

RESEARCH ARTICLE

Isolation and Identification of Two Unreported Sordariomycetes Fungi in Korea: *Pestalotiopsis clavata* and *Botryotrichum iranicum*

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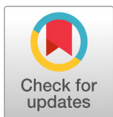
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ABSTRACT

Two fungal strains, designated KNUF-21-006 and KNUF-21-028, were isolated from soil samples collected from Gyeongbuk Province, Korea. The strain KNUF-21-006 was similar to other *Pestalotiopsis* species in terms of morphological characteristics, including whitish to pale brown mycelium, conidial shape, and size. The isolate had aerial hyphae that produced black fruiting bodies on the mycelium. The conidia were fusoid to ellipsoid, four-septate, and appendage-bearing. Phylogenetic analysis using the internal transcribed spacer (ITS) region, translation elongation factor 1-alpha (TEF), and β -tubulin (TUB) gene sequences confirmed that the closest relationship of the isolate at the species level was with *Pestalotiopsis clavata*. The strain KNUF-21-028 exhibits similar morphological characteristics to other *Botryotrichum* species, including white aerial mycelium with sulcate and irregular margins, conidial shape, and size. The conidia were globose, single, and hyaline. Upon molecular analysis—using the ITS region, large subunit (LSU) rRNA gene, and TUB gene sequences—the fungus was identified as *Botryotrichum iranicum*. This is the first record of these fungal species in Korea.

Keywords: *Botryotrichum iranicum*, *Pestalotiopsis clavata*, Soil-inhabiting fungi, Sordariomycetes



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INTRODUCTION

Soil fungi play an important ecological role as decomposers, producing a variety of enzymes that break down organic matter, regulating the nutrient balance. They can be important ecosystem regulators and strongly influence plant productivity and diversity [1]. Globally, Ascomycota is one of the largest and most diverse soil-inhabiting fungal taxa, with a relative proportion of 8% corresponding to Sordariomycetes [2]. The class Sordariomycetes (phylum: Ascomycota, subphylum: Pezizomycotina) was introduced by Eriksson & Winka in 1997, and it is the second largest class in the Ascomycota phylum. There is considerable variability among members of Sordariomycetes, in terms of morphology, habitat, and growth form [3-5]. One of the largest genera is *Pestalotiopsis*, which was first described in 1949 and

currently contains 375 species [6]. The morphological characteristics of the genus *Pestalotiopsis*, which is known to be endogenous to plants and saprotrophic, include conidia with ellipsoidal shape, septate, and appendage-bearing on the apex of the apical cell [7,8]. Recently, Senanayake et al [9], introduced the family Pestalotiopsidaceae to accommodate seven anamorphic genera, including *Pestalotiopsis* which possesses pestalotiopsis-like conidia [9]. *Pestalotiopsis* is distinguished from other genera in this family by the 5-celled, fusiform conidia, with three-colored median cells, hyaline basal cells, and one or more apical appendages. Members of the genus *Pestalotiopsis* are distributed worldwide, they were mainly found on dead or living plant organs, and some species such as *P. terricola*, *P. humicola*, and *P. papuana* were isolated from soil or other substrates [10]. Several *Pestalotiopsis* species have been reported to cause serious plant diseases, such as leaf blight in sweet persimmon tree, and canker in *Acanthopanax divaricatus* [11,12]. In contrast to *Pestalotiopsis*, another member of the class Sordariomycetes, the genus *Botryotrichum* comprises only 25 species. This genus belongs to the family Chaetomiaceae and is known to contain saprotrophic fungi. The morphological characteristics of this genus include conidia that are globose to subglobose, solitary, and unicellular [13]. Members of the genus *Botryotrichum* are commonly found in soils, especially in ones with high levels of organic matter [14]. *B. piluliferum* has been reported among the soil fungal communities in countries like Canada [15], however, there are still no reports of plant pathogenic species of the genus *Botryotrichum*.

The aim of this study was to identify unreported fungal species isolated from soil samples in Korea. In order to contribute to enlarging our knowledge about fungal diversity, we present the morphological and molecular characteristics of two species of the class Sordariomycetes, KNUF-21-006 and KNUF-21-028, previously unreported in Korea and now identified as *Pestalotiopsis clavata* and *Botryotrichum iranicum*, respectively.

