

Complete chloroplast genome of *Asarum chungbuensis* (C.S. Yook & J.G. Kim) B.U. Oh 2005 (Aristolochiaceae), a Korean endemic species

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ABSTRACT

Asarum chungbuensis, a species endemic to Korea, has a limited distribution across the Korean Peninsula and is used in traditional medicine. Despite its importance, the genome structure, genetic composition, and phylogenetic relationships based on its chloroplast genome have not been documented. In this study, the complete chloroplast genome of *A. chungbuensis* was newly assembled. The chloroplast genome is 190,179 base pairs (bp) long, and the overall GC content (%) of the plastid was 36.8%. The chloroplast genome size of *A. chungbuensis* is longer than that of the normal chloroplast genome (160 kb) because of an inverted small single-copy (SSC) duplication that incorporates the SSC into an inverted repeat (IR) region. By extension, this duplication event causes this chloroplast genome to lack an SSC, unlike other formal structures. The chloroplast genome, with a tripartite structure, consisted of a single-copy region of 93,351 bp with a 34.6% GC content and two IR regions, each with a length of 48,414 bp and a 38.8% GC content. Additionally, it was found to have 113 genes, including 79 PCG genes, 30 tRNA genes, and four rRNA genes. Phylogenetic analysis revealed that *A. chungbuensis* was grouped with *A. heterotropoides* var. *seoulense*, which diverged from the clade comprising *A. koreanum* and *A. patens*. The newly sequenced *A. chungbuensis* chloroplast genome could provide valuable genomic information for determining unique genome structures, especially for the assessment of genetic diversity, phylogenetic relationships, species conservation, and biogeographic studies of the genus *Asarum*.

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Introduction

The genus *Asarum* Linnaeus (Aristolochiaceae) is mainly distributed across temperate zones of the Northern Hemisphere (Hou 1984; Kelly 1998; Kelly 2001; Kopyt'ko et al. 2013; Yoo et al. 2021). Approximately, 80% of *Asarum* species in *Asiasarum* flourish in East Asia, spanning across China, Japan, Vietnam, and Korea, whereas approximately 15 species are distributed in North America (Yoo et al. 2021). *Asarum* species contains various bioactive compounds, such as flavonoids, amides, phenylpropanoids, phenanthrenes, lignans, and various other compounds, which have significant pharmacological effects. The roots of *Asarum* have been used in traditional medicine across China, Japan, Europe, North America, and Korea for centuries (Liu and Wang 2022).


Asarum chungbuensis, identified by C.S. Yook & J.G. Kim and described by B.U. Oh 2005, is an endemic species distributed only in South Korea and is the smallest Korean *Asarum* species and is distinguished from other taxa by the presence of calyx tubes and sepals (Yamaki et al. 1996; Yook and Kim 1996; Oh 2008). However, due to high morphological variation within the section *Asiasarum*, there is significant taxonomic confusion regarding species identification and classification (Yamaki et al. 1996).


Despite the significance of this endemic species, the chloroplast genome of *A. chungbuensis* has not been reported until now. Previous study based on the complete chloroplast genome of Korean *Asarum* (Yoo et al. 2021) showed that species in the genus *Asarum* have a tetrapartite genome structure with an expanded inverted repeat (IR) region, or a tripartite genome structure (functionally bipartite structure with a palindromic IR region). Because of the diverse structural characteristics of its genome, a phylogenetic study of this species, revealing the phylogenetic relationships among *Asarum* species, requires in-depth analysis that can detect irregular repeats. In this study, we successfully assembled the chloroplast genome of *A. chungbuensis* and analyzed its structure and phylogenetic position. Revealing the complete chloroplast genome of *A. chungbuensis* would greatly aid in understanding the unique genomic structure of *Asarum*.

