





The complete mitochondrial genome of *Wiesnerella denudata* (Mitt.) Steph. (Wiesnerellaceae, Marchantiophyta): large number of intraspecific variations on mitochondrial genomes of *W. denudata*

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ABSTRACT

Wiesnerella denudata (Mitt.) Steph. is a thallose liverwort distributed in Asian subtropical to tropical regions. It is the only one species in genus *Wiesnerella* and family Wiesnerellaceae. To investigate intraspecific variations on mitochondrial genomes of *W. denudata*, we completed mitochondrial genome of *W. denudata*. Its length is 185,640 bp, longer than that of the previously sequenced mitochondrial genome by 71 bp and contains 73 genes (41 protein-coding genes, 3 rRNAs, 28 tRNAs, and 1 pseudogene). A total of 149 single nucleotide polymorphisms (SNPs) and 3,033 insertions and deletions are identified, much higher than those of *Marchantia polymorpha* subsp. *ruderalis* and *Riccia fluitans*. Phylogenetic trees show that *W. denudata* is clustered with *Monosolenium tenerum* belonging to Monosoleniaceae.

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Wiesnerella denudata (Mitt.) Steph. was described from the East Himalayas by Mitten as *Dumortiera denudata* (Mitten 1860). Later Stephani transferred *D. denudata* to *W. denudata* (Stephani 1898). Inoue described a new family Wiesnerellaceae based on the monotypic genus with one species by four to six irregular valves of capsule dehiscence and specialized asexual structure absent (Inoue 1976). As such, this species has changed in the genus and the family. Recent phylogenetic study shows that Wienerellaceae was related to Ricciaceae (Crandall-Stotler et al. 2009). Whole organelle genome sequences will provide better resolution for uncovering phylogenetic relationship. We completed mitochondrial genome sequences of *W. denudata* for understanding its phylogenetic position.

The thallus of *W. denudata* collected in Jeju city, Korea (Voucher specimen in Jeonbuk National University Herbarium (JNU), Korea; S.S. Choi, CS-201126; 33.48318N, 126.73896E) was used for extracting DNA with DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). Genome sequencing was performed using NovaSeq6000 at Macrogen Inc., Korea. Mitochondrial genome was completed by Velvet v1.2.10 (Zerbino and Birney 2008), SOAPGapCloser version v1.12 (Zhao et al. 2011), BWA version v0.7.17 (Li 2013), and SAMtools version v1.9 (Li et al. 2009) under the environment of Genome Information System (GeIS; <http://geis.infoboss.co.kr>; Park et al., in preparation). Geneious R11 11.0.5 (Biomatters Ltd, Auckland, New Zealand) was used for annotation based on *W. denudata* mitochondrial genome (MK230933; Dong et al. 2019).

The mitochondrial genome of *W. denudata* (GenBank accession is MT745951) is 185,640 bp, which is longer than the previously sequenced mitochondrial genome (MK230933) by 71 bp. It contains 73 genes (41 protein-coding genes, 3 rRNAs, 28 tRNAs, and 1 pseudogene) and overall GC content is 42.9%. *Nad7* gene was revealed as pseudogene in both *W. denudata* mitochondrial genomes which is similar to the other Marchantiales species (Dong et al. 2019; Kwon et al. 2019a, 2019b; Min et al. 2020).