MATERIALS AND METHODS

Sample collection and fungal isolation

Soil samples were collected from Yeongchuk Mountain, Ulju-gun, Gyeongbuk Province (35°30'53.1"N 129°03'08.1"E), and Daro stream, Cheongdo-gun, Hwayang-eup, Gyeongbuk Province (35°39'47.9"N 128°42'58.0"E), Korea. The soil samples were collected between the soil surface and 20 cm below the surface, stored in a sterile plastic bag, brought to the laboratory, and stored at 4°C until analysis. Serial dilution of the soil samples was performed by weighing 1 g of soil and suspending it in 10 mL of sterile distilled water to prepare serial dilutions (10^{-1} to 10^{-5}). Each soil serial dilution was vortexed, and 0.1 mL of each dilution was spread onto potato dextrose agar plates (PDA; Difco, Detroit, MI, USA). Well-developed individual colonies were isolated and cultured again on fresh PDA plates and incubated at 25°C until the mycelium developed. The pure cultures were preserved in 20% glycerol at -80°C for future studies. Stock cultures of both strains, namely, KNUF-21-006 (NJLNFGC000000047) and KNUF-21-028 (NJLNFGC000000042) were deposited in the National Institute of Biological Resources (NIBR) as metabolically inactive cultures.

Morphological characterization

Morphological characteristics of the isolated strains KNUF-21-006 and KNUF-21-028 were studied by growing them on PDA for 7 days of incubation at 25°C [16,17]. Cultural characteristics, such as colony color, texture, growth, shape, and size were recorded. The microscopic characteristics were observed using a light microscope (BX-50; Olympus, Tokyo, Japan).

Genomic DNA extraction, PCR amplification, and sequencing

Fungal strains were grown on PDA plates, and the scraped mycelia were used for genomic DNA extraction using a HiGene™ Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea) following the manufacturer's instructions. For molecular identification of each strain, target regions, primers, and PCR conditions were based on previous reports [16,17]. The internal transcribed spacer (ITS) region, translation elongation factor 1-alpha (TEF) gene, and beta-tubulin (TUB) gene were used for the identification of strain KNUF-21-006; and ITS, TUB, and large subunit (LSU) rRNA gene were applied for the identification of strain KNUF-21-028. PCR amplification was carried out using the ITS1F and ITS4 primers to amplify the ITS region, NL1 and NL4 primers for LSU, EF1-728F and EF2 for TEF, and TUB amplification was carried out with T1 and Bt2b primers for KNUF-21-006 and T1 and T2 primers for KNUF-21-028 [18-21]. The thermal cycling conditions were as follows: for every primer pair, an initial denaturation step of 95°C for 5 min and a final extension step of 72°C for 10 min were used. For ITS 35 amplification cycles of 95°C for 30 s, 55°C for 45 s, and 72°C for 90 s; for TEF 35 cycles of 95°C for 30 s, 55°C for 50 s, and 72°C for 90 s; for LSU 35 cycles of 95°C for 45 s, 50°C for 45 s, and 72°C for 60 s; for TUB 35 cycles of 95°C for 30 s, 55°C for 50 s (KNUF-21-006) or 60°C for 30 s (KNUF-21-0028), and 72°C for 60 s. The amplified PCR products were purified using EXOSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced at Solgent Co., Ltd. (Daejeon, Korea). Closely related strains were identified using the BLAST search program on the National Center for Biotechnology Information (NCBI) website. The obtained sequences were submitted to the NCBI GenBank database (accession numbers are listed in Table 1).

Phylogenetic analysis

To construct the phylogenetic trees, we analyzed the ITS, TUB, and TEF sequences for strain KNUF-21-006 and ITS, LSU, and TUB sequences for strain KNUF-21-028, retrieving allied sequences from the NCBI GenBank database (Table 1). Sequences were edited using BioEdit v.7.0.5.3 [22] and datasets of the corresponding markers were first analyzed separately and then concatenated into a combined dataset for each strain. Phylogenetic trees were constructed using the maximum likelihood algorithm, with the Kimura model and MEGA software (version 7.0) for 1,000 replications [23].

Table 1. List of species used in phylogenetic analyses along with their GenBank accession numbers.

