

## The complete mitochondrial genome of an Antarctic moss, *Andreaea regularis* Müll. Hal. 1890 (Andreaeaceae)

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### ABSTRACT

In the present study, we determined the complete mitochondrial genome of *Andreaea regularis* Müll. Hal. 1890, a lantern moss of the genus *Andreaea* Hedw. (Andreaeaceae). The *A. regularis* mitochondrial genome, with a total length of 118,833 bp, consists of 40 protein-coding genes, 3 ribosomal RNA genes, and 24 transfer RNA genes. A phylogenetic tree constructed with 19 complete mitochondrial genomes composed of liverworts, hornworts, and 15 mosses showed that Andreaeales formed the closest sister to Sphagnales before divergence of the remaining moss groups, indicating *A. regularis* being one of the earliest mosses. Our findings could be beneficial to investigate the bryophyte evolution.

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### Introduction


Species of *Andreaea* Hedw., the type genus of the rock moss order Andreaeales, are frequently referred to as the lantern mosses due to their peculiar appearance, including dehiscent sporangia and colors varying from dark-reddish brown to black depending upon the developmental stage (Schofield 1985; Ochyra et al. 2008). The *Andreaea* is primarily known as one of the groups of mosses that split the earliest; they are distributed across cold temperate regions but also found in Antarctica (Greene et al. 1970; Ochyra et al. 2008). To our knowledge, only one mitochondrial genome of the Andreaeales, that of *A. wangiana*, is available (Huang et al. 2019); this lineage split later than another moss lineage, the Sphagnales (Liu et al. 2019). Herein, we describe the initial sequencing, annotation, and characterization of the complete mitochondrial genome of *A. regularis*, contributing to the fundamental genetic knowledge of the genus *Andreaea*. We also performed a phylogenetic analysis with other closely related moss species to understand the evolutionary relationships of this Antarctic moss species.

### Materials and methods

The *A. regularis* sample was collected in January 2012 by Dr. Hyoungseok Lee from a population growing under natural conditions near the King Sejong Antarctic Station (62° 13' 26.58" S; 58° 47' 05.82" W) on Barton Peninsula of King George Island. A specimen was deposited at KOPRI Herbarium (<https://kvh.kopri.re.kr>, Han-Gu Choi, [hchoi82@kopri.re.kr](mailto:hchoi82@kopri.re.kr)) under the

voucher number KOPRI-MO00902 and identified as *A. regularis* by Dr. Young-Jun Yoon. Total genomic DNA extraction and genomic library construction were conducted using a TruSeq DNA Sample Prep Kit (Illumina, San Diego, CA, USA). Whole-genome sequencing was performed using Illumina MiSeq 2 × 300 bp (Illumina) followed by *de novo* assembly with CLC Genomics Workbench V7.5 (CLC bio, Aarhus, Denmark). All contigs were blasted to the *Physcomitrium patens* mitochondrial genome (GenBank accession number: NC\_007945), and PCR and Sanger sequencing were performed to verify contig ends or ambiguous regions in the contig. To validate the accuracy of the assembly, we mapped trimmed raw sequence reads to the assembled mitochondrial genome to assess the coverage depth (Supplementary Figure 1). Genome annotation was performed using Geneious Prime (version 2022.1.1; Kearse et al. 2012), with *P. patens* (NC\_007945) and *A. wangiana* (NC\_046759) as references. The complete mitochondrial genome of *A. regularis* has been deposited in GenBank under accession number OP067134. A circular mitochondrial map was generated using OGDRAW v1.3.1 (Greiner et al. 2019). To evaluate evolutionary relationships, 18 mitochondrial genomes were downloaded from NCBI GenBank: NC\_037508 (Bowman et al. 2017), NC\_043889, NC\_024521 (Liu et al. 2014), KU725510 (Shaw et al. 2016), KU725489 (Shaw et al. 2016), NC\_046759 (Huang et al. 2019), NC\_024290 (Bell et al. 2014), NC\_039775 (Goryunov et al. 2021), MK131350, NC\_024518 (Liu et al. 2014), NC\_024514 (Liu et al. 2014), NC\_007945 (Terasawa et al. 2007), NC\_026975, NC\_027515 (Yoon et al. 2016), NC\_047462 (Cho et al. 2019), NC\_027974, NC\_012651 (Li et al. 2009), NC\_037476 (Dong et al. 2018). Each bryophyte species used for