The previous mitochondrial genome contains 203 polymorphic sites and 1,174 not determined bases occupying 0.74% of mitochondrial genome. Except these bases, 149 single nucleotide polymorphisms (SNPs; 0.080%) and 3,033 insertions and deletions (INDELs; 1.62%) were identified. Number and ratio of identified sequence variations are much higher than those of *Marchantia polymorpha* subsp. *ruderalis* (7 SNPs; 0.0038%; Kwon et al. 2019b), *Riccia fluitans* (18 SNPs; 0.0097% and 19 INDELs; 0.010%; Min et al. 2020), *Dumortiera hirsuta* (12 SNPs; 0.0067% and 24 INDELs; 0.013%; Dong et al. 2019; Kwon et al. 2019a), and *Monosolenium tenerum* (14 SNPs; 0.0075% and 7 INDELs; 0.0037%; Dong et al. 2019). They are even higher than those of vascular plants, including *Liriodendron tulifipera* (365 SNPs; 0.066%; 2,117 INDELs; 0.38%; Park et al. 2019) and *Arabidopsis thaliana* (64 SNPs; 0.017% and 1,089 INDELs; 0.30%; Park et al., in preparation). However, those of *Scapania ampliata* (823 SNPs; 0.057% and 2,242 INDELs; 1.56%; Choi et al., under revision) display similar level.

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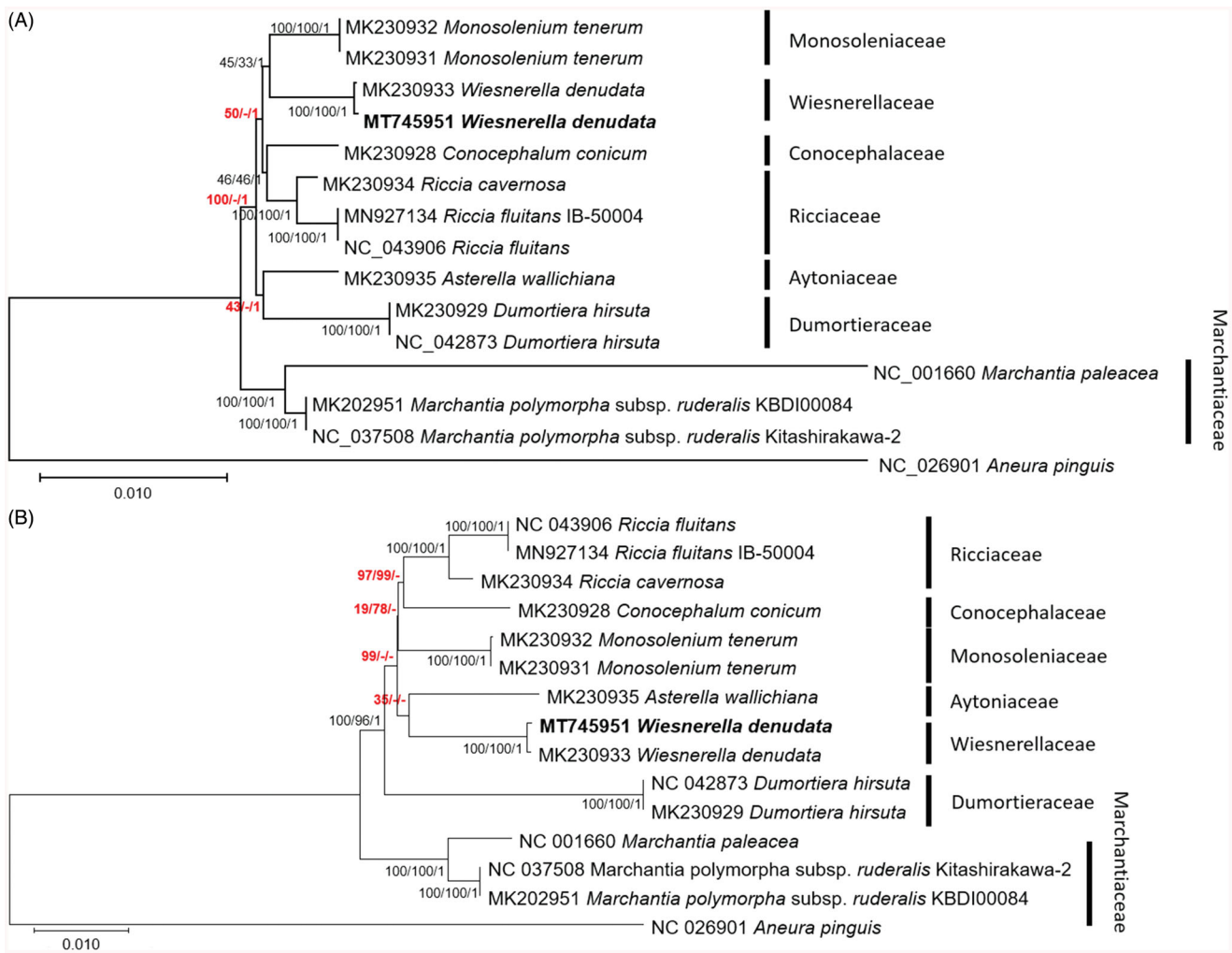