Species	Strain	GenBank accession numbers			
		ITS	LSU	TEF	TUB
<i>Pestalotiopsis lushanensis</i>	NB3-5	MW142353	-	MW147695	MW147688
<i>Pestalotiopsis lushanensis</i>	NB3-3	MW142352	-	MW147694	MW147687
<i>Pestalotiopsis pini</i>	MEAN 1094	MT374681	-	MT374694	MT374706
<i>Pestalotiopsis pini</i>	MEAN 1092	MT374680	-	MT374693	MT374705
<i>Pestalotiopsis grevilleae</i>	CBS 114127	NR147548	-	KM199504	KM199407
<i>Pestalotiopsis unicolor</i>	MFLUCC 12-0275	JX398998	-	JX399063	JX399029
<i>Pestalotiopsis jiangxiensis</i>	LC4399	KX895009	-	KX895227	KX895341
<i>Pestalotiopsis chamaeropsis</i>	CBS 186.71	KM199326	-	KM199473	KM199391
<i>Pestalotiopsis chamaeropsis</i>	CBS 113607	KM199325	-	KM199472	KM199390
<i>Pestalotiopsis australis</i>	CBS 114193	KM199332	-	KM199475	KM199383
<i>Pestalotiopsis australis</i>	CBS 111503	KM199331	-	KM199557	KM199382
<i>Pestalotiopsis leucadendri</i>	CBS 121417	MH553987	-	MH554412	MH554654
<i>Pestalotiopsis clavata</i>	MFLUCC 12-0268	JX398990	-	JX399056	JX399025
<i>Pestalotiopsis clavata</i>	KNUF-21-006	ON409988	-	ON456329	LC710316
<i>Pseudopestalotiopsis cocos</i>	CBS 272.29	MH855069	-	KM199553	KM199467
<i>Acheatomium luteum</i>	CBS 544.83	KX976572	KX976697	-	KX976913
<i>Botryotricum atrogriseum</i>	CBS 130.28	KX976589	KX976714	-	KX976931
<i>Botryotricum atrogriseum</i>	CBS 604.69	KX976590	KX976715	-	KX976932
<i>Botryotricum iranicum</i>	ABRIICC 10152	MN134583	MN134576	-	MN128435
<i>Botryotricum iranicum</i>	ABRIICC 10153	MN134584	MN134577	-	MN128436
<i>Botryotricum iranicum</i>	KNUF-21-028	ON422304	ON422305	-	LC710317
<i>Botryotrichum domesticum</i>	UAMH 11929	MH899168	MH899169	-	MH899172
<i>Botryotricum peruvianum</i>	CBS 460.90	KX976595	KX976720	-	KX976937
<i>Botryotricum peruvianum</i>	CBS 421.93	KX976596	KX976721	-	KX976938
<i>Botryotricum piluliferum</i>	CBS 105.14	KX976598	KX976723	-	KX976940
<i>Botryotricum piluliferum</i>	CBS 654.79	KX976597	KX976722	-	KX976939

The isolated strains and their ITS, LSU, TEF, and TUB accession numbers are shown in bold. ITS: Internal transcribed spacer region; LSU: Large subunit rRNA; TEF: Translation elongation factor 1- α ; TUB: Beta-tubulin.

RESULTS AND DISCUSSION

Pestalotiopsis clavata Maharachch. & K.D. Hyde, Fungal Diversity 56 (1): 108 (2012) [MB#800524] (Fig. 1)

Specimen collection: Yeongchuk Mountain, Ulju-gun, Gyeongbuk Province (35°30'53.1"N 129°03'08.1"E), isolated from soil.

Morphological characteristics of KNUF-21-006

The colonies have dense, aerial mycelium, whitish to pale brown in color, with black fruiting bodies; the reverse of the culture was pale brown to whitish brown. The colony diameter reached 70 mm on PDA after 7 days at 25°C (Figs. 1A and B). Conidiogenous cells, hyaline, short, thin-walled, discrete ampulliform to

lageniform. Conidia straight to slightly curved, fusoid to ellipsoid, four-septate, $15.6\text{--}25.6 \times 5.1\text{--}7.4 \mu\text{m}$ ($n=20$), basal cell hyaline, thin-walled, with conic to obconic with obtuse end. The conidia have three median cells, doliform, olivaceous to brown; wall rugulose, together $11.5\text{--}21.2 \mu\text{m}$ ($n=20$) long second cell from base $4.0\text{--}6.7 \mu\text{m}$ ($n=20$); third cell $3.5\text{--}7.5 \mu\text{m}$ ($n=20$); fourth cell $4.0\text{--}7.0 \mu\text{m}$ ($n=20$); apical cell $4.0\text{--}6.4 \mu\text{m}$ ($n=20$) long; basal appendage mostly 1-3 present, $6.8\text{--}18.6 \mu\text{m}$ ($n=20$) long (Figs. 1C and D). These morphological characteristics matched well with those typical of the genus *Pestalotiopsis* and closely agreed with the characteristics of *P. clavata* [16] (Table 2). The culture and morphological characteristics of the isolated strain KNUF-21-006 suggested that it is most closely related to *P. clavata* [16].



Fig. 1. Cultural and morphological characteristics of *Pestalotiopsis clavata* KNUF-21-006. Cultures were grown at 25°C for 7 days on potato dextrose agar (PDA) in darkness. A, B. front and reverse view of the colony, C, D. conidia with appendage. Scale bars: C, D= $10 \mu\text{m}$.

Table 2. Morphological characteristics of KNUF-21-006 and the reference species *Pestalotiopsis clavata* cultured on potato dextrose agar medium at 25°C .

