




The complete chloroplast genome of *Porella gracillima* Mitt. (Porellaceae) and phylogenetic relationships to other bryophytes

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ABSTRACT

Porella gracillima Mitt. (Jungermanniidae, Porellaceae), a bryophyte is widespread in temperate Asia and North America. In Korea, *P. gracillima* is mainly observed in shaded and dried rocks or tree trunks on mountains. Here, we determined the complete chloroplast (cp) genome sequence of *P. gracillima* to provide useful genetic information in the phylogenetic relationship, phylogeographic history, and conservation of the species. The complete cp genome of *P. gracillima* was assembled using NGS Illumina HiSeqX platform. The cp genome was 121,867 bp in length (GC contents, 33.7%) and showed a typical quadripartite structure, consisting of a large single copy (LSC) of 83,406 bp, a small single copy (SSC) of 19,692 bp, and two inverted repeats (IRs) of 9,385 bp. Phylogenetic analysis shows that Porellaceae was a sister group of Radulaceae, which agrees with the findings of the previous phylogenetic studies. Our cp genome data of *P. gracillima* may contribute to a better understanding of the evolution of the *Porella* in Porellaceae and will help to infer its molecular identification, thereby providing a guideline for conservation.

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Introduction

The genus *Porella* L. is very common and widely distributed and is the largest genus in the Porellaceae. The *Porella gracillima* Mitt. is distributed throughout temperate Asia, with Sichuan and Yunnan provinces in China to the south and the Altai Mountains to the west (Hattori 1969, 1971; Hattori and Zhang 1985). In Korea, they are known to be distributed 13 taxa (Bum 2023) and found in partially shaded open limestone areas, where they are mainly observed growing in shaded and on dried rocks or tree trunks (Hentschel et al. 2008). The leaves are very glossy even when dry, and are relatively hard and brownish to yellowish green. Underleaves are separated from each other, developing decurrent next to the stem, and have a triangular shape similar to an ovoid (Bakalin and Klimova 2019). In this study, we determined the complete chloroplast genome sequence of *P. gracillima* to provide genetic resources to show phylogenetic relationships.

Materials and methods

Plant material and DNA extraction

The specimen was collected from Ulleungdo island, Ulleung-gun, Gyeongsangbuk-do, Korea (E37.48783 N130.87781; Figure 1) by Seung-Jin Park. The voucher specimen was



deposited at Honam National Institute of Biological Resource (HNIBR, <https://hnibr.re.kr/>, moss89@hnibr.re.kr) under the voucher number PSJ-21090809. The total genomic DNA was extracted from dried leaf samples using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany).

Genome sequencing, assembly, and annotation


Genome sequencing was paired-end sequence using the Illumina HiSeqX platform (Macrogen, Korea) and de novo assembly, the annotation was performed in Geneious prime® v.2023.0.1. after BLAST search using the chloroplast genome sequence of *P. perrottetiana* (Genbank accession: MH064507) as a reference sequence. tRNAs were verified using tRNAscan-SE v.2.0 (Schattner et al. 2005). The complete chloroplast genome sequence and annotation results of *P. gracillima* were submitted to GenBank with accession number OQ658673.

Phylogenetic analyses

To analyze the phylogenetic relationship of *Porella* L. and other bryophytes, Maximum Likelihood (ML) tree was constructed using the other cp genome sequences of 43 bryophytes taxa (46 accessions). To build the phylogenetic tree, we first checked synonyms and typos in gene names, and

