RESEARCH NOTE

Grovesiella abieticola (Tympanidaceae): an Unrecorded Endophytic Fungus from South Korea

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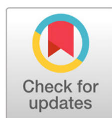
ABSTRACT
We report on an unrecorded endophytic fungus, *Grovesiella abieticola* (Zeller & Goodd.) M. Morelet & Gremmen isolated from *Abies koreana* of Mt. Halla in Jeju. In this study, we used a culture method to determine its conidia and compared relative species using an internal transcribed spacer (ITS) 1, 2, and 5.8S ribosomal DNA sequence. Thus, we present the cultural characteristics and morphology of *G. abieticola* in this paper.

Keywords: *Abies koreana*, Endophytic fungi, *Grovesiella abieticola*, Korea

There were four fungal species in the genus *Grovesiella* M. Morelet. But two fungal species - *G. abieticola* and *G. grantii* - are remained in this genus since *G. ericae* and *G. ledi* have been reclassified as *Erissonopsis ericae* and *Godronia ledi*, respectively [1, 2]. Until now, there has been no description of *Grovesiella* spp. in Korea. The genus *Grovesiella* is defined as a solid black apothecium and club-shaped ascus with eight ascospores. In the asexual stage, it will characteristically have cylindrical macroconidia with septate and oval microconidia without septate [3].

Alpine conifers are vulnerable to climate change and are directly affected by global warming [4]. Therefore, the Ministry of Environment (National Institute of Ecology) is conducting an effective conservation process for alpine conifers through basic ecological studies on vulnerable species including *Abies koreana*. Based on this context, this study focuses on the biodiversity of endophytic fungi in alpine conifers. So we try to elucidate the classification and ecological matters of endophytic fungi on fir. Through this, we have discovered an unrecorded endophytic fungus *G. abieticola* in *A. koreana* and reported it.

Needle leaves of *A. koreana* were harvested from Mt. Halla (33° 20' N, 126° 31' E, 1,952 m) in the Jeju Special Self-governing Province of Korea in 2018. The samples were transported in a zipper bags, and fungi were isolated within 24 hours. All samples were washed with tap water and cut into 1-cm pieces. Surface sterilization was performed; they were immersed in 96% ethyl alcohol for 1 minute, sodium hypochlorite for 3 min, and then 96% ethyl alcohol for 30 seconds; finally, they were washed twice with sterilized water. Each sample was placed on potato dextrose agar (PDA, MBcell, Seoul, Korea) and incubated in the dark



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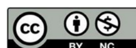

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for 4 weeks at 25℃ to isolate endophytic fungi [5]. PDA and maltose extract agar (MEA, MBcell, Seoul, Korea) media were used for the pure culture of endophytic fungi. The fungi's macroscopic and microscopic features were measured by light microscopy (DM2500, Leica Microsystems, Wetzlar, Germany). Finally, this unrecorded endophytic fungus was deposited in the Korean Collection for Type Cultures (KCTC).

Genomic DNA was extracted from the fungus using a plant tissues genomic DNA extraction kit (Xi'an Tianlong Science & Technology, Shaanxi, Taiwan) following the manufacturer's instructions. Polymerase Chain Reaction (PCR) was performed using primers ITS1 (internal transcribed spacer 1) and LR3, which can selectively amplify from the ITS1 region to the D2 region of 28S ribosomal DNA [6, 7]. The conditions of PCR were as follows: Pre-denaturing for 5 minute at 94℃ with 1 cycle, denaturing for 30 seconds at 94℃, annealing for 30 sec at 50℃, extending for 1 min at 72℃ in 30 cycles, and then finally stabilizing for 10 min at 72℃ in 1 cycle. The PCR product was confirmed by electrophoresis using 1.5% agarose gel.

DNA sequencing was applied to Macrogen (Seoul, Korea), and the analyzed DNA sequence was then identified based on similarity with the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool (BLAST). A maximum-likelihood tree was generated by MEGA 10.0.5 based on the Kimura-2 parameter distance model with the 1,000-times bootstrap method [8].