Characteristic	KNUF-21-006 ^a	<i>Pestalotiopsis clavata</i> ^b
Colony	Whitish to pale brown; dense; aerial mycelium with black fruiting bodies; reverse pale brown to whitish brown; reaching 7 cm diameter after 7 days	Whitish to pale brown; dense; aerial mycelium, black fruiting bodies; reverse pale brown to brown; reaching 7 cm diameter after 8 days
Conidiophore	Hyaline; short; thin-walled; ampulliform to lageniform	Ampulliform to lageniform; smooth; thin-walled; hyaline; short
Conidia	Straight to slightly curved; fusoid to ellipsoid; 4-septate; $15.6\text{--}25.6 \times 5.1\text{--}7.4 \mu\text{m}$	Fusoid to ellipsoid; straight to slightly curved; 4-septate; $20\text{--}27 \times 6.5\text{--}8 \mu\text{m}$

^a Fungal strain studied in this paper, ^b Maharachchikumbura et al [16].

Molecular phylogeny of the KNUF-21-006

Sequences containing 611, 720, and 483 bp were obtained, which corresponded to the ITS region, TEF, and TUB genes, respectively. A BLAST search of the NCBI database revealed high similarities between the ITS regions of KNUF-21-006 and *Pestalotiopsis lushanensis* NB3-5 (MW142353; 100%), *P. clavata* MFLUCC 12-0268 (JX398990; 99.8%), *P. pini* MEAN 1092 (MT374680; 99.7%), and *P. grevilleae* CBS 114127 (NR_147548; 99.3%). Based on TUB gene sequence similarities, the closest relatives of the isolate were identified as *P. lushanensis* NB3-5 (MW147688; 100%), *P. pini* MEAN 1092 (MT374705; 100%), and *P. clavata* MFLUCC 12-0268 (JX399025; 99.7%). Based on the TEF gene sequence of KNUF-21-006, 100, 97.2, and 96.5% similarity was

observed with the closely related *P. clavata* MFLUCC 12-0268 (JX399056), *P. pini* MEAN 1092 (MT374693), and *P. lushanensis* NB3-5 (MW147695), respectively. Separate analysis of ITS, LSU, or TUB sequences did not allow accurate identification of the novel strain at the species level. Considering that the sequences of ITS, TUB, and TEF are available in GenBank for almost all *Pestalotiopsis* species and that phylogenetic analysis based on combined ITS, TUB, and TEF sequences allowed the precise differentiation of *Pestalotiopsis* species investigated in previous studies [16], we also conducted a phylogenetic analysis using concatenated ITS, TUB, and TEF sequences (Table 1). The maximum likelihood phylogenetic tree based on concatenated sequences (Fig. 2) demonstrated that KNUF-21-006 occupies a position within the genus *Pestalotiopsis*, and the clustering of the isolate with *P. clavata* indicates that this is the closest relationship at the species level. To our knowledge, this is the first report on this fungal species in Korea.

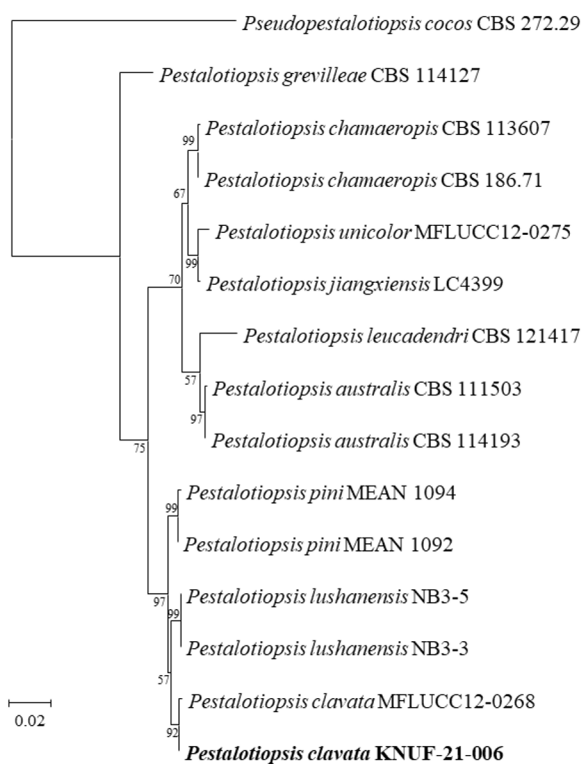


Fig. 2. Maximum likelihood phylogenetic tree based on a combined dataset of partial sequences of internal transcribed spacer (ITS) regions, beta-tubulin (TUB), and elongation factor (TEF) genes showing the phylogenetic position of strain KNUF-21-006 among *Pestalotiopsis* species and its closest relationship with *Pestalotiopsis clavata*. Bootstrap values greater than 50% (percentage of 1,000 replications) are shown at branching points. The strain isolated in this study is in bold. The tree was rooted using *Pseudopestalotiopsis cocos* CBS 272.29 as an out-group. Bar, 0.02 substitutions per nucleotide position.

