

## Nanopore sequencing of chloroplast genome of *Scapania undulata* (L.) Dumort., 1835 (Scapaniaceae, Jungermanniales)

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### ABSTRACT

We performed long-read sequencing of the *Scapania undulata* (L.) Dumort. chloroplast genome with Oxford Nanopore Technology using improved flow cells (10.3). *S. undulata* is a leafy liverwort living on rocks in or near water in mountains. The size of the complete plastome sequence is 117,651 bp long, the large single copy (LSC) region is 80,606 bp long, the small single copy (SSC) region is 19,401 bp long. The nanopore sequencing allowed for the distinction of inverted repeats A and B (IRA, IRB) which were not identical. Guanine cytosine (GC) ratio is 33.7% for the complete chloroplast genome, for LSC and SSC are 31.7% and 30.6%. The phylogeny reconstruction shows that *Scapania undulata* is the sister to *S. ciliata* and *S. griffithii*.

### ARTICLE HISTORY

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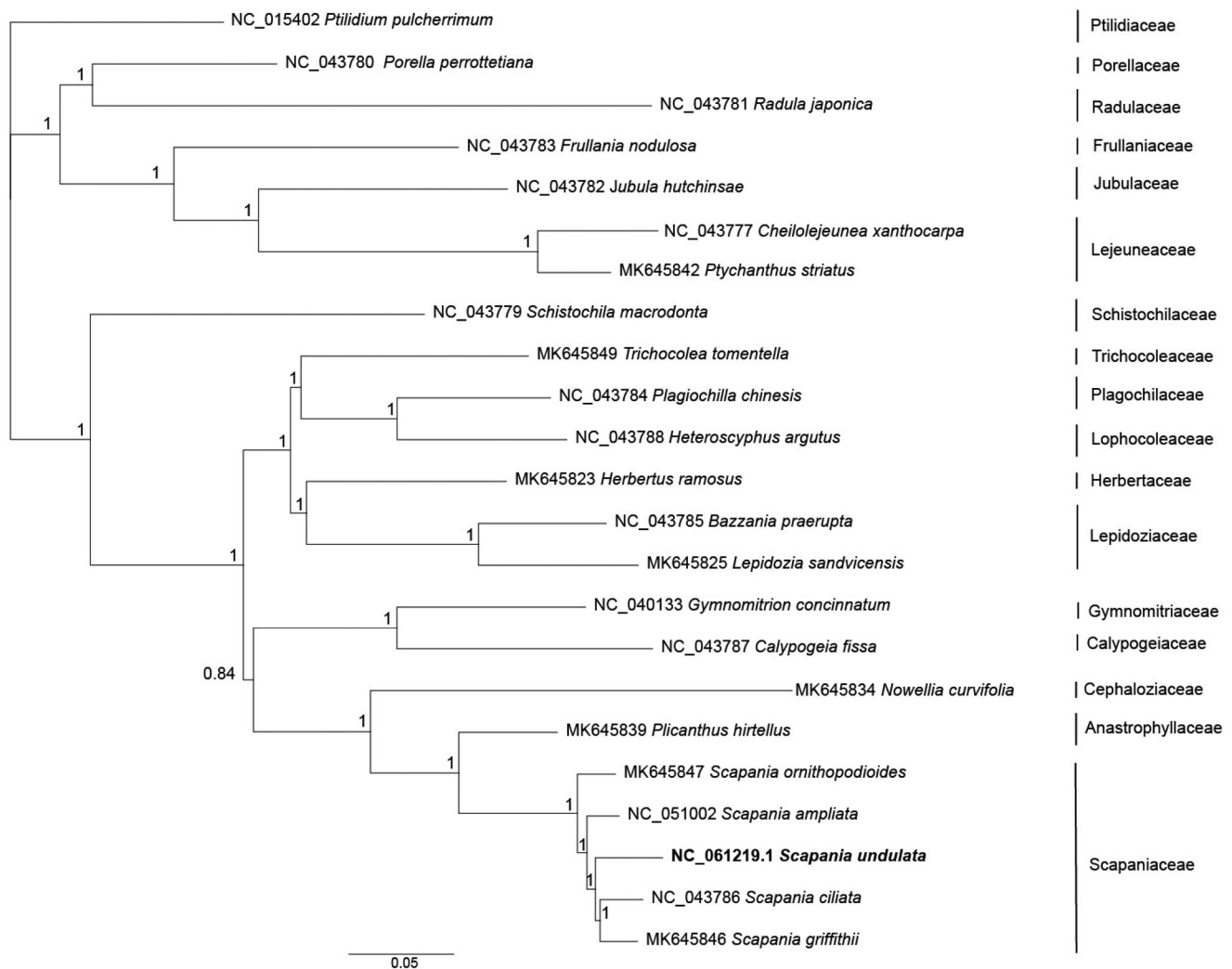
*Scapania undulata* (L.) Dumort. is a widespread species of leafy liverworts which belongs to the genus *Scapania* (Dumort.) Dumort. It's mostly found near mountain springs and on the rocks. Sometimes it can be even found in the sprays of waterfalls or even form submerged patches. It's characteristic due to the often found reddish color of a border on green leaves and dark stems. When placed in the shadow it produces clusters of green gemmae (Lincoln 2008). Here, we assembled and characterized the complete chloroplast genome sequence of *S. undulata* as a resource for future studies on this species including phylogenetics studies and adaptation to different habitats