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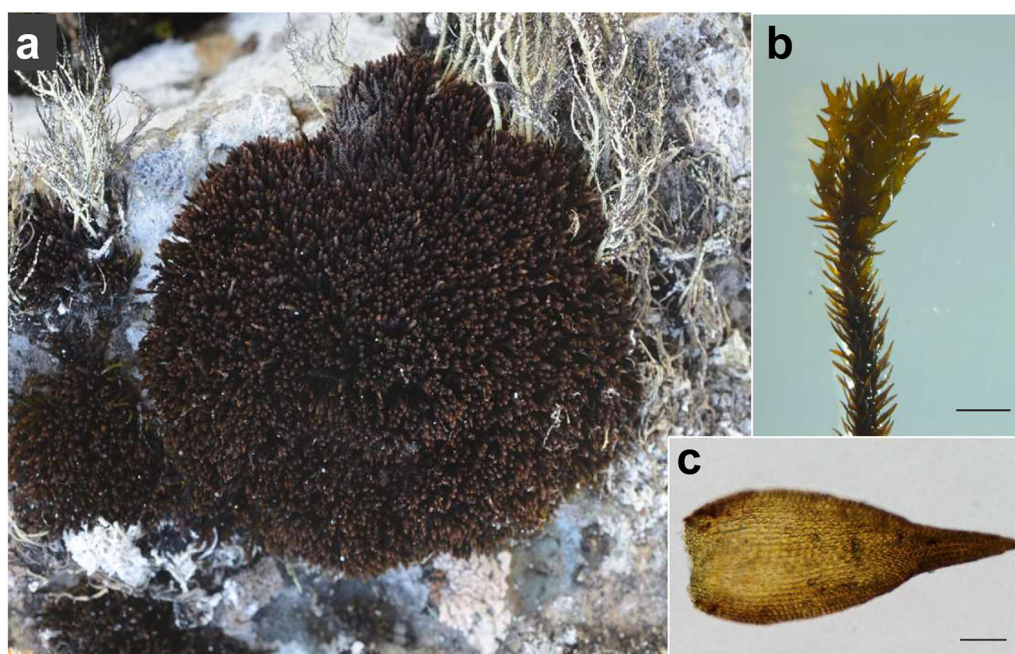
phylogenetic analysis represents their corresponding family and was selected based on available mitochondrial genome data from GeneBank.

## Results and discussion

*Andreaea regularis* is the smallest and most slender of all Antarctic species of the *Andreaea* genus in Antarctica (Ochyra et al. 2008). It differs morphologically from other *Andreaea* species in terms of its stems, at 5~25 mm in length, and ecostate leaves that are lanceolate or ovate-lanceolate, gradually tapering into a narrow long acumen (Figure 1). The mitochondrial genome of *A. regularis* has a total length of 118,833 base pairs and consists of 40 protein-coding genes (PCGs), 3 ribosomal RNA genes (rRNAs), and 24 transfer RNA genes (tRNAs) (Figure 2). There were 10 PCGs (*atp1*, *atp6*, *ccmFC*, *cob*, *cox3*, *nad2*, *nad4*, *nad4L*, *nad9*, *sdh3*) containing one intron, two PCGs (*nad1*, *nad7*) having two introns, three PCGs (*atp9*, *cox2*, *nad5*) having three introns, and one PCG (*cox1*) having four introns. These cis-splicing genes were verified to be corrected and annotated with multiple sequence alignment (Supplementary Figure 2). The genomic nucleotide composition is 29.4% A, 20.1% C, 21.8% G, and 28.7% T. The start codons for all PCGs comply with ATN rules, except for *nad9*, *rpl16*, and *tatC*; the two former genes employ GTG, and the latter uses ACG. All of the PCGs end with the stop codons TAG, TAA, or TGA, except for *ccmFC*, which uses CAA. The lengths of the tRNAs range from 71 to 84 bp, totaling 1,799 bp. The lengths of the three rRNAs (*rrn5*, *rrn18*, and *rrn26*) are 122, 1,695, and 3,442 bp, respectively, totaling 5,259 bp. A comparative analysis of the mitochondrial genomes of *A. regularis* and *A. wangiana*, which was the only Andreaeaceae mitochondrial genome previously available in GenBank, revealed a significant collinearity for 40 PCGs,