***Botryotrichum iranicum* A. Alidadi, Mycological Progress 19 (12): 1578 (2020) [MB#831975]**

Specimen collection: Daro stream, Cheongdo-gun, Gyeongbuk Province (35°39'47.9"N 128°42'58.0"E), isolated from soil.

Morphological characteristics of KNUF-21-028

The strain KNUF-21-028 was cultured on PDA medium for 7 days at 25°C to study its cultural and morphological characteristics. The colony diameter reached 24 mm on PDA plates, off-white color, with irregular margins, and canaliculate, dense, smooth aerial mycelium; the back color was flavous (Figs. 3A and B). Conidiophores were septate and hyaline and were produced endobasidially or apically from hyphae. Conidia were globose, single, hyaline, asperate, 8.3–16.8 µm (n=20), and the hilum was clearly visible. No sexual morphs were observed (Figs. 3C and D). These morphological characteristics matched well with those typically described for the genus *Botryotrichum* and closely agreed with the characteristics of *B. iranicum* [17] (Table 3). The culture and morphological characteristics of the isolated strain KNUF-21-028 suggested that it was most closely related to *B. iranicum* [17].

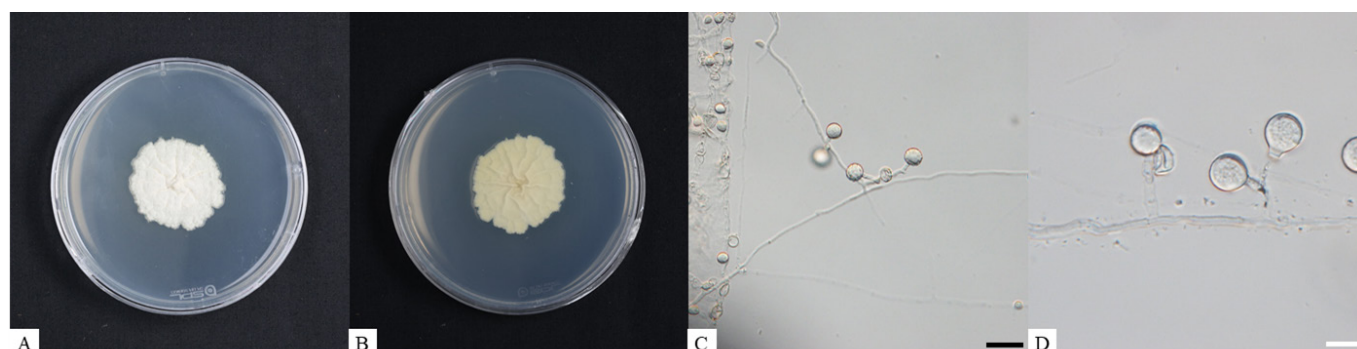


Fig. 3. Cultural and morphological characteristics of *Botryotrichum iranicum* KNUF-21-028. Cultures were grown at 25°C for 7 days on potato dextrose agar (PDA) in darkness. A, B: front and reverse view of the colony. C, D: conidia. Scale bars: C=20 µm, D=10 µm.

Table 3. Morphological characteristics of KNUF-21-028 and the reference species *Botryotrichum iranicum* cultured on potato dextrose agar medium at 25°C.

Characteristic	KNUF-21-028 ^a	<i>Botryotrichum iranicum</i> ^b
Colony	Off-white color; irregular margins; canaliculate; dense; smooth aerial mycelium; reverse flavous; reaching 2.4 cm diameter after 7 days	Orange; white irregular margin; white aerial mycelium; reaching 2.4 cm diameter after 7 days
Conidiophore	Septate; hyaline; produced endobasidially or apically from hyphae	Laterally or terminally from hyphae; septate; hyaline
Conidia	Globose; single; hyaline; asperate; 8.3–16.8 µm; hilum clearly visible	Solitarily; globose to subglobose or obpyriform, hyaline, roughened; 9.5–23.5×9.5–18 µm; hilum clearly visible

^a Fungal strain studied in this paper, ^b Source of description [17].

