

The complete chloroplast genome of an Arctic lycophyte, *Huperzia arctica* (Tolm.) Sipliv. 1973 (Lycopodiaceae)

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ABSTRACT

Huperzia arctica (Tolm.) Sipliv. 1973 is a lycophyte species belonging to the Lycopodiaceae family, which is widely distributed in the Arctic region of Svalbard, Norway. To determine its taxonomic position, we sequenced the chloroplast genome of *H. arctica*. The complete chloroplast genome of *H. arctica* is 153,956 bp in length with 122 annotated genes, including 87 protein-coding genes, 31 tRNA genes, and 4 rRNA genes. To evaluate its evolutionary position, we performed phylogenetic analysis using 36 conserved orthologous protein-coding gene sequences from the chloroplast genomes of *H. arctica* and publicly available data from other Lycopodiaceae. *H. arctica* formed a monophyletic group with four other *Huperzia* species: *H. lucidula*, *H. serrata*, *H. crispata*, and *H. javanica*. However, it appeared as a separate species with a highly supported bootstrap value.

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Introduction

Huperzia arctica (Tolm.) Sipliv. 1973 is the only lycophyte species in the family Lycopodiaceae among the 185 vascular plant taxa native to the Arctic region of Svalbard, Norway (Elven and Elvebakk, 1996), and in the mountainous regions of Russia (Kharkevich et al. 2003). It grows to a height of 5–10 cm with shallow roots. Stems emerge from the base, stand upright, and bifurcate several times. The narrowly triangular leaves are light green to yellowish green in color and are irregularly arranged in 8–10 rows along each shoot. The plant reproduces both sexually and asexually. Sexual reproduction occurs through the development of a yellowish sporangium bearing spores at the base of ordinary leaves on the annual shoot, while vegetative reproduction is achieved through the formation of numerous bulbils near the leaf apex (Rønning 1996; Kharkevich et al. 2003). The species is commonly found in rocky crevices, among shrubs and moss, and is known to prefer acidic soils (Kukkonen 2000; Svalbardflora 2023).





Plants of the genus *Huperzia*, including *Huperzia serrata*, have traditionally been used as medicinal plants in East Asia, and studied for their pharmacological components, particularly alkaloids (Ma and Gang 2004; Ma et al. 2007). *H. arctica* is a perennial plant that thrives in tundra regions above latitude 66.3° in the Arctic, with its aboveground parts covered by snow during the winter (Rønning 1996; Kukkonen 2000). Although *H. arctica* has the potential to produce


pharmacologically active compounds, there has been no research to date regarding its secondary metabolites adapted to the Arctic's extreme environment.

The family Lycopodiaceae is classified into three subfamilies: Lycopodielloideae, Lycopodioideae, and Huperzioideae (PPG I 2016). Huperzioideae is divided into three genera: *Huperzia*, *Phlegmariurus*, and *Phylloglossum*. These Huperzioideae genera demonstrate ecological and geographical adaptations to different habitats and morphological



Figure 1. Reference image of *Huperzia arctica*. Perennial plants, 5–10 cm tall, sporangia are yellow and produced at the leaf base, and spores are yellow as well. Bulbils are located at the apices of stems. The photograph was taken by Youngsim Hwang in Ny-Ålesund, Svalbard (78°54'49.0" N; 11°58'53.6" E) on 17 July 2022.

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diversification. Specifically, *Huperzia* represents temperate and alpine terrestrial species, *Phlegmariurus* represents tropical epiphytic species, and *Phylloglossum* represents southern temperate semi-aquatic species (Field et al. 2016). The genus *Huperzia* was established in 1801 and consists of 25 species that are distributed worldwide (PPG I 2016). Taxonomically, *Huperzia* plants in northern Europe are divided into three species: *Huperzia selago*, *Huperzia appressa*, and *H. arctica*. Recently *H. arctica* and *H. appressa* were merged within *H. selago* ssp. *arctica* (Kukkonen 2000). Currently, *H. arctica* is treated as a separate species, or as a synonym of either *H. appressa* (Desv.) Á.Löve & D.Löve (WFO 2023) or *H. selago* subsp. *appressa* (La Pylaie ex Desv.) D.Löve (Hassler 2023); it

has unclear taxonomic relationships. Therefore, this study evaluated the phylogenetic position of *H. arctica* by analyzing its chloroplast genome, which has not been reported previously.

