

RESEARCH NOTE

Unreported Endophytic Fungi Isolated from the Leaves of Evergreen Woody Plants in Korea: *Muyocopron lithocarp* and *Didymocyrtis cladoniicola*

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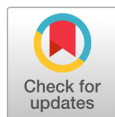
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ABSTRACT

In this study, endophytic fungi were isolated from the leaves of evergreen woody plants in Hansando Island in South Korea. The fungal isolates were identified based on morphological characteristics and phylogenetic analysis of the DNA sequences of the internal transcribed spacer and the large subunit regions of rDNA. It was confirmed that two endophytic fungal species, *Muyocopron lithocarp* and *Didymocyrtis cladoniicola*, isolated in this study have not previously been reported in South Korea.

Keywords: Ascomycota, *Chamaecyparis obtuse*, Hansando, *Ligustrum japonicum*,



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Fungal endophytes are fungi that inhabit the tissues of healthy plants without any disease symptom [1]. Leaves of evergreen woody plants have a wider parenchyma and intercellular space area per unit area compared with that of leaves from deciduous trees [2]. Endophytes inhibit plant pathogens of evergreens through production of ethyl acetate or antifungal metabolites [3]. Endophytes found in evergreen trees differ from those found in deciduous trees in terms of species composition and diversity [4]. In this study, two endophytic fungal species, *Muyocopron lithocarp* and *Didymocyrtis cladoniicola* were isolated from the healthy leaves of *Ligustrum japonicum* Thunb. and *Chamaecyparis obtuse* (Siebold & Zucc.) Endl, two evergreen woody plants native to Hansando Island. The fungal species were discovered while studying the biodiversity of endophytic fungi in evergreen woody plants; they have not previously been recorded in South Korea. Based on the molecular and morphological characteristics, the species were confirmed by comparison with the first reported descriptions.

The leaves of *L. japonicum* and *C. obtuse* used in the study were collected from Hansando Island (N34° 46' 77", E128° 29' 05") in April, 2019. Samples were transported to the laboratory using polyethylene bags and separated within 24 h. For surface sterilization of leaves, the surface was washed with distilled water and 1% NaClO solution for 1 min, and then with 70% ethanol for 2 min [5]. The sterilized leaves were cut

Table 1. Morphological characteristics of endophytic fungal strains isolated in this study

	<i>Muyocopron li thocarp</i>		<i>Didymocyrtis cladoniicola</i>	
	Mapook et al. [10]	KNUE19E011	Trakunyingcharoen et al. [13] Piccolo et al. [14]	KNUE19E070
Colonies				
Cultural condition	MEA, 18°C, 14 d	MEA, 25°C, 7 d	MEA, 25°C, 7 d	MEA, 25°C, 7 d
Color	Surface grayish to light brown, reverse brown to dark brown	Surface beige reverse ocher pale yellow at the edges	Initially white and later olive grey, dark brown to black in reverse	Surface white, with yellow at the center; reverse pale yellow
Size	20mm in diam	18–22mm in diam	60–80 mm in diam	26–28 mm in diam
Shape	Flat	Radial furrow	Flat, powdery to velvety	Mycelia covered entirely
Conidia				
Shape	-	-	Irregularly arranged, hyaline, oval with obtuse ends	Irregularly arranged, hyaline, oval with obtuse ends
Size	-	-	(5.04–) 6.12 (–9.69) × (3.15–) 4.56 (–5.84) μm	(5.04–) 6.12 (–9.69) × (3.15–) 4.56 (–5.84) μm

MEA, malt extract agar

into 1.5–2 cm length and placed on potato dextrose agar (PDA) medium. The mycelium extending from the leaf was cut with a knife and repeatedly transferred to PDA medium to isolate it [6]. The fungal isolates retained were cultured in PDA medium and malt extract agar (MEA) medium at 25°C for 7 d. Genomic DNA was extracted according to the manufacturer's protocol for the DNeasy plant mini kit (Qiagen, Germantown, MD, USA). The extracted DNA was subjected to polymerase chain reaction (PCR) using the primer pairs ITS1F/ ITS4 and LR0R/LR16 [7, 8]. Each primer pair was used to amplify the internal transcribed spacer (ITS) region and the large subunit (LSU) region of ribosomal DNA, respectively. ITS and LSU sequences were identified based on their similarity with the sequences recorded in the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>) using the basic local alignment search tool (BLAST). By combining the ITS and LSU regions, a neighbor-joining tree was created using the MEGA 7.0.26 program [9]. The analysis model used the Kimura-2 parametric distance model, and reliability was evaluated with the 1,000-times bootstrap method.