Materials and methods

Taxon sampling, DNA extraction, sequencing, assembly, and annotation

The *A. chungbuensis* was collected from its native habitat (Mt. Samak, Seo-Myeon, Chuncheon-Si, Gangwon Province;

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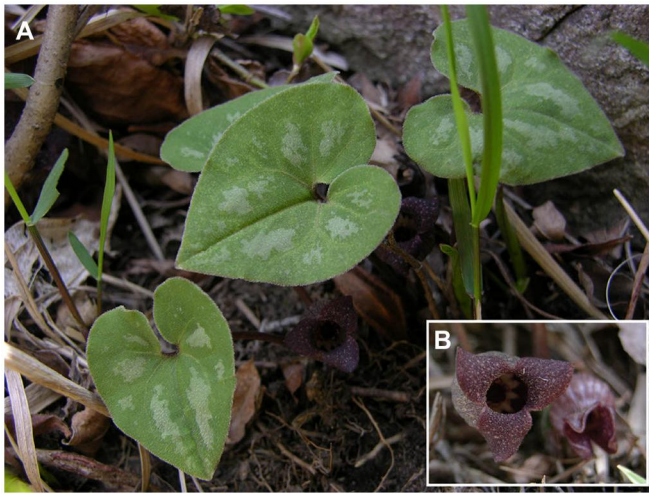


Figure 1. Morphology of *Asarum chungbuensis*. (A) Whole individual with faintly patterned leaves and (B) flower. The photos were taken by Sa-Beom Jang at the Korea National Arboretum (KNA) with the author's approval for use; Mt. Samak, Seo-Myeon, Chuncheon-Si, Gangwon Province; E 37.8277, N 127.6581667.

location: E 37.8277, N 127.6581667; **Figure 1**), and a voucher specimen deposited in the Korea National Arboretum (KNA) Herbarium with the voucher number ESK20-217 (contact: Dong-Chan Son, sdclym@korea.kr). After sampling, fresh leaves were immediately dried with silica gel at room temperature (25 °C, 77 °F) to prevent DNA degradation. Genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA), and Illumina sequencing library construction (insertion size, 550 bp) conducted using the TruSeq Nano DNA Kit. Sequencing was conducted using the Illumina MiSeq platforms (Illumina Inc., San Diego, CA) by Macrogen Inc. (Seoul, South Korea), in which 15,136,278 paired-end chloroplast-derived reads were generated. The generated raw reads draft assembled into a complete chloroplast sequence using GetOrganelle v.1.7.7.0 with the 'embplant_pt' option for de novo assembly, and finally completed using Geneious v.10.2.6 (Kearse et al. 2012). Verification of genes and tRNA was conducted using GeSeq online (Tillich et al. 2017) at <https://chlorobox.mpimp-golm.mpg.de/geseq.html> and manually edited by comparison with the published chloroplast genome sequences of the genus *Asarum* using Geneious. A circular map of the complete chloroplast genome was generated using OGDRAW (OrganellarGenomeDRAW) (Greiner et al. 2019) available at <https://chlorobox.mpimp-golm.mpg.de/OGDraw.html>. To verify assembly correctness, we examined the genome coverage using a plot generated by the Draw_SequencingDepth.py script (Ni et al. 2023) (**Supplementary Figure 1**).

Phylogenetic analysis

The dataset for phylogenetic analysis based on 79 protein-coding gene (PCG) sequences from 22 related species was compiled into a single file with a 60,000 bp size and aligned using PhyloSuite v1.2.3 (Zhang et al. 2020) with MAFFT

(Kato et al. 2002). To analyze the phylogenetic relationships of the *Asarum* genus, 21 other complete chloroplast genomes from Aristolochiaceae plants (eight from the *Asarum* genus) and one outgroup taxon from Winteraceae (*Drimys granadensis*) were obtained from NCBI. Considering the biogeographic relationship, native species distributed in South Korea among related species of *A. chungbuensis* were selected as the tip with priority. Maximum-likelihood (ML) analysis was performed using an IQ-tree (<https://iqtree.org>) based on PCGs with 10,000 replications to infer the phylogenetic position of *A. chungbuensis*; the best-fit model of evolution used in the analysis was GTR + R3 + F.