Materials and methods

The *H. arctica* sample was collected in July 2022 by Youngsim Hwang from a population growing under natural conditions near the Korean Dasan Arctic Station (78°54'49.0" N; 11°58'53.6" E) in Ny-Ålesund, Svalbard (Figure 1). A specimen was deposited at the Korea Polar Research Institute (KOPRI) Herbarium (<https://kvh.kopri.re.kr>, Han-Gu Choi,

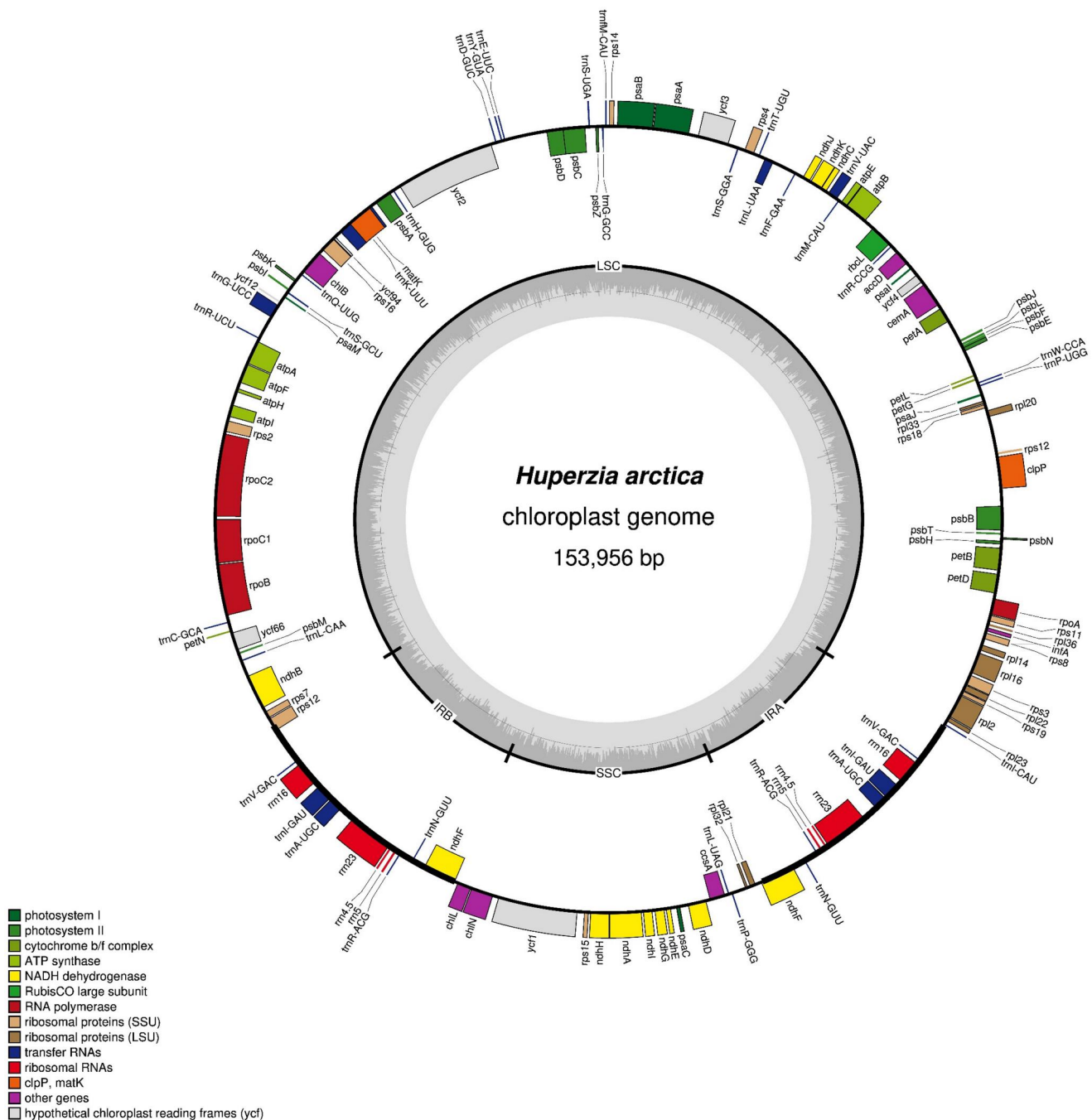


Figure 2. Map of the chloroplast genome of *Huperzia arctica*. Genes lying outside the outer circle are transcribed clockwise, while those inside the circle are transcribed counterclockwise. Genes belonging to different functional groups are color-coded. The innermost darker gray corresponds to GC content, while the lighter gray corresponds to AT content. IR, inverted repeat; LSC, large single copy region; SSC: small single copy region.

hchoi82@kopri.re.kr) under voucher number KOPRI-PL00138 and identified as *H. arctica* by Youngsim Hwang.