3 rRNAs, and 24 tRNAs. Another unique finding of this analysis was the presence of additional base pairs within three different intergenic regions of *A. regularis*: (1) 104 bp between *trnQ-UUU* and *trnL-UAG*, (2) 111 bp between *trnL-UAA* and *nad5*, and (3) 110 bp between *nad6* and *cox2*, partially explaining why the mitogenome size of *A. regularis* is larger than that of *A. wangiana* (Supplementary Table 1).

A total of 19 complete mitochondrial genomes, consisting of two hornworts, two liverworts, and 15 mosses (including *A. regularis*) were aligned using MUSCLE, followed by the construction of a phylogenetic tree based on the maximum-likelihood method (ML; bootstrap repeat is 10,000) using MEGA X software (Kumar et al. 2018); the model (GTR + G) with the lowest Bayesian information criterion scores was considered to best specify the substitution pattern (Adachi and Hasegawa 1996). As expected, the phylogenetic analysis categorized 19 complete mitochondrial genomes into three different phyla, i.e. Marchantiophyta (liverworts), Anthocerotophyta (hornworts), and Bryophyta (mosses) wherein *A. regularis* is clustered with *A. wangiana* within Bryophyta (Figure 3). More importantly, Andreaeales forms a sister group with Sphagnales (*Sphagnum palustre*, *Eosphagnum rigescens*, and *Flatbergium sericeum*), indicating that Andreaeales is an early diverging lineage in the evolution of mosses. Indeed, Huang et al. (2019) noted that both Sphagnales and Andreaeales represent early diverging mosses based on an evolutionary trend of size reduction of the mitochondrial genome wherein Sphagnales and Andreaeales had relatively higher number of introns and longer intergenic spacers than derived mosses. This notion is further reinforced by our comparative analysis of mitochondrial genome sizes across 15 selected mosses (the same species used for phylogenetic analysis as in Figure 3), revealing that the mitochondrial genome size of *A. regularis* is 21.2 kb smaller than the average size of Sphagnopsida mitochondrial genomes and



**Figure 1.** Species reference images of *Andreaea regularis*; whole plant (a), a shoot (b) and a leaf (c). The pictures were taken by authors in a field on Barton Peninsula of King George Island, Antarctica (a) and in a laboratory (b, c). Scale bar = 1 mm (b) and 0.1 mm (c).



**Figure 2.** Circular map of the *Andreaea regularis* mitochondrial genome. Genes outside the outer circle are transcribed clockwise, and those inside the circle are transcribed counterclockwise. Genes belonging to different functional groups are color coded. The innermost darker gray corresponds to GC content, and the lighter gray corresponds to AT content.

13.7 kb larger than the average size of Bryopsida mitochondrial genomes (Supplementary Table 1). The present study is the first report of the complete mitochondrial genome of *A. regularis* and will be helpful in our understanding of the evolution of mosses, particularly *Andreaea*.