Results

As evidence of the correct assembly of the genome, a coverage depth figure was provided with an average depth of $\times 2151.19$ (maximal sequencing depth, $\times 3089$; minimal sequencing depth, $\times 24$; **Supplementary Figure 1**). Unlike the typical tetrapartite genome structure found in most angiosperm chloroplasts, *A. chungbuensis* exhibits a circular tripartite genome structure (functionally bipartite with a palindromic IR region). The chloroplast genome of *A. chungbuensis* was 190,179 bp in length and composed of a single copy (SC) (93,351 bp) and a pair of IR regions (48,414 bp), following the characteristics of the genus *Asarum* (**Figure 2**). The GC content of the SC region was lower than that of the two IR regions, which is consistent with the molecular characteristics of *A. heterotropoides* var. *seoulense*, a congeneric species. A total of 113 genes were encoded in the chloroplast sequence, comprising 79 PCGs, 30 tRNA genes, and four rRNA genes. A SC comprised 80 genes, including 58 PCGs and 22 tRNA genes, whereas each IR encoded 33 genes, including 21 PCGs (*ccsA*, *ndhA*, *ndhB*, *ndhD*, *ndhE*, *ndhF*, *ndhG*, *ndhH*, *ndhI*, *psaC*, *rpl2*, *rpl22*, *rpl23*, *rpl32*, *rps3*, *rps7*, *rps12*, *rps15*, *rps19*, *ycf1*, and *ycf2*), eight tRNA genes (*trnA-UGC*, *trnI-CAU*, *trnI-GAU*, *trnL-CAA*, *trnL-UAG*, *trnN-GUU*, *trnR-ACG*, and *trnV-GAC*), and four rRNA genes (*rrn16*, *rrn23*, *rrn4.5*, and *rrn5*). In addition, 18 genes with introns were observed, of which *clpP1* and *pafl* contained two introns and others (*atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpl2*, *rpl16*, *rpoC1*, *rps16*, *trnA-UGC*, *trnG-UCC*, *trnI-GAU*, *trnK-UUU*, *trnL-UAA*, and *trnV-UAC*) contained a single intron (**Supplementary Figure 2A**). The *rps12* gene spanned two regions (with the 5'-end of the exon located in the LSC and the 3'-end in the IR), and was demonstrated as a trans-splicing gene (**Supplementary Figure 2B**).

The number on each branch of the ML tree indicates the bootstrap (BS) value. Most nodes were supported by strong BS values. The Aristolochiaceae family formed two major groups with 97 BS values (**Figure 3**): clade (A) *Lactoris* – *Thottea* – *Aristolochia* and clade (B) *Saruma* – *Asarum*. *Asarum* was strongly monophyletic, and *A. chungbuensis* formed a strong sister group with *A. heterotropoides* var. *seoulense*, whereas *A. heterotropoides* formed a strong support group with *A. heterotropoides* var. *mandshuricum*.



Figure 2. Circular chloroplast genome map of *Asarum chungbuensis*. Based on the outer bold line, genes located outside the circle are transcribed clockwise, whereas those inside are transcribed counterclockwise. Relative GC contents for each section are displayed in deep and light gray within the inner circle. IR: inverted repeat region; SC: single copy.

Discussion

The taxonomic position and relationships of *A. chungbuensis* within the genus *Asarum* have been debated due to isonyms, synonyms, and individual morphological variations (Yamaki et al. 1996; Yook and Kim 1996; Oh 2008). Initially described by Yamaki et al. (1996) and Yook and Kim (1996), *A. chungbuensis* poses particular challenges regarding its phylogenetic position. This was because they treated this taxon as a synonym of *A. sieboldii* based on morphology without phylogenetic studies (Yamaki et al. 1996; Yook and Kim 1996; Oh 2008). However, our findings show that *A. sieboldii* and *A. chungbuensis* are distinct species, with *A. chungbuensis* forming a sister group with *A. heterotropoides* var. *seoulense*, contrary to previous morphological classifications.