Figure 1. Neighbor-joining (bootstrap repeat is 10,000), maximum likelihood (bootstrap repeat is 1000), and Bayesian Inference (Number of generations is 1,100,000) phylogenetic trees of (A) concatenation of alignment of conserved 37 genes (B) 15 complete mitochondrial genomes: *Wiesnerella denudata* (MT745951 in this study and MK230933; Dong et al. 2019), *Monosolenium tenerum* (MK230931 and MK230932; Dong et al. 2019), *Conocephalum conicum* (MK230928; Dong et al. 2019), *Riccia cavernosa* (MK230934; Dong et al. 2019), *Riccia fluitans* (NC_043906 and MN927134; Min et al. 2020), *Asterella wallichiana* (MK230935; Dong et al. 2019), *Dumortiera hirsuta* (NC_042873 and MK230929; Dong et al. 2019; Kwon et al. 2019a), *Marchantia polymorpha* subsp. *ruderalis* (NC_037508 and MK202951; Bowman et al. 2017; Kwon et al. 2019b), *Marchantia paleacea* (NC_001660; Oda et al. 1992), and *Aneura pinguis* (NC_026901; Myszczynski et al. 2017) as an out-group. Gray bars with labels indicate specific classes. The numbers above branches indicate bootstrap support values of maximum likelihood, neighbor-joining, and Bayesian Inference phylogenetic trees, respectively. Phylogenetic trees were drawn based on maximum likelihood tree. Red-colored supportive values indicate incongruent among three trees.

Fifteen complete mitochondrial genomes including two *W. denudata* mitochondrial genome were used for drawing neighbor-joining (bootstrap repeat is 10,000), maximum likelihood (bootstrap repeat is 1,000), and Bayesian Inference phylogenetic trees (Number of generations is 1,100,000) with MEGA X (Kumar et al. 2018) and MrBayes v3.2.7a (Huelsenbeck and Ronquist 2001) based on alignments of both 37 conserved protein-coding genes based on annotation of the used mitochondrial genomes and whole mitochondrial genome sequences by MAFFT version v7.450 (Kato and Standley 2013). Phylogenetic trees present that our *W. denudata* mitochondrial genome is clustered with the previous *W. denudata* mitogenome with high supportive values (Figure 1(A)), which is different from the previous study based on chloroplast genome (Choi et al. 2020). It also displays that Wiesnerellaceae is clustered with Monosoleniaceae, not Ricciaceae (Figure 1(A)), which is incongruent to the previous study (Crandall-Stotler et al. 2009). In addition, five

nodes which cover higher level of taxa display incongruency of maximum likelihood tree or supportive values of maximum likelihood are lower than those of Bayesian Inference under the congruent between two trees (Figure 1(A)). In addition, phylogenetic trees constructed based on complete mitochondrial genome display more incongruent among three trees (Figure 1(B)). Maximum likelihood tree presents that *Asterella wallichiana* is clustered with *W. denudata*; while the rest two trees display that *A. wallichiana* is located as third basal group after Marchantiaceae and Dumortieraceae. These incongruencies of phylogenetic trees indicate that additional in-depth phylogenetic analyses should be conducted in near future.

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Data availability statement

Mitochondrial genome sequence can be accessed via accession number MT745951 in NCBI GenBank.

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