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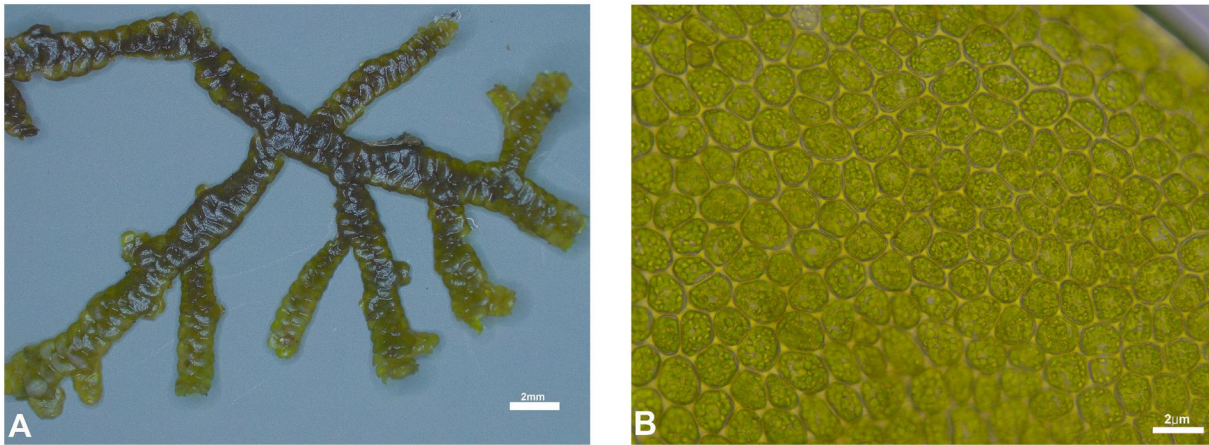


Figure 1. The image of *Porella gracillima*, collected by Seung-Jin Park on Ulleungdo island (E37.48783 N130.87781), was photographed by Jae-Kyeong Lee at the HNIBR laboratory using Leica S9i and Leica DM750. Before taking the photographs, dried specimen was soaked in water for 5 min. This species is distinguished from other *Porella* by its entire ventral lobe and underleaf. (A) Photograph of a dried specimen and (B) Photomicrograph of leaf median cells of *P. gracillima*.