***Muyocopron lithocarp* Boonmee & K.D. Hyde, Phytotaxa 265 (3): 235 (2016) [MB#551618]**

Morphological Characteristics of Strain KNUE19E011. The size of the colony cultured in PDA medium for 7 days was 22–24 mm. The surface of the colony was grayish white in PDA, with an irregular margin and a texture similar to down or cotton (Fig. 1). The reverse surface was yellow, and the edges were pale yellow. After 7 days of culture in MEA medium, the size of the colony was 18–22 mm, the center of the top surface was beige, and the edges were white. Irregular radial furrows appeared on the surface, which was higher than the surface of the medium. The center of the reverse surface was ocher, and the edges were pale yellow (Fig. 1).

Specimen examined. Hansando Island, Tongyoung-si, Korea, N34° 46' 9.41", E128° 30' 24.99", April 5, 2019. *M. lithocarp* KNUE19E011, isolated from needles of *L. japonicum*, NIBRFG0000506585,

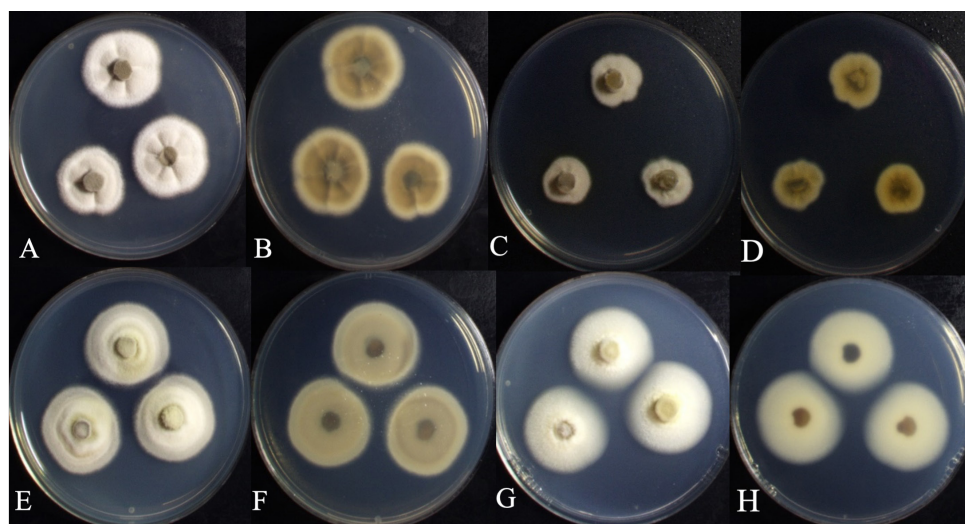


Fig. 1. Cultural characteristics of two endophytic fungal strains. Colonies of *Muyocopron lithocarp* KNUE19E011 grown for 7 days on potato dextrose agar (PDA) (A: Surface, B: Reverse) and malt extract agar (MEA) (C: Surface, D: Reverse); Colonies of *Didymocyrtis cladoniicola* 19E070 grown for 7 days on PDA (E: Surface, F: Reverse) and MEA (G: Surface, H: Reverse).

GenBank No. MZ206175 (ITS), MZ254891 (LSU).

Note. *Muyocopron lithocarp* was first discovered on *Lithocarpus lucidus* in Chiang Rai, Thailand in 2016. Its name was derived from the host from which the fungus was first isolated [10]. Although the morphological characteristics of asci and ascospores of *M. lithocarp* were similar to those of *M. umbilicatum*, they were considered as different species owing to the average size of their ascospores. [10]. The KNUE19E011 strain isolated in this study was confirmed as *M. lithocarp* by comparing its morphological characteristics with the Boonmee and Hyde's description and phylogenetic analysis. BLAST result showed 100.0% similarity with *M. lithocarp* MK347717.1 for the ITS region and 99.67% similarity with *M. lithocarp* KU726967.1, for LSU. Phylogenetic tree analysis showed that strain 19E001 clustered together with *M. lithocarp* C135 showing 99% bootstrap values (Fig. 2, Table 2).

***Didymocyrtis cladoniicola* (Diederich, Kocourk. & Etayo) Ertz & Diederich [MB#814023] [MB#810830]**

Morphological Characteristics of Strain KNUE19E070. The size of the colony cultured in PDA medium for 7 days was 22–25 mm, the top surface was beige, and the center and edges were light olive. The back of the PDA medium was olive-colored, and the edges were pale yellow. The surface of the colony has a texture similar to down, and the edges were smooth and round (Fig. 1B). The size of the colony cultured for 7 days in MEA medium was 26–28 mm. The top of the MEA medium was white, and the center was light yellow. As in PDA medium, the surface of the colony was covered with mycelium with a texture similar to that of down. The conidia were smooth, oval, and colorless or light brown. The diameter was $2.86 (1.54\text{--}3.72) \times 5.38 (3.24\text{--}6.59) \mu\text{m}$.