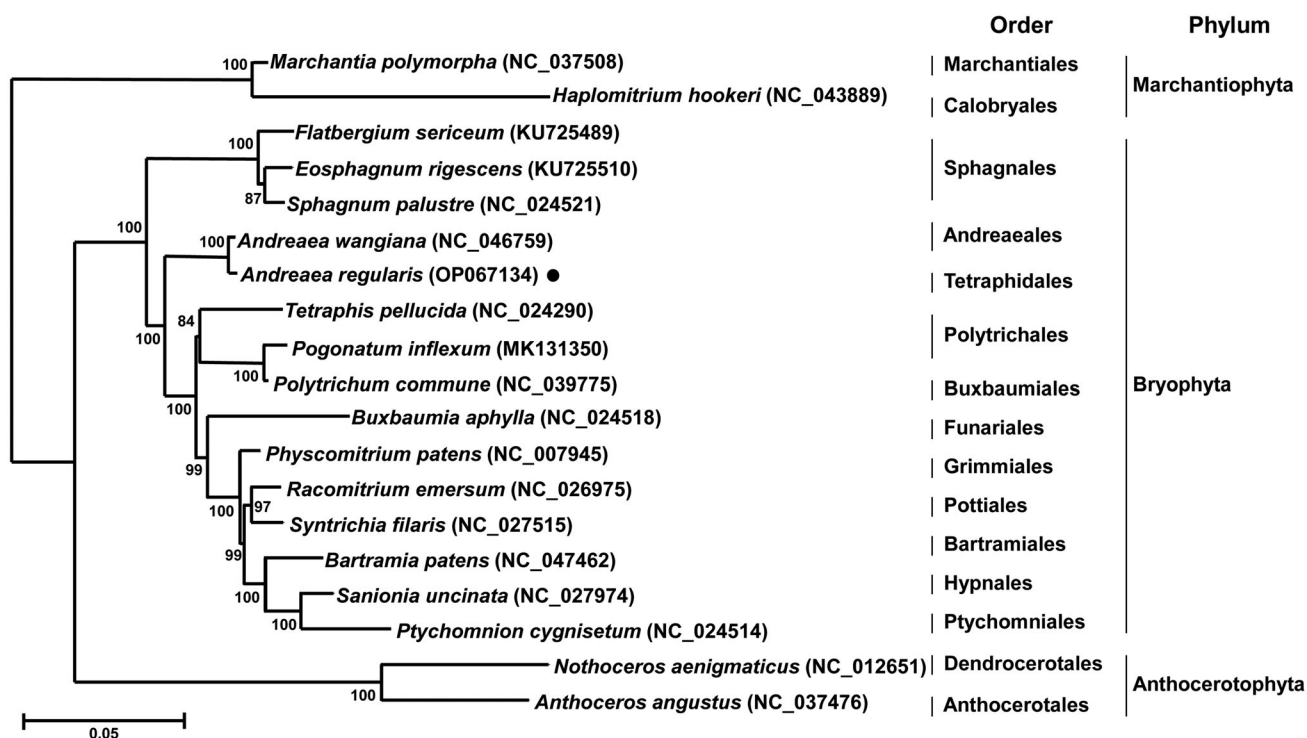
### Ethical approval

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 Minister of Foreign Affairs of the Republic of Korea.

### Author contributions

HsL, KM and HdL jointly performed the analysis and wrote the manuscript. YY identified a specimen. PK, YY, and HsL extracted DNA and assembled the mitochondrial genome. SS and KM annotated the mitochondrial genome and carried out the phylogenetic analysis. All authors have reviewed and approved the manuscript.



**Figure 3.** Maximum likelihood-based phylogenetic tree of 19 complete mitochondrial genomes representing 15 different orders from three phyla. The numbers on each internal node indicate bootstrap values based on 10,000 iterations.

## 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. OP067134. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA911323, SRP412618, and SAMN32178634, respectively.

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