Porella gracillima

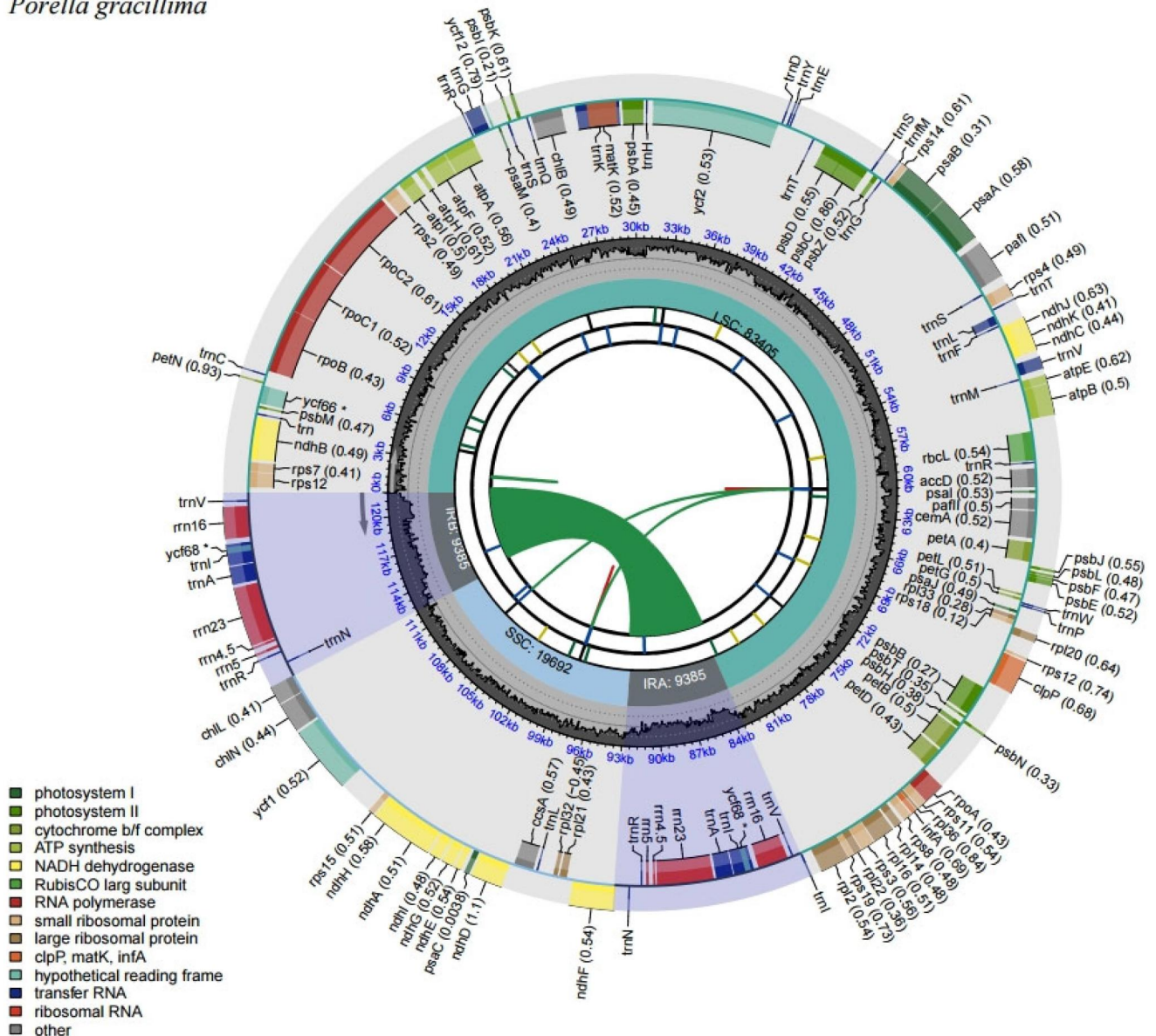


Figure 2. Map of the chloroplast genome of *Porella gracillima* generated by CPGview. From the center outward, the first circle shows the dispersed repeats with red (forward) and green (reverse) arcs. In the second circle, long tandem repeats are denoted by short blue bars. The third circle shows the short tandem repeats or microsatellite sequences. And fourth circle shows the small single-copy (SSC), inverted repeat (IRA and IRB), and large single-copy (LSC) regions. The GC content is represented on the fifth circle, and genes are represented on the sixth circle.