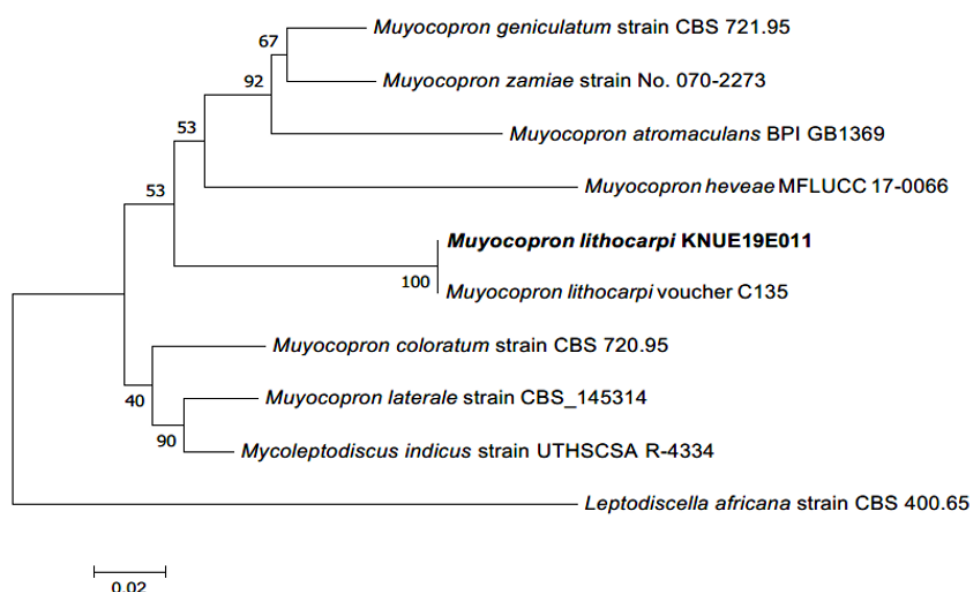


Fig. 2. Neighbor-joining phylogenetic tree based on a concatenated alignment of internal transcribed spacer (ITS) and large subunit (LSU) rDNA sequences. *Leptodiscella africana* was used as an outgroup. Numbers on branches indicate bootstrap values (1,000 replicates). The fungal strain isolated in this study is in bold.

Table 2. Fungal taxa used in the phylogenetic analyses

Fungal Species	Strain Number	GeneBank Accession Number	
		Internal transcribed spacer (ITS)	Large subunit (LSU) rDNA
<i>Didymocyrtis cladoniicola</i>	CBS 128027	MH877976.1	MH877976.1
<i>Leptodiscella africana</i>	CBS 400.65	MH858635.1	MH870275.1
<i>Leptosphaeria pedicularis</i>	CBS 126582	JF740223.1	MH875624.1
<i>Leptosphaeria rubefaciens</i>	CBS 387.80	JF740242.1	JF740311.1
<i>Leptosphaeria slovacica</i>	CBS389.80	JF740247.1	JF740315.1
<i>Leptosphaerulina nitida</i>	CBS 450.84	MH861755.1	MH873454.1
<i>Muyocopron atromaculans</i>	BPI GB1369	NR_164053.1	NG_066446.1
<i>Muyocopron coloratum</i>	CBS 720.95	MH862554.1	MH874184.1
<i>Muyocopron geniculatum</i>	CBS 721.95	MH862555.1	MH874185.1
<i>Muyocopron heveae</i>	MFLUCC 17-0066	NR_168784.1	MH986832.1
<i>Muyocopron laterale</i>	CBS_145314	MK487749.1	MK492727.1
<i>Muyocopron lithocarpus</i>	C135	MK347717.1	MK347931.1
<i>Muyocopron zamiae</i>	No. 070-2273	MW883426.1	MW883425.1
<i>Mycoleptodiscus indicus</i>	UTHSCSA R-4334	GU220382.1	GU220383.1

Specimen examined. Hansando Island, Tongyoung-si, Gyeongsangnam-do, Korea, N34° 47' 57.70", E128° 28' 27.55", April 5, 2019. *D. cladoniicola* KNUE19E070, isolated from needles of *Chamaecyparis obtusa*, NIBRFG0000506594, GenBank No. MZ206178 (ITS), MZ221116 (LSU).

Notes. *Didymocyrtis cladoniicola* was first discovered on the thallus of *Cladonia pyxidata*, a lichen species, in USA [11]. *D. cladoniicola* is generally found in lichens, however, the species isolated in this study had been isolated as a leaf endophytic fungi from *Geum peckii*, a herbaceous plant belonging to the Rosaceae [12]. BLAST results showed a 98.47% similarity with *D. cladoniicola* LT796877.1 for the ITS region, and a 99.67% similarity with *D. cladoniicola* LN907456.1 for the LSU region. Phylogenetic tree analysis showed that strain 19E070 clustered together with *D. cladoniicola* CBS 128023 and CBS 128025 showing 94% bootstrap values (Fig. 3, Table 2).