Molecular phylogeny of the KNUF-21-028

From the results of the sequencing analysis, 523, 471, and 650 bp sequences were obtained from the ITS, LSU, and TUB, respectively. A BLAST search of the NCBI database revealed similarities of 99.8% between the ITS regions of KNUF-21-028 and *Botryotrichum iranicum* ABRIICC 10152 (MN134583), *B. iranicum* ABRIICC 10153 (MN134584) and *B. artogriseum* CCF 5752 (LR584032); 100% similarity to *B. piluliferum* CBS 579.63 (MH858364) was observed. Based on the sequence similarity of the LSU region, the closest relatives of the isolate were identified as *B. iranicum* ABRIICC 10152 and ABRIICC 10153 (MN134576 and MN134577), *B. piluliferum* CBS 579.63 (MH869989), and *B. verrucosum* CBS 116.64 (LT993567), all with 100% similarity. The TUB gene sequence of KNUF-21-028 shared 100% identity with closely related *B. iranicum* ABRIICC 10152 and ABRIICC 10153 (MN128435 and MN128436), and only 96.4% identity with *B. artogriseum* CCF 5752 (LR584034). The comparison of ITS, LSU, and TUB loci clearly indicated that isolate KNUF-21-028 belongs to the genus *Botryotrichum*, but comparative analysis using the sequence of only one gene did not allow precise identification of the novel strain at the species level. Considering that sequences of the ITS, LSU, and TUB regions are available in GenBank for almost all *Botryotrichum* species, phylogenetic analyses based on combined ITS, LSU, and TUB sequences allowed us to accurately differentiate *Botryotrichum* species investigated in previous studies [17]. We also conducted a phylogenetic analysis using concatenated ITS, LSU, and TUB sequences (Table 1). The topology of the maximum likelihood phylogenetic tree based on concatenated sequences (Fig. 4) confirmed that KNUF-21-028 belongs to the genus *Botryotrichum* and showed that this isolate is most closely related to *B. iranicum* at the species level. To our knowledge, this is the first record of this species in Korea.

Previous studies have reported the isolation of *Pestalotiopsis clavata* from viable leaves in the Hunan and Yunnan Provinces, China [16]. Some *Pestalotiopsis* species are of great importance due to their plant pathogenicity [24]. Plant diseases caused by *Pestalotiopsis* species have been also reported in Korea, including gray blight in tea plant caused by *P. theae*, leaf blight in sweet persimmons caused by *P. diospyri*, and canker in *Acanthopanax divaricatus* caused by *P. ellipsospora* [12,13,16]. Out of the *Pestalotiopsis* species isolated in Korea, 11 have been reported as pathogenic [25]. Non-pathogenic *Pestalotiopsis* species have been found in Korea as endophytic fungi [26], associated with persimmon tree bark [7], in indoor air samples [27] or soil samples [28]. The species-rich genus *Pestalotiopsis* includes numerous plant pathogens as well as endophytes, which synthesize more than 300 secondary metabolites possessing various biological activities [29]. Seventy new bioactive secondary metabolites have been reported from *P. fici* including pestalofone F, pestalodiol C, and chloropistolide A showing high cytotoxicity against HeLa and MCF-7 cells [30]. Antifungal pestalofide A and pestaphthalide A were isolated from *P. foedans* [31] and anti-angiogenic dihydroxanthone AGI-B4 is produced by *P. clavispora* [32]. *P. clavata* is not yet known as the producer of bioactive compounds; therefore, isolate KNUF-21-006 could be considered as the indigenous strain for further study of this species in Korea.