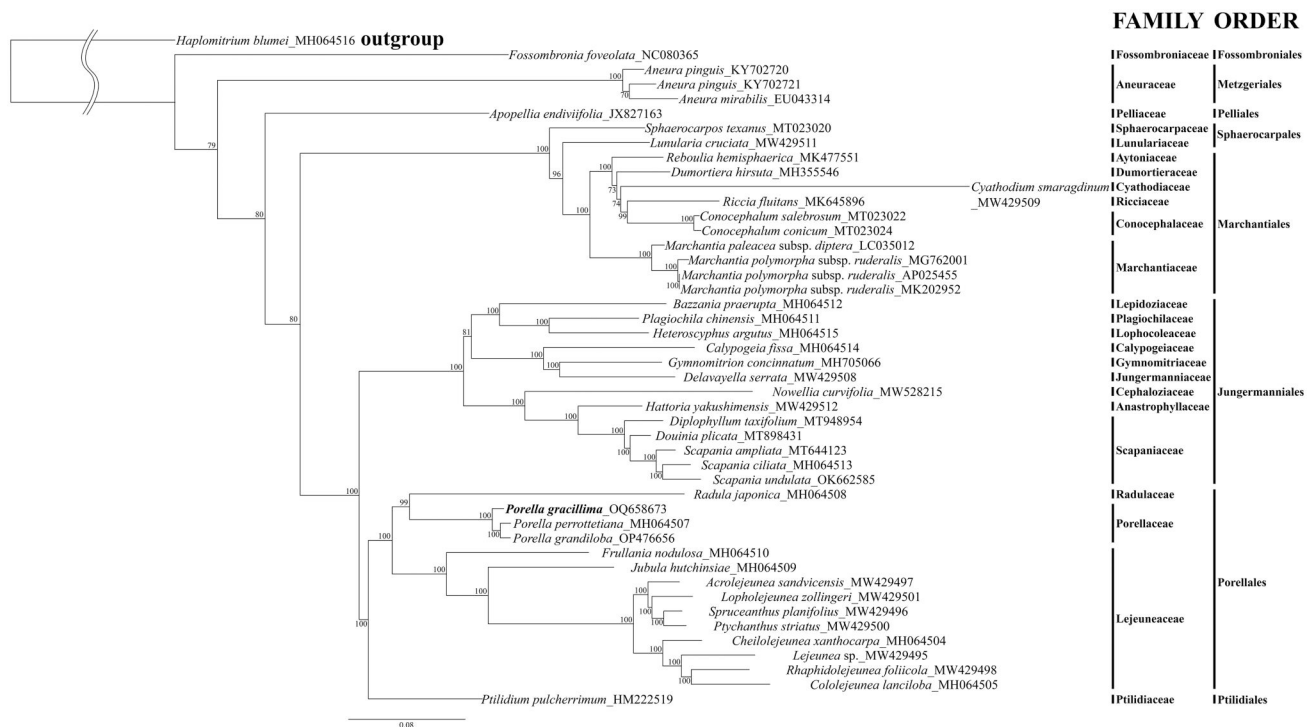


Figure 3. Maximum likelihood tree inferred from complete cpDNA of 43 cp genomes from the other bryophytes. The focal species was marked in bold. The GenBank accession numbers were indicated after the species names with an underscore. The number near the branches indicates bootstrap values. The following sequences were used: *Haplomitrium blumei* MH064516 (Yu et al. 2019) as an outgroup species, and *Fossombronia foveolata* NC080365 (Pauksztos et al. 2023), *Aneura pinguis* KY702720, KY702721 (Myszczyński et al. 2017), *A. mirabilis* EU043314 (Wickett et al. 2008), *Apopellia endiviifolia* JX827163 (Grosche et al. 2012), *Sphaerocarpos texanus* MT023020, *Lunularia cruciata* MW429511 (Frangedakis et al. 2021), *Reboulia hemisphaerica* MK477551 (Kwon, Min, Kim, et al. 2019), *Dumortiera hirsuta* MH355546 (Kwon et al. 2019), *Cyathodium smaragdinum* MW429509 (Frangedakis et al. 2021), *Riccia fluitans* MK645896 (Kwon, Min, Xi, et al. 2019), *Conocephalum salebrosus* MT023022, *C. conicum* MT023024, *Marchantia paleacea* subsp. *diptera* LC035012, *M. polymorpha* subsp. *ruderalis* MG762001, *M. polymorpha* subsp. *ruderalis* AP025455, *M. polymorpha* subsp. *ruderalis* MK202952, *Bazzania praeurupta* MH064512 (Yu et al. 2019), *Plagiochila chinensis* MH064511 (Yu et al. 2019), *Heteroscyphus argutus* MH064515 (Yu et al. 2019), *Calypogeia fissa* MH064514 (Yu et al. 2019), *Gymnomitrium concinatum* MH705066 (Myszczyński et al. 2018), *Delavayella serrata* MW429508 (Frangedakis et al. 2021), *Nowellia curvifolia* MW528215 (Frangedakis et al. 2021), *Hattoria yakushimensis* MW429512 (Frangedakis et al. 2021), *Diplophyllum taxifolium* MT948954 (Bum et al. 2020), *Douinia plicata* MT898431 (Bum et al. 2020), *Scapania ampliata* MT644123 (Choi et al. 2020), *S. ciliata* MH064513, *S. undulata* OK662585, *Radula japonica* MH064508 (Yu et al. 2019), *Porella gracillima* OQ658673 (this paper), *P. perrottetiana* MH064507, *P. grandiloba* OP476656 (Lee et al. 2023), *Frullania nodulosa* MH064510 (Yu et al. 2019), *Jubula hutchinsiae* MH064509 (Yu et al. 2019), *Acrolejeunea sandvicensis* MW429497 (Frangedakis et al. 2021), *Lopholejeunea zollingeri* MW429501 (Frangedakis et al. 2021), *Spruceanthus planifolius* MW429496 (Frangedakis et al. 2021), *Ptychanthus striatus* MW429500 (Frangedakis et al. 2021), *Cheilelejeunea xanthocarpa* MH064504 (Yu et al. 2019), *Lejeunea* sp. 17-8794 MW429495 (Frangedakis et al. 2021), *Rhaphidolejeunea foliicola* MW429498 (Frangedakis et al. 2021), *Cololejeunea lanciloba* MH064505 (Yu et al. 2019), *Ptilidium ciliare* var. *pulcherrimum* HM222519 (Frangedakis et al. 2021).

