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The complete chloroplast genome sequence of a morphotype of *Goodyera schlechtendaliana* (Orchidaceae) with the column appendages

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ABSTRACT

Goodyera schlechtendaliana is a common orchid species in East Asia. A distinctive population of *G. schlechtendaliana* with the lateral appendages of the column in their flowers was found in southwestern Korea. In this study, we presented complete chloroplast genome of this morphotype as a part of systematic study of the *Goodyera*. The chloroplast genome is 153,882 bp in length and contains 134 genes (83 CDSs, 8 rRNAs, and 39 tRNAs). Phylogenetic analysis showed that the morphotype of *G. schlechtendaliana* with column appendages is sister to a normal form of *G. schlechtendaliana* with long branch, supporting that this distinctive morphotype has a potential to be a new species.

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Goodyera schlechtendaliana Rchb. f. (Orchidaceae: Orchidoideae) is a common orchid widely distributed in East Asia ranging from the Himalayas, Sumatra, China, Taiwan, Korea, to Japan (Chen et al. 2009). It is characterized by having white variegated marking on the adaxial surface of the leaves that are arranged in basal rosette in Orchidaceae. We found a population of plants similar to *G. schlechtendaliana* but having distinct morphology that would be potentially a new species on remote islands in southwestern Korea. This plant is different from *G. schlechtendaliana* in having larger leaves, longer inflorescence, and the lateral appendages of the column. As part of the systematic study to understand the phylogenetic position of this morphotype in *Goodyera* we determined its complete chloroplast genome.

A plant used in this study was collected on Hongdo Island in Heuksan-myeon, Shinan-gun, Jeollanam-do province, Korea (voucher specimen: Oh 7169, deposited in Daejeon University Herbarium, TUT). Total genomic DNA was extracted from fresh leaves using a DNeasy Plant Mini kit (QIAGEN, Hilden, Germany). Paired-end sequencing was performed using HiSeq4000 (Illumina, San Diego, CA) and *de novo* assembly was performed using Velvet version 1.2.10 (Zerbino and Birney 2008) and gap sequences were filled by SOAPGapCloser version 1.12 (Zhao et al. 2011). Geneious R11 version 11.0.5 (Biomatters Ltd., Auckland, New Zealand) was used for genome annotation.

The chloroplast genome of the morphotype of *G. schlechtendaliana* with the column appendages (GenBank accession number, MK134679) is 153,882 bp in length and composed of

four subregions: 82,733 bp of large single copy (LSC), 18,019 bp of small single copy (SSC) regions, and 26,535 bp of a pair of inverted repeats (IRs). It contains 134 genes (83 CDS, 8 rRNAs, and 39 tRNAs); 20 genes (eight CDS, four rRNAs, and eight tRNAs) are in each IR region. The overall GC content of the morphotype of *G. schlechtendaliana* was 37.2% and those in the LSC, SSC, and IR regions were 34.9, 29.7 and 43.3%, respectively.

Complete chloroplast genomes from five species of *Goodyera* and two outgroups, *Anoectochilus emeiensis* (NC033895) and *Ludisia discolor* (NC030540), were included in phylogenetic analysis using the maximum likelihood method. Sequence alignment was conducted by MAFFT version 7.388 (Katoh and Standley 2013), and phylogenetic tree was constructed by IQ-TREE version 1.6.6 (Nguyen et al. 2014) with 1000 pseudoreplicates of bootstrapping.

The phylogenetic tree shows that the morphotype of *G. schlechtendaliana* with column appendages from Korea is sister to *G. schlechtendaliana* with 100% bootstrap support. A long branch leading to the morphotype of *G. schlechtendaliana* with column appendages suggests that there is large amount of evolutionary divergence between the two samples (Figure 1). The chloroplast genome data are consistent with morphology, supporting that the morphotype of *G. schlechtendaliana* from Korea could potentially be treated as a new species. In addition, the chloroplast genome will be a useful resource for understanding phylogenetic relationship of the genus *Goodyera*.

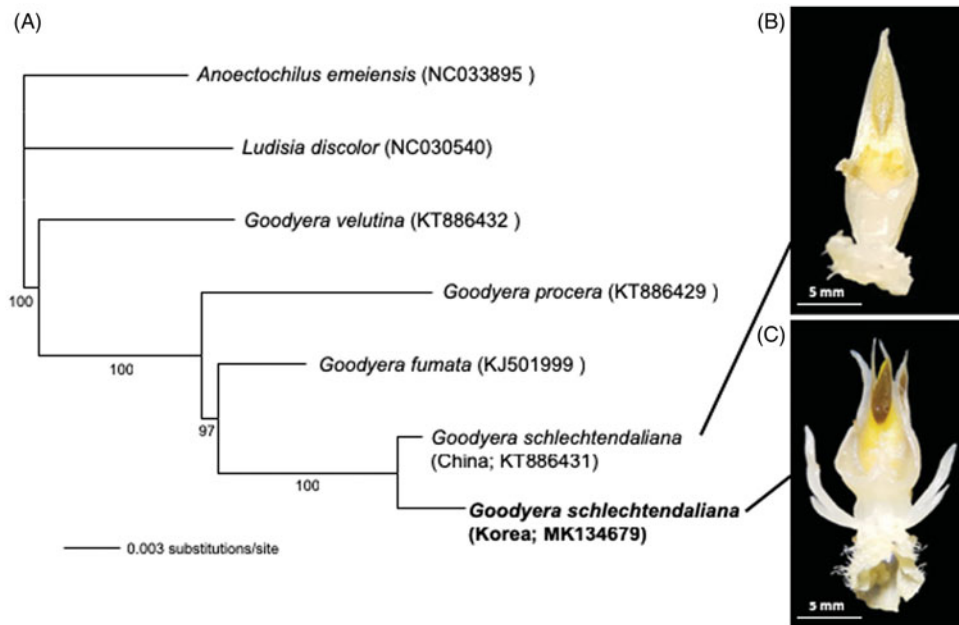


Figure 1. A maximum-likelihood tree (A) using chloroplast genomes of the morphotype of *G. schlechtendaliana* with the column appendages from Korea (this study) and previously published related taxa: *G. schlechtendaliana* from China (KT886431), *Goodyera fumata* (KJ501999), *Goodyera procera* (KT886429), *Goodyera velutina* (KT886432), *Ludisia discolor* (NC030540), and *Anoectochilus emeiensis* (NC033895). The number below the branch indicates bootstrap value. The columns of *G. schlechtendaliana* (B) and the morphotype of *G. schlechtendaliana* with lateral appendages (C) are shown.

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