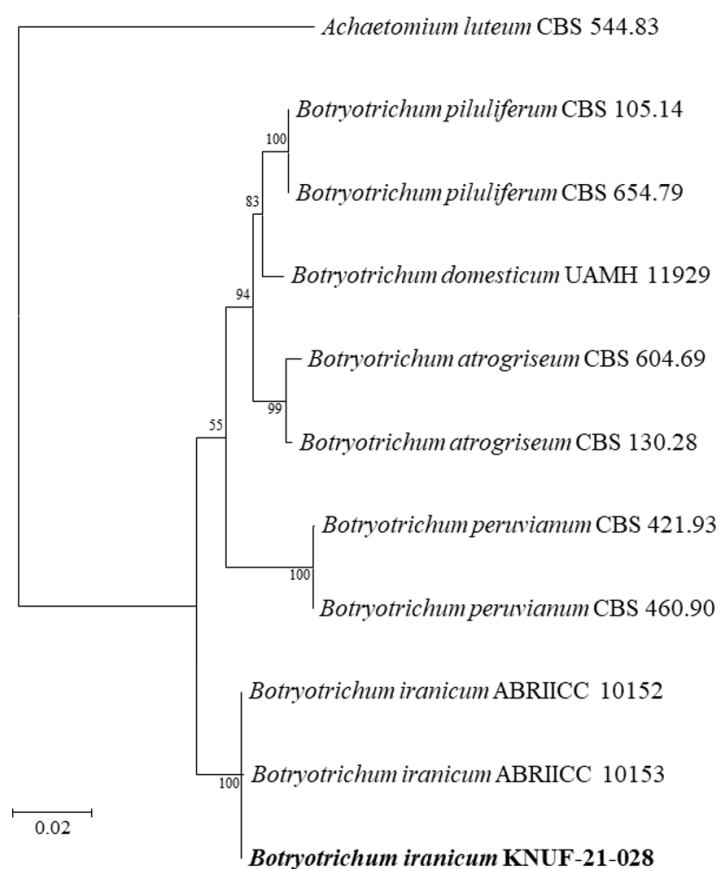


Fig. 4. Maximum likelihood phylogenetic tree based on a combined dataset of partial sequences of internal transcribed spacer (ITS) regions, large subunit (LSU) region, and beta-tubulin (TUB) genes showing the phylogenetic position of strain KNUF-21-028 among *Botryotrichum* species and its closest relationship with *Botryotrichum iranicum*. Bootstrap values greater than 50% (percentage of 1,000 replications) are shown at branching points. The strain isolated in this study is in bold. The tree was rooted using *Achaetomium luteum* CBS 544.83 as an out-group. Bar, 0.02 substitutions per nucleotide position.

Most *Botryotrichum* species have been isolated from outdoor environments, such as *B. iranicum*, first discovered in a soil sample in Iran [17]. There are no reports of plant pathogenicity for representatives of the genus *Botryotrichum*, which comprises just 25 species. The genus *Botryotrichum* includes significantly fewer species than *Pestalotiopsis*, consequently, the number of isolated and identified secondary metabolites from its representatives is not so large. Among them, *B. piluliferum* is more studied and known as the producer of oxisterigmatocystins E, G, and H displaying antimalarial activity towards *Plasmodium falciparum* and cytotoxicity against KB, MCF-7, and NCI-H187 cell lines [33], altersolanol C, diorcinol, and botryobutenolide A possessing antibacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis* [34].

Although the genus *Botryotrichum* was first reported in Korea in 1995 with two unidentified strains isolated from traditional Nuruk [35], until now, there are no detailed reports of any *Botryotrichum* species. Just like *P. clavata*, *B. iranicum* has not yet been investigated for the production of bioactive compounds; therefore the isolate KNUF-21-028 can be considered as the indigenous strain for further study of this species in Korea. Overall, further research on *Pestalotiopsis* and *Botryotrichum* species is required for a better understanding of their diversity, geographical distribution, pathogenicity, and ecological and biological roles, especially, under Korean environmental conditions.

To our knowledge, this is the first record of the two species, namely, *Pestalotiopsis clavata* KNUF-21-006 and *Botryotrichum iranicum* KNUF-21-028, in Korea.

CONFLICT OF INTERESTS

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

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REFERENCES

1. Frąc M, Hannula SE, Belka M, Jędryczka M. Fungal biodiversity and their role in soil health. *Front Microbiol* 2018;9:707.
2. Tedersoo S, Bahram M, Pöhlme S, Kõljalg U, Yorou NS, Wijesundera R, Villarreal Ruiz L, Vasco-Palacios AM, Quang Thu P, Suija A, et al. Global diversity and geography of soil fungi. *Science* 2014;346:6213.
3. Eriksson OE, Winka K. Supraordinal taxa of Ascomycota. *Myconet* 1997;1:1-16.
4. Zhang N, Castlebury LA, Miller AN, Huhndorf SM, Schoch CL, Seifert KA, Rossman AY, Rogers JD, Kohlmeyer J, Volkmann-Kohlmeyer B, et al. An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. *Mycologia* 2006;98:1076-87.
5. Barr ME. *Pestalosphaeria*, a new genus in the Amphisphaeriaceae. *Mycologia* 1975;67:187-94.
6. Steyaert RL. Contribution à l'étude monographique de *Pestalotia* de Not. et Monochaetia Sacc. (*Truncatella* gen. nov. et *Pestalotiopsis* gen. nov. *Bull Bot Gard State Bruss* 1949;19:285-354.
7. Das K, Lee SY, Jung HY. *Pestalotiopsis kaki* sp. nov., a novel species isolated from persimmon tree (*Diospyros kaki*) bark in Korea. *Mycobiology* 2021;49:54-60.
8. Maharachchikumbura SSN, Hyde KD, Groenewald JZ, Xu J, Crous PW. *Pestalotiopsis* revisited. *Stud Mycol* 2014;79:121-86.
9. Senanayake IC, Maharachchikumbura SSN, Hyde KD, Bhat JD, Jones EBG, McKenzie EHC, Qin Dai D, Daranagama DA, Dayarathne MC, Goonasekara ID, et al. Towards unraveling relationships in Xylariomycetidae (Sordariomycetes). *Fungal Divers* 2015;73:73-144.
10. Liu F, Bonthond G, Groenewald JZ, Cai L, Crous PW. Sporocadaceae, a family of coelomycetous fungi with appendage-bearing conidia. *Stud Mycol* 2019;92:287-415.