Total genomic DNA was purified using a BiomedicVR Plant gDNA Extraction Kit (Biomedic Co., Ltd., Bucheon, Korea) according to the manufacturer's protocol. Genomic library construction was performed using a TruSeq PCR Free DNA Sample Prep Kit (Illumina, San Diego, CA, USA), and paired-end whole-genome sequences were produced using the Illumina NovaSeq platform. We obtained 14,770,255 filtered reads with a mean length of 144.5 bp, and the total read length of raw data was 2.4 Gb. The raw sequencing data were trimmed followed by *de novo* assembly with CLC Assembly Cell v4.2.1 (CLC bio, Aarhus, Denmark). Among the assembled contigs, chloroplast genome sequences were retrieved, aligned, and merged into a single sequence using the *Huperzia lucidula* chloroplast genome sequence as a reference (Wolf et al. 2005). The assembly was finalized through a manual check and sequence errors were corrected by read mapping against the assembled contig. To verify the accuracy of the assembly, we mapped trimmed raw sequence data to the assembled chloroplast genome and the average coverage depth was x472.2 (Supplementary Figure 1).

Genes were annotated using the GeSeq program (Tillich et al. 2017) with reported *Huperzia* chloroplast genomes (AX660566.1, KX426071.1, ON745420.1, MN566837.1, and MH549642.1) as references, and manually curated using the Artemis program (Carver et al. 2012). The complete chloroplast genome of *H. arctica* has been deposited in GenBank under accession number OP714151. A circular map was generated using OGDRAW v1.3.1 (Greiner et al. 2019). From a

total of 13 chloroplast genome sequences including *H. arctica*, nucleotide sequences of 36 conserved orthologous protein-coding genes (*atpA*, *atpB*, *atpE*, *atpF*, *atpH*, *clpP*, *ndhC*, *petA*, *petD*, *petG*, *psaA*, *psaB*, *psaC*, *psal*, *psbA*, *psbB*, *psbC*, *psbE*, *psbH*, *psbl*, *psbJ*, *psbK*, *psbM*, *psbN*, *psbT*, *rbcL*, *rpl14*, *rpl2*, *rpl23*, *rpoA*, *rps3*, *rps4*, *rps7*, *rps8*, *ycf3*, and *ycf4*) were aligned using MAFFT version 7 (<https://mafft.cbrc.jp/alignment/server/>) (Katoh and Standley 2013), followed by the construction of a phylogenetic tree based on the maximum-likelihood method (ML; bootstrap repeat is 1000) based on the GTR + G + I model using MEGA11 (Tamura et al. 2021).

Results and discussion

The complete chloroplast genome sequence of *H. arctica* is 153,956 bp in length with GC content of 36.25%, composed of a large single-copy (LSC) region of 103,996 bp, a small single-copy (SSC) region of 19,669 bp, and 15,145 bp of paired inverted repeat (IR) regions. In total, 122 genes were annotated, consisting of 87 protein-coding genes, 31 tRNA genes, and 4 rRNA genes (Figure 2). There were 16 genes (*atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpl2*, *rpl16*, *rpoC1*, *rps16*, *ycf66*, *trnA-UGC*, *trnG-UCC*, *trnI-GAU*, *trnK-UUU*, *trnL-UAA*, and *trnV-UAC*) containing one intron and three genes (*clpP*, *rps12*, and *ycf3*) having two introns. Twelve cis-spliced genes (*atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpl2*, *rpl16*, *rpoC1*, *rps16*, *ycf66*, *clpP*, and *ycf3*) and one trans-spliced gene (*rps12*) were verified to be corrected and annotated with multiple sequence alignment (Supplementary Figure 2).