Grovesiella abieticola (Zeller & Goodd.) M. Morelet & Gremmen

[MB#314737]

The colony diameter was <1.0 mm on PDA and MEA after 7 days. Mycelium hardly grew in either media. However, the colony diameter was 49.5-51.2 mm on PDA after 300 days and the mycelium was dense. The surface color is brownish black (Munsell color notation: 5YR 3/1) to brownish gray (Munsell color notation: 7.5GY 6/2) [9] with undulate margin, velvety texture at the center, whitish aerial hyphae at the margin, and no exudates. The reverse color is bluish black (Munsell color notation: 10PB 3/2) to dark grayish blue (Munsell color notation: 5PB 3/2) [9]. Macroconidia are 31.5-42.5×1.6-2.8 μm (n=20), cylindrical with rounded-to-pointed ends, hyaline and dyed well with lactophenol cotton blue and have 2-3 septa inside. Microconidia are 5.0-7.3×2.6-3.1 μm (n=20), ovoid, hyaline, and dyed well with lactophenol cotton blue without a septum inside (Fig. 1 and 2).

Specimen examined: Mt. Halla, Jeju special self-governing province, Korea, 2018.5.14., isolated from leaves of *Abies koreana*, strain NIE7113, KCTC no. 56675, GenBank no. MT157258.

Note: According to the Index of Fungi (1971-1980), three species-*G. abieticola*, *G. ericae*, and *G. ledi*- have been reported in the genus *Grovesiella* [1]. After that, Funk discovered *G. grantii* on *Abies grandis* (Douglas ex D. Don) Lindl. in 1977 [2], however in 1971 Morelet reclassified *G. ericae* as *Erikssonopsis ericae* [10] and in 1885 Krasten also reclassified *G. ledi* as *Godronia ledi* [11]. Therefore, a total of two species have been remained in this genus to date. This genus has few molecular studies compared to other taxa outside the ITS barcode region. The ITS barcode region in this species showed 96.3% similarity with *G. abieticola* (KX358852.1). In this study, large subunit ribosomal nucleic acid (LSU rDNA) were aligned and loaded into NCBI but there are no reference sequences to compare with this study. The distribution region

of *G. abieticola* has been reported in the United States, Canada, France, and the United Kingdom (Scotland). In addition, host plants in which *G. abieticola* has been found include *Abies alba* Mill., *A. amabilis* Douglas ex J. Forbes, *A. balsamea* (L.) Mill., *A. concolor* (Gordon) Lindley ex. Hildebrand, *A. grandis* (Douglas ex D. Don) Lindley, *A. lasiocarpa* (Hooker) Nuttall, *Picea abies* (L.) H. Krast., and *A. procera* Rehder. Host plants are all woody plants [3, 12]. The present study confirmed for the first time that this fungus is distributed in Korea (Asia) and can be isolated from *A. koreana* in the genus *Abies*. Further observation of the fungus' presence is also needed in other Korean fir plants such as *A. holophylla* Maxim. and *A. nephrolepis* (Truatv.) Maxim. Finally, Sieber and Kowalski commented on *Agyriellopsis caeruleoatra* Höhn isolated from *A. alba* in Switzerland as the same species as *G. abieticola* [12] but there were no morphological characteristics or DNA barcode regions for reference, so further studies are needed on the identity of *A. caeruleoatra*.

