed using IQ-TREE 2.2.0 (Minh et al. 2020) under the TVM + F + I nucleotide substitution model, which was determined to be the best-fit model by ModelFinder (Kalyaanamoorthy et al. 2017), with 1000 standard bootstraps.

Results

A total of ~2.26 gigabase pairs (Gbp) of raw data were generated, and ~2.07 Gbp of clean reads were assembled after quality control. The average coverage was 7483, and the mapped read depth across the entire chloroplast genome was ≥3900, highlighting the reliability of the genome assembly (Supplementary Figure S1). The complete chloroplast genome of *S. quelpaertensis* was 139,730 bp in length and consisted of four regions: a large single-copy region (LSC) of 83,351 bp, a small single-copy region (SSC) of 12,788 bp, and two inverted repeats (IRs) of 21,796 bp each (Figure 2 and Supplementary Table S1). The chloroplast genome had a G + C content of 38.86%, with the IRs having a higher G + C content (44.23%) than the LSC and SSC regions (36.90% and 33.30%, respectively).

The chloroplast genome sequence contained 131 genes, including 86 protein-coding, 37 tRNA, and 8 rRNA genes. The 86 protein-coding genes could be functionally categorized into four groups: photosynthesis-related (45), self-replication-related (31), other functional (6), and unknown (4) genes (Supplementary Table S2). A total of 10 protein-coding genes (*ndhA*, *ndhB*s (x2), *petB*, *petD*, *atpF*, *rpl16*, *rpl2*s (x2), and *rps16*) and 7 tRNA genes (2 *tRNA-Ala(UGC)*s, 2 *tRNA-Ile*(GAU)s, *tRNA-Leu*(UAA), *tRNA-Lys*(UUU), and *tRNA-Val*(UAC)) were identified to contain one intron each (Supplementary Table S2 and Figure S2). Additionally, the *ycf3* gene contained 2 introns, and 2 *rps12* genes were *trans*-splicing genes, with exon 1 and exons 2–3 in the LSC and IR regions, respectively (Supplementary Figure S2).

Pairwise sequence alignment of the *S. quelpaertensis* chloroplast genome against a collection of DNA nucleotides from chloroplast ORF or intergenic DNA nucleotide sequences from multiple *S. palmata* vouchers revealed several gaps and mismatches (Supplementary Table S3). Of the 11 vouchers examined, 5 (45.5%) and 8 (82.7%) exhibited gaps and mismatches in at least one DNA region, respectively. The