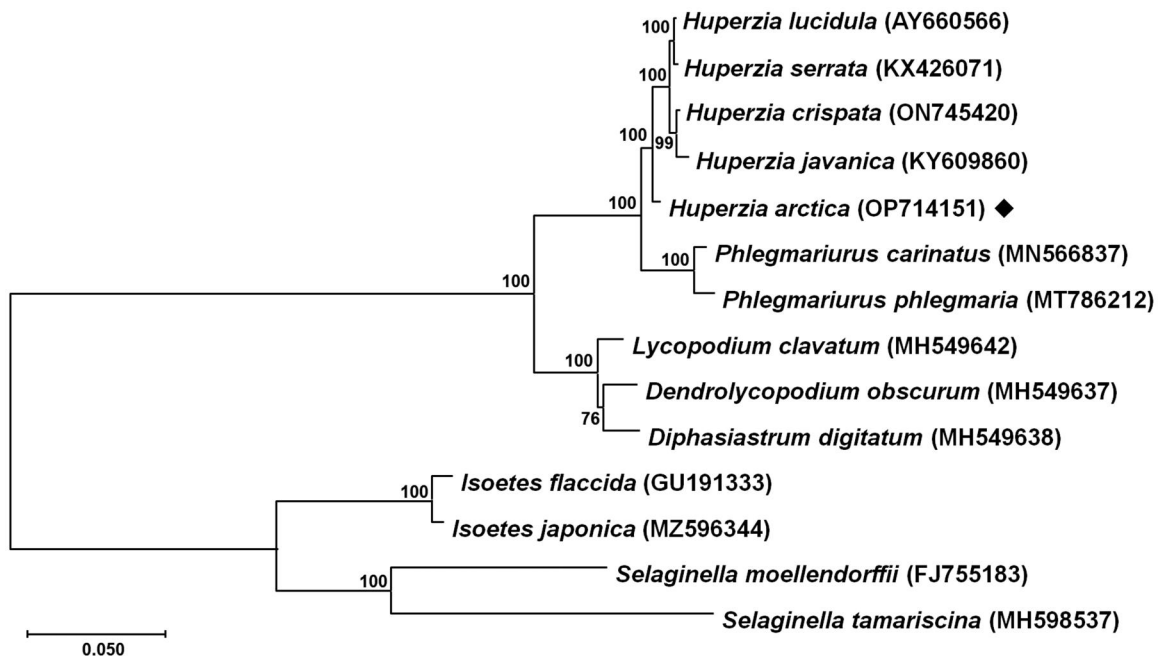


Figure 3. Maximum-likelihood phylogenetic tree based on 14 complete chloroplast genomes. The tree was constructed based on the GTR + G + I model using MEGA11 with nucleotide sequences of 36 conserved orthologous protein-coding genes. The numbers on each internal node indicate bootstrap values based on 1000 iterations. The following sequences were used: *Huperzia lucidula* (AY660566) (Wolf et al. 2005), *H. serrata* (KX426071) (Guo et al. 2016), *H. crispata* (ON745420) (Yin et al. 2022), *H. javanica* (KY609860) (Zhang et al. 2017), *H. arctica* (OP714151) (this study), *Phlegmariurus carinatus* (MN566837) (Luo et al. 2019), *P. phlegmaria* (MT786212) (Tang et al. 2020), *Lycopodium clavatum* (MH549642) (Mower et al. 2019), *Dendrolycopodium obscurum* (MH549637) (Mower et al. 2019), *Diphasiastrum digitatum* (MH549638) (Mower et al. 2019), *Isoetes flaccida* (GU191333) (Karol et al. 2010), *I. japonica* (MZ596344) (Lin et al. 2022), *Selaginella moellendorffii* (FJ755183) (Smith 2009), *S. tamariscina* (MH598537) (Zhang et al. 2019). *Isoetes flaccida*, *I. japonica*, *Selaginella moellendorffii*, *S. tamariscina* were used as outgroup taxa. Scale bar refers to a phylogenetic distance of 0.05 nucleotide substitutions per site.

To evaluate the evolutionary relationships, 13 chloroplast genome sequences including *H. arctica*, eight complete chloroplast genomes of Lycopodiales, two of Isoetales, and two of Selaginellales were used. As shown in Figure 3 which is consistent with a previous study on the molecular phylogenetics of the family Lycopodiaceae (Field et al. 2016), *H. arctica* formed a monophyletic clade with four other *Huperzia* species, and the subfamily Huperzioidae (*Huperzia* and *Phlegmariurus*) was also monophyletic with perfect bootstrap support in the phylogenetic tree (Figure 3).

Conclusions

Our results indicate that while *H. lucidula*, *H. serrata*, and *Huperzia crispata* are closely related to each other, *H. arctica* is also closely related but appears as a separate and independent species. These findings will be crucial for determining the taxonomic position of *H. arctica* and provide a genetic foundation for future studies on this species, particularly in relation to adaptation to extreme Arctic environments.

Ethical approval

The materials used in this study are not included in the IUCN Red List of Threatened Species, and the sampling site is not located in any protected area. The field study was conducted with the permission granted by the Governor of Svalbard (RIS-ID 11964).

Authors' contributions

YS and HL planned experiments. YH and YKL sampled plants. YH and HL extracted DNA, assembled the chloroplast genome and carried out the phylogenetic analysis. YH, YKL, YS, and HL wrote the manuscript. All authors have reviewed and approved 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 that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov> under the accession no. OP714151. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA941097, SRP425714, and SAMN33589810, respectively.

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