

## The complete mitochondrial genome of *Riccia fluitans* L. (Ricciaceae, Marchantiophyta): investigation of intraspecific variations on mitochondrial genomes of *R. fluitans*

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### ABSTRACT

*Riccia fluitans* L. is the most common species in *Riccia* genus. To investigate intraspecific variations on mitochondrial genomes of *R. fluitans*, we completed mitochondrial genome of *R. fluitans*. Its length is 185,640 bp, longer than that of NC\_043906 by 19 bp and it contains 74 genes (42 protein-coding genes, 3 rRNAs, 28 tRNAs, and 1 pseudogene). 18 single nucleotide polymorphisms (SNPs) and 19 insertions and deletions are identified, higher than that of *Marchantia polymorpha* subsp. *ruderalis*. One non-synonymous SNP is found in *ccmFN*. Phylogenetic trees show that *R. fluitans* is clustered with *Dumortiera hirsuta*, requiring additional mitogenome to clarify the phylogenetic relationship.

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*Riccia fluitans*; mitochondrial genome; Ricciaceae; intraspecific variation; *ccmFN*

*Riccia fluitans* L., the most common species in *Riccia* genus, is distributed all over the world (Manju et al. 2012). In order to investigate its organellar genome sequences, its chloroplast genome was sequenced previously (Kwon, Min, Xi, et al. 2019). In addition, the mitochondrial genome of *R. fluitans* was also uncovered to understand RNA editing events on mitochondrial genomes of early land plants (Myszczyński et al. 2019). To understand intraspecific variations of mitochondrial genome of *R. fluitans*, we completed its mitochondrial genome sequences.

The thallus of *R. fluitans* collected in Namyangju city, Korea (Voucher in InfoBoss Cyber Herbarium (IN); W. Kwon, IB-50004; 37.583486 N, 127.238337E) was used for extracting DNA with DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). Genome sequencing was performed using HiSeqX at Macrogen Inc., Korea. Mitochondrial genome was completed by Velvet 1.2.10 (Zerbino and Birney 2008), SOAPGapCloser 1.12 (Zhao et al. 2011), BWA 0.7.17 (Li 2013), and SAMtools 1.9 (Li et al. 2009). Geneious R11 11.0.5 (Biomatters Ltd, Auckland, New Zealand) was used for annotation based on *R. fluitans* mitochondrial genome (NC\_043906; Myszczyński et al. 2019).

The mitochondrial genome of *R. fluitans* (GenBank accession is MN927134) is 185,640 bp, which is longer than NC\_043906 by 19 bp. It contains 74 genes (42 protein-coding genes, 3 rRNAs, 28 tRNAs, and 1 pseudogene) and overall GC content is 42.4%. *nad7* gene was revealed as pseudogene in both *R. fluitans* mitochondrial genomes.

From alignment of two mitochondrial genomes, 18 single nucleotide polymorphisms (SNPs; 0.0096%) and 19 insertions

and deletions (INDELs; 0.010%) were identified. Number and ratio of sequence variations are higher than those of *Marchantia polymorpha* subsp. *ruderalis* (7 SNPs; 0.0038%; Kwon et al. 2019c); however, it is still less than those of vascular plants, such as *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), addressing hypothesis that the number of variations on bryophytes mitochondrial genomes is less than those of vascular plants. Only one non-synonymous SNP is found in *ccmFN* and the remaining variations are in intergenic space.