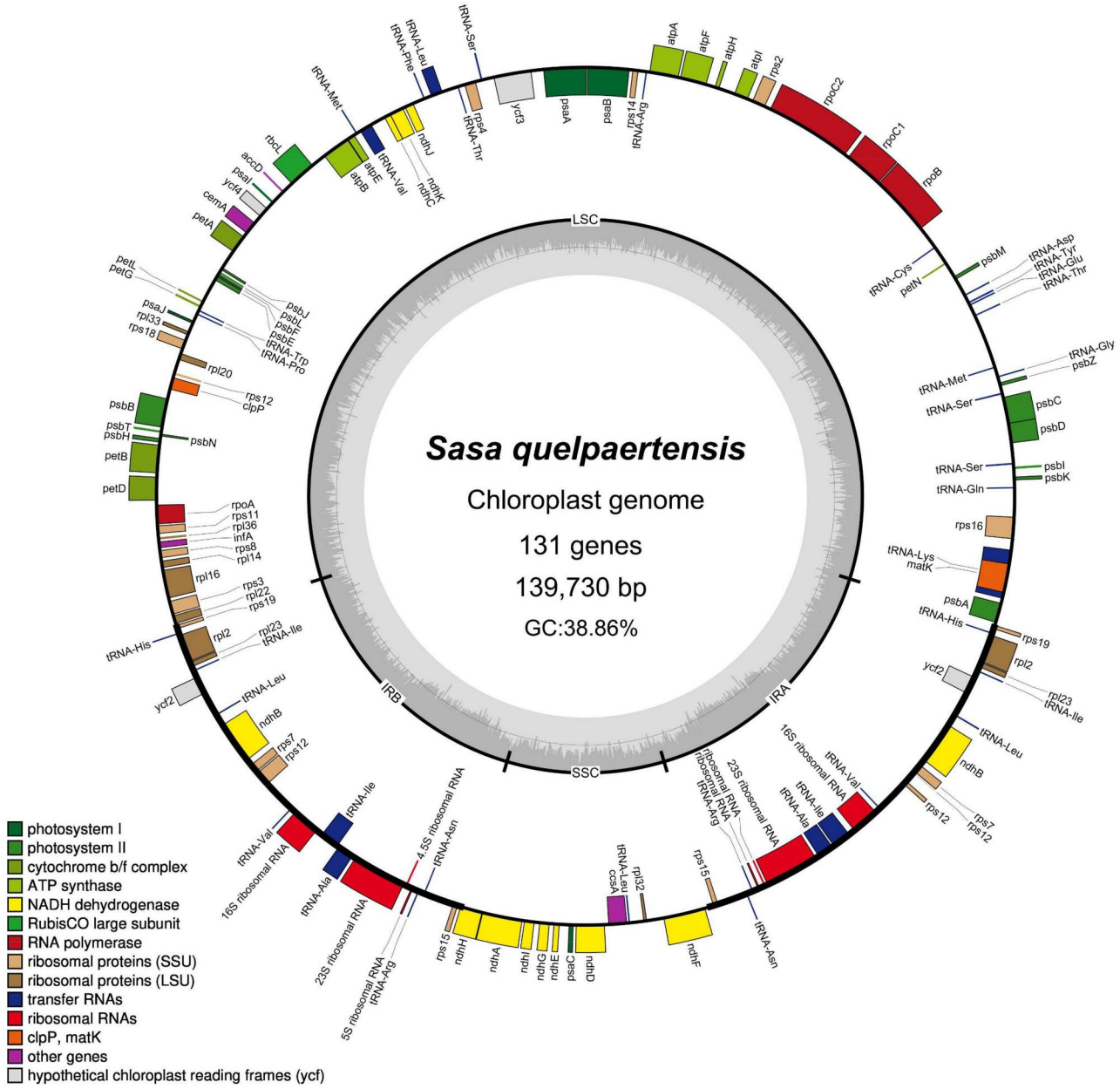

Figure 2. Circular map of the chloroplast genome of *S. quelpaertensis*. The chloroplast genome contains small single-copy and large single-copy regions, which are separated by inverted repeats (IRs; IRA, and IRB). Genes inside the map are transcribed in the clockwise direction, and those shown outside are transcribed in the counterclockwise direction. Genes with similar functions are indicated in the same color.

average identity in all pairwise alignments was 99.83%, with the lowest value being 99.21%.

The phylogenetic analysis showed that *S. quelpaertensis* was closely related to *S. veitchii* and *Sasella kogasensis* and these species together formed a well-supported clade (Figure 3). The *S. quelpaertensis* clade was closely related to another clade consisting of *Acidosasa purpurea*, *Pleioblastus maculatus*, *Indosasa sinica*, *Pseudosasa cantorii,* and *Oligostachyum shiuyingianum*, supporting the classification of these species within the *Arundinaria* clade (Ma et al. 2017).

Discussion and conclusion

We report the first complete chloroplast genome sequence of *S. quelpaertensis* (Jeju-Joritdae), an endemic species found on Mount Halla, South Korea. The plastid genome of *S. quelpaertensis* was found to be 139,730 base pairs in size and contains 131 genes.

Pairwise alignment of the *S. quelpaertensis* chloroplast genome with *S. palmata* DNA entries showed a high level of identity in their chloroplast gnome, but also the presence of DNA variants, indicating that they have diverged over time. These results provided molecular evidence supporting the hypothesis that *S. quelpaertensis* is a distinct species from *S. palmata. S. quelpaertensis* has been suggested to be synonymous with *S. palmate*, but there is no clear molecular evidence to support this claim (Chang et al. 2014). Future molecular taxonomic investigations based on their entire chloroplast genomes will be able to clarify the relationship between these two species.