11. Yun YH, Ahn GR, Kim SH. First report and characterization of *Pestalotiopsis ellipsospora* causing canker on *Acanthopanax divaricatus*. *Mycobiology* 2015;43:366-70.
12. Kwon JH, Ahn GH, Park CS. Leaf blight of sweet persimmon tree in the field and fruit rot in the storage caused by *Pestalotia diospyri*. *Kor J Mycol* 2004;32:130-3.
13. Wang XW, Houbraken J, Groenewald JZ, Meijer M, Andersen B, Nielsen KF, Crous PW, Samson RA. Diversity and taxonomy of *Chaetomium* and *Chaetomium*-like fungi from indoor environments. *Stud Mycol* 2016;84:145-224.
14. Elkhateeb WA, Daba GM. *Botryotrichum* and *Scopulariopsis* secondary metabolites and biological activities. *J Biotech and Bioprocessing* 2022;3:1-4.
15. Widden P. Microfungal community structure from forest soils in southern Quebec, using discriminant function and factor analysis. *Can J Bot* 1986;64:1402-12.
16. Maharachchikumbura SSN, Guo LD, Cai L, Chuksatiro E, Wu WP, Sun X, Crous PW, Bhat DJ, McKenzie EHC, Bahkali AH, et al. A multi-locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species. *Fungal Divers* 2012;56:95-129.
17. Alidadi A, Vala SA, Jouzani GS. *Botryotrichum iranicum* sp. nov. and *Trematosphaeria magenta* sp. nov. as two new species from Iran. *Mycol Prog* 2020;19:1575-86.
18. White T, Bruns T, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. *PCR Protocols: A guide to methods and applications*. San Diego: Academic Press; 1990. p. 315-22.
19. Rehner SA, Samuels GJ. Molecular systematics of the Hypocreales: a teleomorph gene phylogeny and the status of their anamorphs. *Can J Bot* 1995;73:S816-23.
20. O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proc Natl Acad Sci* 1998;95:2044-9.
21. Carbone I, Kohn LM. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 1999;91:553-6.
22. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 1999;41:95-8.
23. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870-4.
24. Park SK, Lee SY, Lee JJ, Back CG, Ten L, Lee HB, Jung HY. First report of *Neopestalotiopsis australis* isolated from soil in Korea. *Kor J Mycol* 2016;44:360-4.
25. The Korean Society of Plant Pathology. *List of plant diseases in Korea*. 6th ed. Seoul: World Science; 2022.
26. Park H, Shim JS, Kim JS, Choi HS, Eom AH. Five previously unreported endophytic fungi isolated from the leaves of woody plants in Korea. *Kor J Mycol* 2017;45:345-54.
27. Ahn GR, Noh H, Kim JY, Ko HG, Kim SH. Unrecorded fungi isolated from *Lentinula edodes* cultivation houses in Korea. *J Mushrooms* 2020;18:72-8.
28. Ahn GR, Kim SH. Undescribed fungal species found in Ulleungdo, Korea. *Kor J Mycol* 2015;43:53-7.
29. Aguilar-Pérez MM, Torres-Mendoza D, Vásquez R, Rios N, Cubilla-Rios L. Exploring the antibacterial activity of *Pestalotiopsis* spp. under different culture conditions and their chemical diversity using LC-ESI-Q-TOF-MS. *J Fungi* 2020;6:140.
30. Liu L. Bioactive metabolites from the plant endophyte *Pestalotiopsis fici*. *Mycology* 2011;2:37-45.

31. Ding G, Liu S, Guo L, Zhou Y, Che Y. Antifungal metabolites from the plant endophytic fungus *Pestalotiopsis foedans*. *J Nat Prod* 2008;71:615-8.
32. Alade GO, Moody JO, Bakare AG, Awotona OR, Adesanya S, Lai D, Debbab A, Proksch P. Metabolites from endophytic fungus; *Pestalotiopsis clavispora* isolated from *Phoenix reclinata* leaf. *Future J Pharm Sci* 2018;4:273-5.
33. Rajachan OA, Kanokmedhakul K, Soyong K, Kanokmedhakul S. Mycotoxins from the fungus *Botryotrichum piluliferum*. *J Agric Food Chem* 2017;65:1337-41.
34. Ebrahim W, Ebada SS. Antimicrobial metabolites from extremophilic fungus *Botryotrichum piluliferum* strain WESH19. *Chem Nat Compd* 2021;57:654-8.
35. Park JW, Lee KH, Lee CY. Identification of filamentous molds isolated from Korean traditional Nuruk and their amyolytic activities. *Kor J App Microbiol Biotechnol* 1995;23:737-46.