The individuals were collected in the Karkonosze mountains (50.82848 N, 15.55381 E) and deposited in the herbarium of the Department of Botany and Nature Protection under the voucher number OLS-H-SC21091 (contact: Jakub Sawicki, [jakub.sawicki@uwm.edu.pl](mailto:jakub.sawicki@uwm.edu.pl)). DNA extraction was performed with the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). DNA quantitation was estimated by Qubit 4.0 fluorometer and Qubit dsDNA BR assay kit (Thermo Fisher Scientific, Waltham, U.S.). Genomic DNA by Ligation kit (SQK-LSK109) and MinION R10.3 flow cell (Nanoporetech, Oxford, UK) were used for genome sequencing. Guppy 3.2.10 allowed for successful high-accuracy base-calling and the chloroplast genome was assembled with Minimap2 (Li 2018) with mean coverage of 217.4X. The genomes were aligned using Mauve 2.4.0 (Darling et al. 2010) and phylogenetic relationships were analyzed using MrBayes plugin as implemented in Geneious 10.2.6 software (Biomatters Ltd, Auckland, New

Zealand). The early divergent leafy liverwort, *Ptilidium pulcherrimum*, was used as root.

The complete chloroplast genome of *Scapania undulata* (GenBank accession is NC\_061219.1) is 117,651 bp long. Guanine cytosine (GC) ratio is 33.7%. The large single copy (LSC) region is 80,606 bp long (GC ratio 31.7%) and the small single copy (SSC) region includes 19,401 bp (GC ratio 30.6%). Long-read sequencing enabled separation reads containing sequences of inverted repeat A and B and these regions revealed not being identical. The 4 SNPs between IRB and IRA were found with the use of long-read sequencing. IRB is 8815 bp long while the IRA is 8817 bp long. The genome contains 130 genes which includes 86 protein-coding genes, 8 ribosomal RNAs (rRNAs) and 36 transfer RNAs (tRNAs); nine genes (four rRNAs and five tRNAs) are duplicated in IR regions. The plastome of *S. undulata* shows the same content and order of genes as previously sequenced members of this genus (Choi et al. 2020; Dong et al. 2021). The plastome structure seems to be conserved among liverworts, with few exceptions, where few SSC genes were lost or moved to IRs (Yu et al. 2019; Sawicki et al. 2020; Dong et al. 2021), however no gene lost was detected in plastome of *Scapania undulata*.

For the construction of the phylogenetic tree twenty two chloroplast genome sequences of other species were compared with *S. undulata*. Analysis shows no differences in comparison to the previous studies (Dong et al. 2021). *Scapania undulata* resolved as a sister group to *S. ciliata* and *S. griffithii*. The phylogenetic relationships inside clade of



**Figure 1.** Phylogenetic relationships of liverworts based on plastome sequences obtained using Bayesian inference. The posterior probability values are given at nodes.

Scapaniaceae is similar to the one presented in the past (Heinrichs et al. 2012) (Figure 1).

## Ethical approval

None permission are required according to national law – species and collecting site are not under protection.

## Authors contributions

KC, BS and KZ performed the experiments investigations and writing the original draft. KK and JS prepared the resources, supervised the project, and made revisions to the manuscript.

## Disclosure statement

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

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## Data availability statement

The chloroplast genome sequence is deposited in GenBank of NCBI under accession number NC\_061219.1. Raw reads are deposited in SRA under accession PRJNA843916 (Biosample SAMN28772361 of Bioproject PRJNA843898).

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