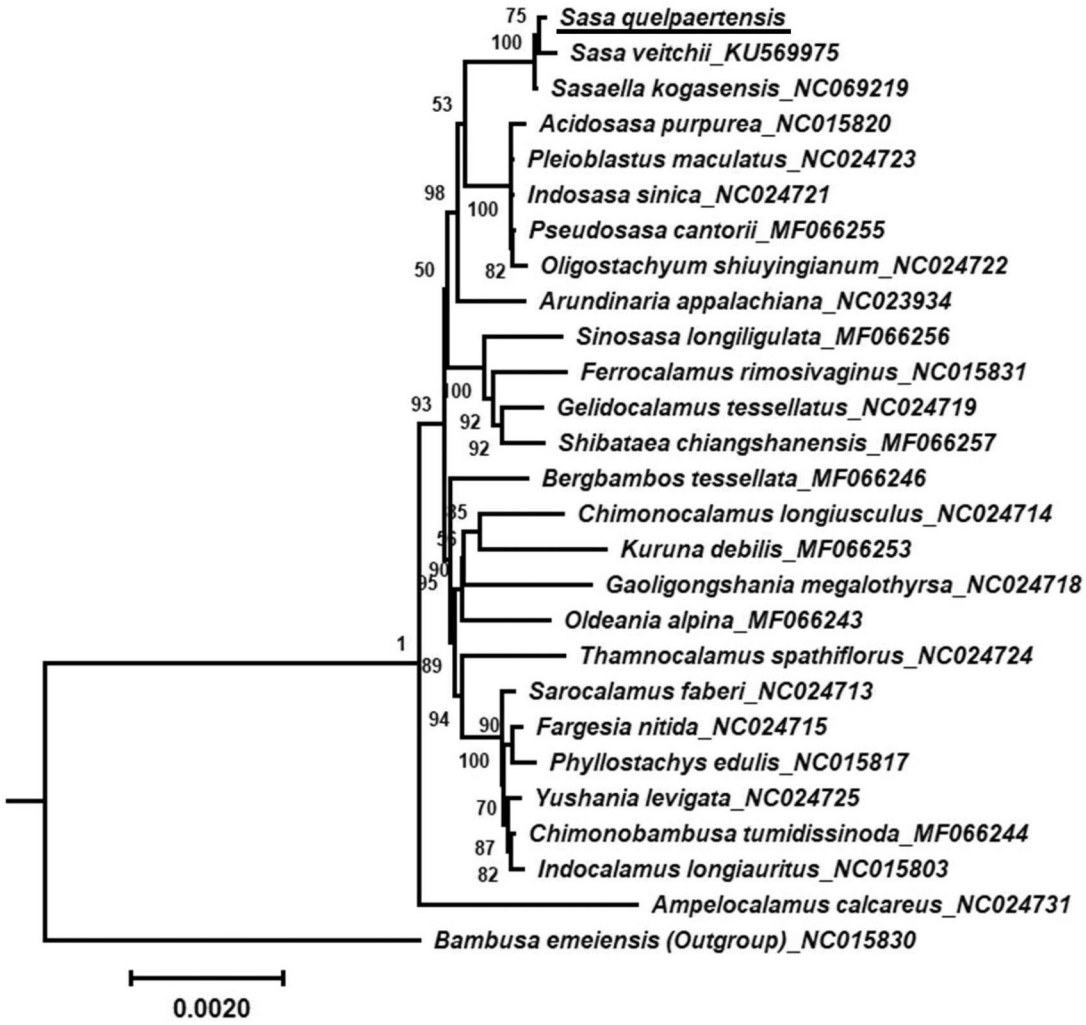

Figure 3. Maximum-likelihood tree of *S. quelpaertensis*. The phylogenetic tree of *S. quelpaertensis* (underlined) and 26 relative species was constructed using the IQ-Tree software based on 73 protein-coding genes shared by all genomes. Bootstrap values are shown next to the nodes. The following sequences were retrieved from the NCBI database (some of them have not been published yet): *Sasa veitchii* (KU569975), *Sasaella kogasensis* (NC069219; Zheng, Lin, et al. 2020), *Acidosasa purpurea* (NC015820; Li et al. 2006), *Pleioblastus maculatus* (NC024723; Li et al. 2006), *Indosasa sinica* (NC024721; Li et al. 2006), *Pseudosasa cantorii* (MF066255; Li et al. 2006), *Oligostachyum shiuyingianum* (NC024722; Li et al. 2006), *Arundinaria appalachiana* (NC023934; Triplett et al. 2010), *Sinosasa longiligulata* (MF066256; Tong et al. 2023), *Ferrocalamus rimosivaginus* (NC015831; Li et al. 2006), *Gelidocalamus tessellatus* (NC024719; Li et al. 2006), *Shibataea chiangshanensis* (MF066257; Li et al. 2006), *Bergbambos tessellata* (MF066246; Soderstrom and Ellis 1982), *Chimonocalamus longiusculus* (NC024714; Li et al. 2006), *Kuruna debilis* (MF066253; Soderstrom and Ellis 1988), *Gaoligongshania megalothyrsa* (NC024718; Zhang et al. 2014), *Oldeania alpina* (MF066243; Wimbush 1947), *Thamnocalamus spathiflorus* (NC024724; Janzen 1976), *Sarocalamus faberi* (NC024713; Stapleton et al. 2004), *Fargesia nitida* (NC024715; Jian et al. 2013), *Phyllostachys edulis* (NC015817; Liu et al. 2023a), *Yushania levigata* (NC024725; Li et al. 2006), *Chimonobambusa tumidissinoda* (MF066244; Li et al. 2006), *Indocalamus longiauritus* (NC015803; Li et al. 2006), *Ampelocalamus calcareus* (NC024731; Zhang et al. 2018), and *Bambusa emeiensis* (outgroup; NC015830; Li et al. 2021). The scale bar represents an evolutionary distance of 0.002.

Phylogenetic analysis of the plastid genome revealed that *S. quelpaertensis* is most closely related to *S. veitchii* and *S. kogasensis*. However, further research is needed to clarify the phylogenetic relationships among other species in the *Sasa* genus based on the chloroplast genome. This approach could provide clearer insights into phylogenetic relationships compared to the results obtained from the short sequences of the chloroplast intron located between *rbcL* and *ORF106* in 16 *Sasa* species (Sasaki et al. 2007). In conclusion, our findings provide a valuable genetic resource for future studies of the *Sasa* genus and its related genera.

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Author contributions

Jin Hee Kim and Jeongsik Kim conceived the study and collected the samples. Jin Hee Kim conducted experiments, Jeongsik Kim performed data analysis, and Jin Hee Kim and Jeongsik Kim wrote the manuscript. All authors provided comments and final approval.

Disclosure statement

No potential conflict of interest was reported by the authors.

Ethical approval

Since *S. quelpaertensis* is not classified as an endangered or protected species, specific permission for its collection was not required. This study, including the collection of plant material, was conducted in compliance with the guidelines provided by Jeju National University.

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Data availability statement

The genome sequence data supporting the findings of this study are openly available in GenBank of NCBI under accession no. OR188081. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA972468, SRR24554619, and SAMN35082091, respectively.

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