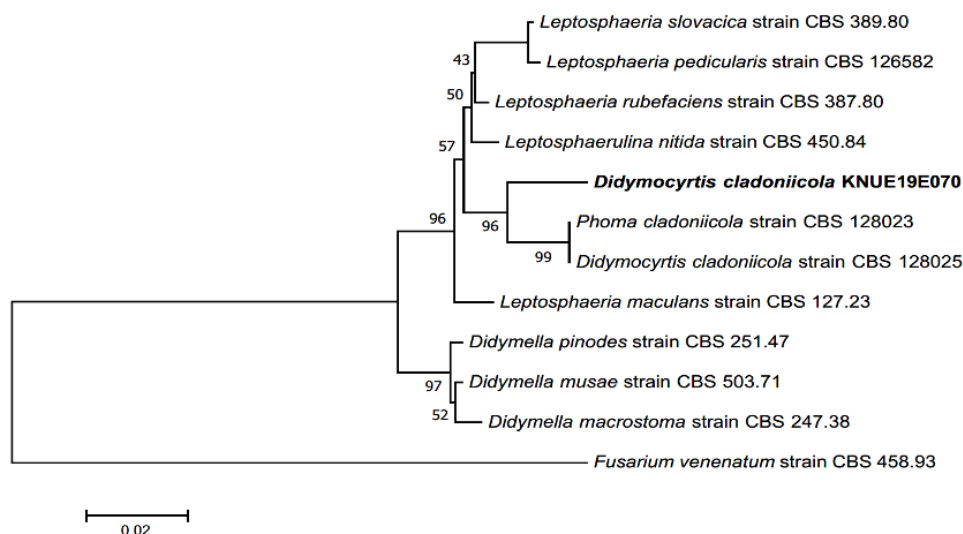


Fig. 3. Neighbor-joining phylogenetic tree based on a concatenated alignment of internal transcribed spacer (ITS) and large subunit (LSU) rDNA sequences. *Fusarium venenatum* was used as an outgroup. Numbers on branches indicate bootstrap values (1,000 replicates). The fungal strain isolated in this study are in bold.

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REFERENCES

1. Carroll G. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecology* 1988;69:2-9.
2. Villar R, Ruiz-Robledo J, Uberta JL, Poorter H. Exploring variation in leaf mass per area (LMA) from leaf to cell: an anatomical analysis of 26 woody species. *Am J Bot* 2013;100:1969-80.
3. Liu CH, Liu TT, Yuan FF, Gu YC. Isolating endophytic fungi from evergreen plants and determining their antifungal activities. *Afr J Microbiol Res* 2010;4:2243-8.
4. Matsumura E, Fukuda K. A comparison of fungal endophytic community diversity in tree

- leaves of rural and urban temperate forests of Kanto district, eastern Japan. *Fungal Biol* 2013;117:191-201.
5. Park H, Eom AH. Three unreported endophytic fungi isolated from conifer leaves of *Pinus densiflora* in Korea. *Kor J Mycol* 2019;47:35-42.
 6. Kim CK, Eo JK, Eom AH. Diversity of foliar endophytic fungi isolated from *Lindera obtusiloba* in Korea. *Kor J Mycol* 2012;40:136-40.
 7. Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Mol Ecol* 1993;2:113-8.
 8. Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol Res* 1990;172:4238-46.
 9. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870-4.
 10. Mapook A, Hyde KD, Dai DQ, Li J, Jones EG, Bahkali AH, Boonmee S. *Muyocoprionales*, ord. nov., (Dothideomycetes, Ascomycota) and a reappraisal of *Muyocoprion* species from northern Thailand. *Phytotaxa* 2016;265:225-37.
 11. Diederich P, Kocourkova J, Etayo J, Zhurbenko M. The lichenicolous *Phoma* species (coelomycetes) on *Cladonia*. *Lichenologist* 2007;39:153-63.
 12. Adams SJ, Robicheau BM, LaRue D, Browne RD, Walker AK. Foliar endophytic fungi from the endangered eastern mountain avens (*Geum peckii*, Rosaceae) in Canada. *Plants* 2021;10:1026.
 13. Trakunyingcharoen T, Lombard L, Groenewald JZ, Cheewangkoon R, Toanun C, Alfenas AC, Crous PW. Mycoparasitic species of *Sphaerellopsis*, and allied lichenicolous and other genera. *IMA fungus* 2014;5:391-414.
 14. Piccolo SL, Mondello V, Giambra S, Conigliaro G, Torta L, Burruano S. *Arthrinium phaeospermum*, *Phoma cladoniicola* and *Ulocladium consortiale*, new Olive pathogens in Italy. *J Phytopathol* 2014;162:258-63.