Furthermore, the genera *Asarum* and *Aristolochia*, both present in Korea, exhibit clear physiological and morphological differences (Chung 1965; Ryang et al. 2004; Simpson 2010). Herbaceous *Asarum* typically grows in shaded mountainous areas, whereas *Aristolochia* with climbing habit is found in open fields, highlighting their ecological and morphological divergence.

Conclusions

This study successfully assembled and characterized the chloroplast genome of *A. chungbuensis*, a species endemic to Korea, with a total length of 190,179 bp. The tripartite genome structure of *A. chungbuensis*, one of the characteristic

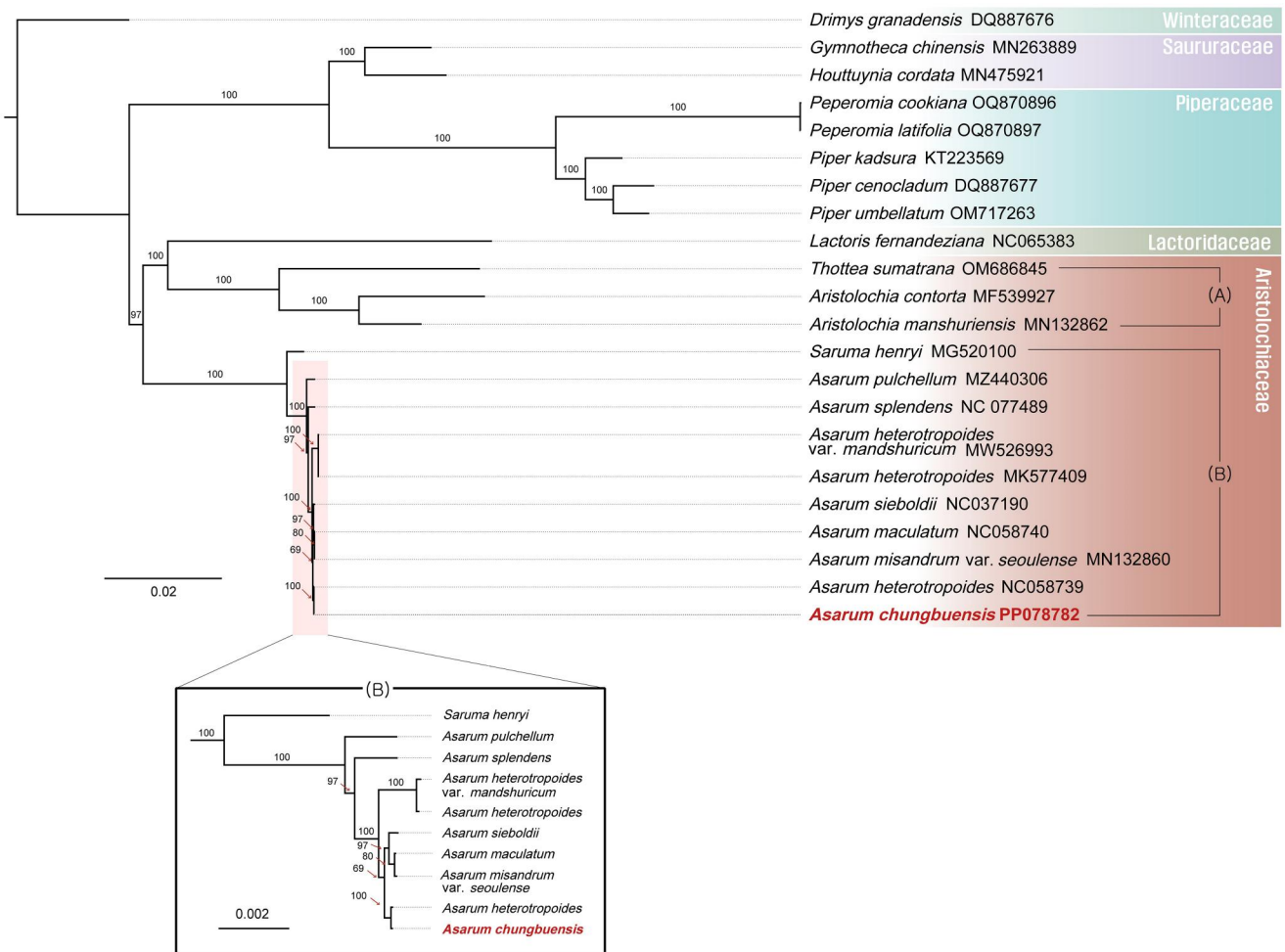


Figure 3. Phylogenetic tree with maximum-likelihood analysis of *Asarum chungbuensis* and 21 other related plastid genome sequences. Bootstrap values are indicated above the branches. GTR + R3 + F was selected as the best evolutionary model by ModelFinder. The scale bar located at the bottom left corner represents the evolutionary distance (unit length, 0.02). Below are the sequences used in constructing this phylogenetic tree: *Drimys granadensis* DQ887676 (Cai et al. 2006), *Piper cenocladum* DQ887677 (Cai et al. 2006), *P. kadsura* KT223569 (Lee et al. 2015), *P. umbellatum* OM717263 (unpublished), *Peperomia cookiana* OQ870896 (Heyduk et al. 2023), *Pe. latifolia* OQ870897 (unpublished), *Saruma henryi* MG520100 (Sinn et al. 2018), *Aristolochia manshuriensis* MN132862 (Kim and Lim 2019), *Ar. contorta* MF539927 (Zhou et al. 2017), *Gymnotheca chinensis* MN263889 (Jin et al. 2019), *Houttuynia cordata* MN475921 (Yu et al. 2019), *Lactoris fernandeziana* NC_065383 (Jost et al. 2022), *Thottea sumatrana* OM686845 (Jost et al. 2022), *Asarum heterotropoides* MK577409 (Zhao et al. 2019), *A. heterotropoides* var. *seoulense* MN132860 (unpublished), *A. heterotropoides* var. *mandshuricum* MW526993 (unpublished), *A. pulchellum* MZ440306 (unpublished), *A. sieboldii* NC_037190 (Lim et al. 2018), *A. misandrum* NC_058739 (unpublished), and *A. maculatum* NC_058740 (unpublished).

structures observed in *Asarum* species, provides important phylogenetic information supporting its distinct taxonomic position and synapomorphy as an endemic species, having independent lineage. Overall, this study provides new phylogenetic insight into *Asarum* and accumulating genomic resources for this rare species.

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Author contributions

Ye-Rim Choi designed the study, interpreted the data set and drafted the original manuscript. Sang-Chul Kim designed the study and interpreted the data set. Young-Ho Ha designed the study and collected the plant samples and performed the experiments. Tae-Hee Kim designed the study and performed the experiments. Hyuk-Jin Kim designed the study and provided financial support. All authors have revised and approved the manuscript.

Ethical approval

All plant materials used in this study complied with national and international standards, as well as local laws and regulations. The use of all plant materials did not pose any risk to other natural species. The study did not involve endangered or protected species, and sample collection did not require special permission from relevant authorities.

Disclosure statement

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

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Data availability statement

The complete chloroplast genome of *Asarum chungbuensis* that supports the findings of this study was deposited in NCBI GenBank under the accession no. PP078782. The associated BioProject, SRA, and BioSample accession numbers are PRJNA1060900, SRR27480213, and SAMN39248553, respectively.

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