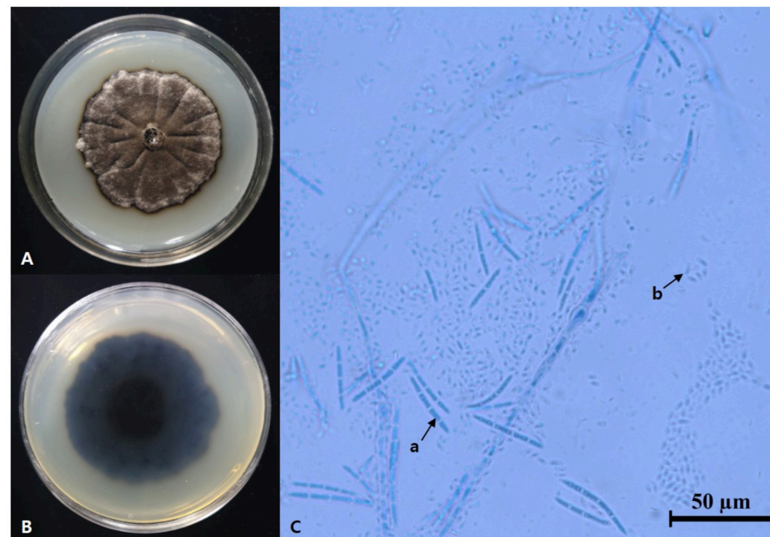

Fig. 1. Cultural characteristics of *Grovesiella abieticola* strain NIE7113 isolated from *Abies koreana*. A and B, Front and reverse sides of the colony on PDA after 300 days. C, (a) Macroconidia and (b) microconidia. Scale bars: C=50 µm.

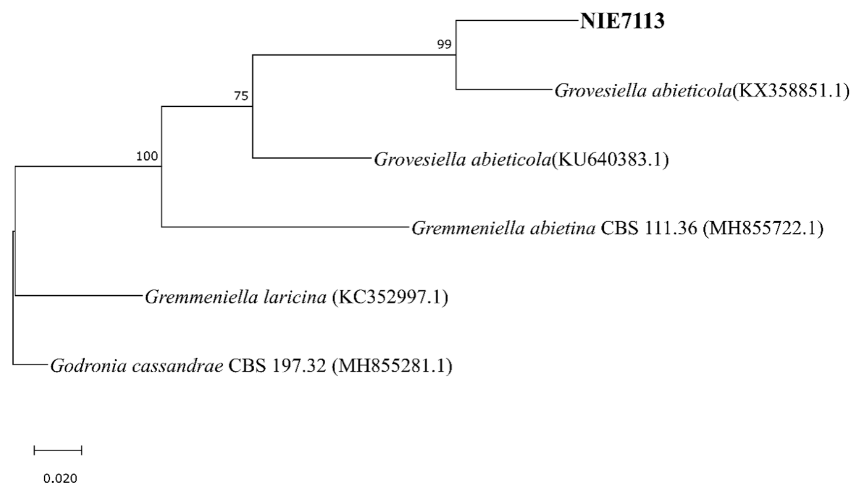

Fig. 2. Phylogenic tree of *Grovesiella abieticola* strain NIE7113 isolated from *Abies koreana*. The internal transcribed spacer (ITS) region including the 5.8S ribosomal DNA region was used for sequence analysis to confirm the topological appropriation of the fungal isolates. *Godronia cassandrae* was used as an out-group, and bootstrap values are shown at the branches (1,000 replicates).

Table 1. Morphological characteristics of *Grovesiella abieticola* NIE7113 isolated from needle leaves of *Abies korenana*

Strain	*G. abieticola* NIE7113	*G. abieticola* [12]	*G. grantii* [2]
Colony	PDA, 25℃, 300 days	PDA, 20℃, 30 days	MEA, 20℃, 14 days
Color	Brownish black to brownish gray; reverse bluish black to dark grayish blue	Dark brown to olivaceous; reverse black	Light-grey to bluish; reverse dark bluish
Size	49.5-51.2 mm in diameter for 300 days	22.8-45.6 mm in diameter for 30 days	10 mm in diameter for 14 days
Shape	Velvety texture at the center to whitish aerial mycelium, margin undulate	Floccose aerial mycerium	Compact, heaped up aerial mycelium, margin regular
Conidia	Macroconidia hyaline, 2-3 septa, cylindrical with rounded-to-pointed ends, 31.5-42.5×1.6-2.8 µm in diameter	Macroconidia hyaline, septate, slightly protuberant, 20.0-30.0×2.0-2.2 µm in diameter	No observation
	Microconidia hyaline, ovoid, aseptate, 5.0-7.3×2.6-3.1 µm in diameter	Microconidia hyaline, holoblastic, aseptate, smooth, cylindrical, apex obtuse, base truncate, 3.0-6.0×1.5-1.8 µm in diameter	No observation

PDA: Potato dextrose agar; MEA: Maltose extract agar.

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