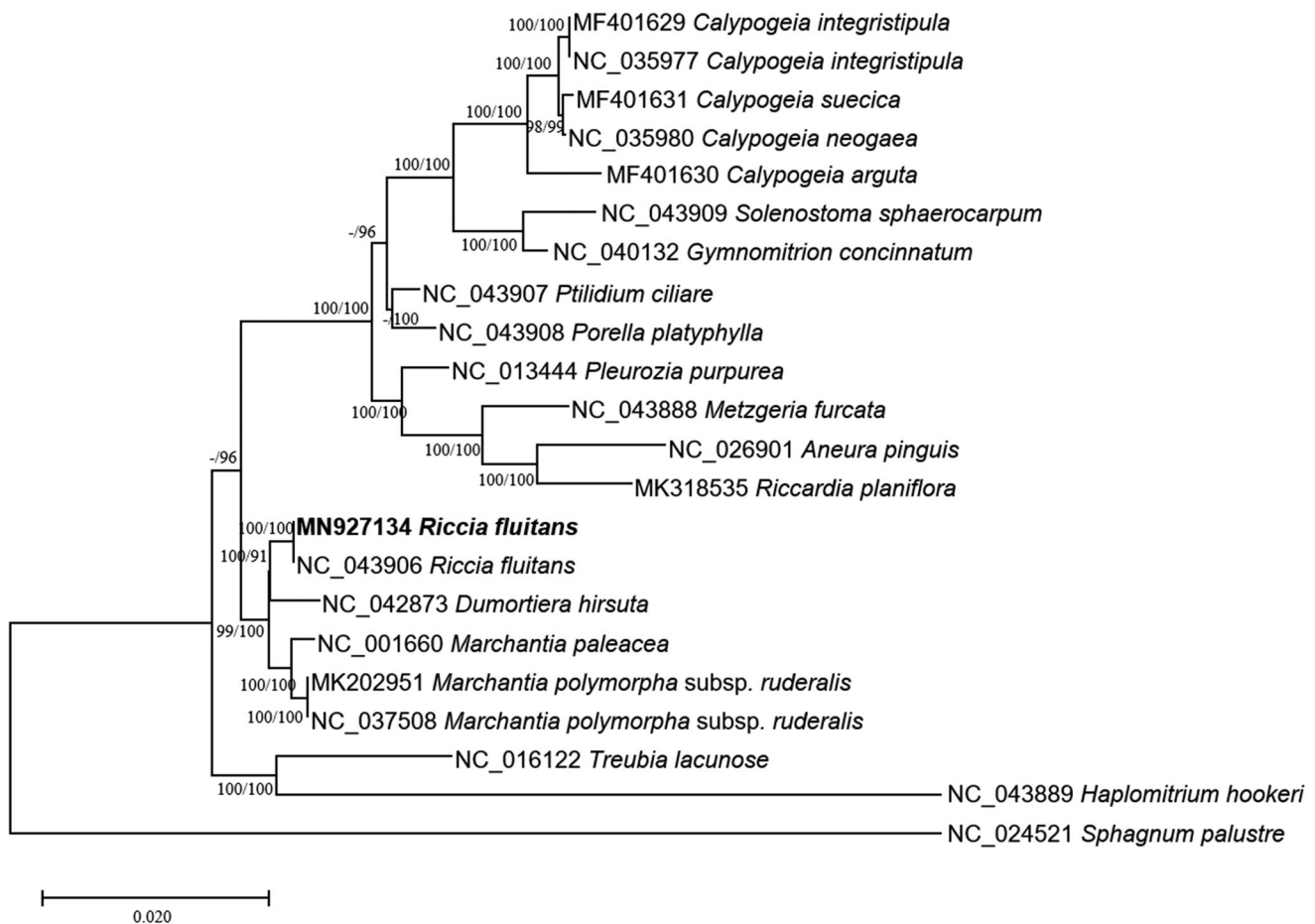
Twenty-two complete mitochondrial genomes including *R. fluitans* mitochondrial genome were used for drawing neighbor joining (bootstrap repeat is 10,000) and maximum likelihood (bootstrap repeat is 1,000) trees based on alignments of 26 conserved protein-coding genes based on annotation of all used mitochondrial genomes by MAFFT 7.450 (Katoh and Standley 2013) and MEGA X (Kumar et al. 2018). Phylogenetic trees present that *R. fluitans* is clustered with *Dumortiera hirsuta* (Figure 1), which is not congruent with the tree that *D. hirsuta* is clustered with *Marchantia* genus (Kwon et al. 2019a) but agrees with three of four trees which display that *R. fluitans* is clustered with *D. hirsuta* (Kwon, Min, Xi, et al. 2019). Because of incongruence in the topology of *R. fluitans*, *D. hirsuta*, and *Marchantia* genus, additional mitochondrial genomes such as *Reboulia hemisphaerica* clustered with *D. hirsuta* (Kwon et al. 2019b; Kwon, Min, Kim, et al. 2019) will be required to clarify their phylogenetic relationship.

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**Figure 1** Neighbor joining (bootstrap repeat is 10,000) and maximum likelihood (bootstrap repeat is 1,000) phylogenetic trees of 22 complete mitochondrial genomes: *Riccia fluitans* (MN927134 in this study and NC\_043906), *Marchantia polymorpha* subsp. *ruderalis* (NC\_037508 and MK202951), *Marchantia paleacea* (NC\_001660), *Pleurozia purpurea* (NC\_013444), *Aneura pinguis* (NC\_026901), *Dumortiera hirsuta* (NC\_042873), *Calypogeia neogaea* (NC\_035980), *Calypogeia integristipula* (NC\_035977), *Calypogeia suecica* (MF401631), *Calypogeia arguta* (MF401630), *Calypogeia integristipula* (MF401629), *Riccardia planiflora* (MK318535), *Solenostoma sphaerocarpum* (NC\_043909), *Porella platyphylla* (NC\_043908), *Ptilidium ciliare* (NC\_043907), *Metzgeria furcata* (NC\_043888), *Haplomitrium hookeri* (NC\_043889), *Tritomaria quinquentata* (NC\_037041), *Gymnomitrium concinnatum* (NC\_040132), *Calypogeia integristipula* (NC\_035977), *Treubia lacunose* (NC\_016122), and *Sphagnum palustre* (NC\_024521) as an outgroup. The numbers above branches indicate bootstrap support values of maximum likelihood and neighbor-joining phylogenetic trees, respectively.

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