then finally selected 93 Protein-Coding Genes (PCG). The sequence alignments were performed using MAFFT v7.490 (Kato and Standley 2013). The phylogenetic tree was produced based on the maximum likelihood (ML) with 1,000 bootstrap replicates using the IQ-TREE web server (<http://www.iqtree.org/>, Trifinopoulos et al. 2016). Best-fit model GTR + F + I + G4 was selected using IQ-TREE to construct the phylogenetic tree according to Bayesian Information Criterion (BIC).

Results and discussion

General feature of cp genome

The chloroplast genome of *P. gracillima* was 121,867 bp long (GC contents, 33.7%) and divided into four subregions, which are composed of a large single copy (LSC) of 83,406 bp, a small single copy (SSC) of 19,692 bp, and two inverted repeats (IR) of 9385 bp including 131 genes (84 CDS, eight rRNAs, and 36 tRNAs; Figure 2). Furthermore, eight PCGs (*ndhB*, *rpoC1*, *atpF*, *petB*, *petD*, *rpl16*, *rpl2*, *ndhA*) possess a single intron, three PCGs (*rps12*, *pafl*, *clpP*) has two introns, and then 73 PCGs no intron (Supplementary Figure 1). The

average coverage depth of the genome was 668.4 with a minimum of 248 and a maximum of 961 (Supplementary Figure 2).

Phylogenetic relationship

To the phylogenetic relationship, we used the reported chloroplast genome of 43 bryophytes taxa and *Haplomitrium blumei* as an outgroup (Figure 3). We used 93 PCGs from cp genomes. The concatenated length of the aligned 93 PCGs is a total of 93,620 bp. The result of the phylogenetic analysis shows that Porellaceae was a sister group of Radulaceae, which agrees with the finding of previous phylogenetic studies (Yu et al. 2019; Dong et al. 2021).

Conclusion

In this study, we reported the chloroplast genome of *P. gracillima* for the first time and checked the phylogenetic position with other bryophytes. It consists of 131 genes and 121,867 bp in length. It has been confirmed that Porellaceae is a sister group of Radulaceae. Our cp genome data may

contribute to a better understanding of the phylogenetic relationship of the bryophytes group and the evolution of the genus *Porella* in Porellaceae. Additionally, our study will help to infer its molecular identification, thereby providing a guideline for conservation.

Author contributions

JP conceived and designed the research. SP collected the plant materials. JL and MP performed the experiments and analyzed the data. JL and MP wrote the draft. JP revised the draft. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Ethical statement

Field works have complied with local legislation and appropriate permissions/license were granted while taking samples from protected land. The process and purpose of this experimental research were in line with the rules and regulations of our institute. There are no ethical issues or other conflicts of interest in this study.

Disclosure statement

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

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Data availability statement

The genome data that supported the findings of this study are openly available in GenBank of NCBI at [<https://www.ncbi.nlm.nih.gov>] under accession no. OQ658673. The associated BioProject, SRA, and Bio-sample numbers are PRJNA990926, SRR25119183 and SAMN36277543 respectively.

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