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Three New Records of Ascomycetes Isolates from Field Soils in Korea

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Abstract Three new records of Ascomycota species (*Chaetomium acropullum*, *Phialemonium globosum, Phialemonium atrogriseum*) from field soils in Korea are presented in this study. These newly discovered fungal isolates were isolated from field soils from various places across Gyeongnam, Korea in 2016. All the isolates were identified and described based on morphological characteristics, and rDNA internal transcribed spacer and β-tubulin gene sequence data. Morphological features of these fungal species were studied on different agar media: potato dextrose agar, oatmeal agar, malt extract agar, Czapek yeast extract agar, and yeast extract sucrose agar. Full description and illustrations of their morphological characters are provided. These fungal species have not officially been previously reported in Korea.

Keywords Ascomycota, *Chaetomium acropullum*, *Phialemonium atrogriseum*, *Phialemonium globosum*, rDNA

Ascomycota is the largest phylum of the kingdom Fungi and is a morphologically diverse group consisting of unicellular yeast to complex cup fungi. There are over 64,000 species of ascomycetes [1]. The most common feature of the ascomycetes is the ascus, a microscopic sexual structure containing nonmotile spores called ascospores.

Ascomycetes are monophyletic, meaning they are all descendants of one common ancestor. *Chaetomium*, representing ascomycetous fungi of the Chaetomiaceae family (class: Sordariales), is a species-rich genus with roughly 100 classified species distributed worldwide and found on various substrates as saprotrophs [2]. This genus is distinctive by its superficial ascomata (mostly ostiolate perithecia) covered with several hair types [2]. *C. globosum* has been mainly found in soil, woods, and wet walls [3], and it may cause systemic infection upon inhalation [4]. Some *Chaetomium* species are reported to be associated with *Acremonium*, *Botryotrichum*, *Humicola*, *Paecilomyces*, and *Scopulariopsis* [5]. *C. acropullum* is a psychrotolerant mesophilic fungi first identified in China [6]. The *Phialemonium* genus of fungi belongs to the class Sordariomycetes, order Sordariales, and family Cephalothecaceae. This is an intermediate genus between *Acremonium* and *Phialophora* [7]. The main objective of the present study was to (1) study the *C. acropullum*, *Phialemonium globosum*, and *Phialemonium atrogriseum* isolates on a morphological and molecular level, and (2) to compare these newly recorded species with previously reported species.

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MATERIALS AND METHODS

Soil sample collection and fungal isolation. Soil samples were collected in 2016 in Gyeongnam, Korea from depths of 0–15 cm after scraping and removing leaves and other plant debris on the soil surface. Approximately 200–250 g of soil was collected for each sample. The collected soil samples were prepared by drying at room temperature, grinding, and sieving with an autoclave-sterilized brass sieve of 2-mm aperture size. The fungi were isolated using the dilution technique [8]. Briefly, 1 g soil sample was suspended in 9-mL sterile distilled water and vigorously shaken for 2–3 min. The suspensions of soil were serial diluted in sterile distilled water. From each dilution, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} mL was removed by pipette and streaked

onto petri plates containing potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA), and the plates were incubated for 5–7 days at 25°C until growth of fungal colonies was observed. Morphologically distinct colonies were selected and further purified by sub-culturing on plates containing PDA. The pure cultures were preserved on 20% glycerol stock at 4°C for further studies.

Morphological characterization. Morphological characteristics of isolates KNU16-31, KNU16-137, and KNU16-168 were studied on PDA, oatmeal agar (OA), malt extract agar (MEA), Czapek yeast extract agar (CYA), and yeast extract sucrose agar (YESA). The strains were inoculated in three regions on 9-cm petri dishes and incubated for 10 days at 26°C in darkness. All media used were prepared as described by Samson *et al.* [9]. After incubation, the diameters of the colonies on each agar media were measured. Colony color (obverse and reverse sides) and the degree of sporulation were also observed. Colony colors of the isolates were characterized using the

Table 1. ITS and the β-tubulin gene sequences of *Chaetomium acropullum* used in this study, along with their strains and GenBank accession numbers

No.	Species	Isolate No.	GenBank Accession No.	
			ITS	β-Tubulin
1	*Chaetomium afropilosum*	CBS 145.38	NR144855	-
2	*Chaetomium tenue*	CBS 139.38	NR144853	-
3	*Chaetomium umbonatum*	CBS 293.83	NR144856	-
4	*Chaetomium pseudocochliodes*	CGMCC 3.94	NR144824	-
5	*Chaetomium rectangulare*	IRAN 164	NR144817	-
6	*Chaetomium coarctatum*	MUCL 18697	NR144822	-
7	*Chaetomium cucumericola*	CBS 378.71	NR144858	-
8	*Chaetomium subglobosum*	MUCL 18694	NR144826	-
9	*Chaetomium subaffine*	CBS 637.91	NR144825	-
10	*Chaetomium unguicola*	CBS 128446	NR144852	-
11	*Chaetomium cervicicola*	CBS 128492	NR144848	-
12	*Chaetomium acropullum*	J1-2	KP994323	-
13	*Chaetomium pilosum*	CBS 335.67	NR144862	-
14	*Chaetomium megalocarpum*	CBS 149.59	NR144832	-
15	*Chaetomium ascotrichoides*	CBS 113.83	NR144835	-
16	*Chaetomium madrasense*	CBS 315.74	KC109751	-
17	*Chaetomium contagiosum*	CBS 128494	NR144846	-
18	*Chaetomium nozdrenkoae*	CBS 163.62	KP970636	-
19	*Chaetomium jatrophae*	MMI00056	JQ246354	-
21	*Chaetomium pseudoglobosum*	CBS 574.71	-	KT214750
21	*Chaetomium tenue*	CBS 139.38	-	KT214745
22	*Chaetomium umbonatum*	CBS 293.83	-	KT214752
23	*Chaetomium afropilosum*	CBS 145.38	-	KT214751
24	*Chaetomium citrinum*	CBS 693.82	-	KT214764
25	*Chaetomium subaffine*	CBS 637.91	-	JN256199
26	*Chaetomium pseudocochliodes*	CGMCC 3.94	-	JN256195
27	*Chaetomium spiculipilium*	CBS 373.66	-	KC109774
28	*Chaetomium cochlioides*	CBS 155.52	-	KC109772
29	*Chaetomium cervicicola*	CBS 128492	-	KT214735
30	*Chaetomium nozdrenkoae*	CBS 163.62	-	KT214736
31	*Chaetomium grande*	C57	-	HM365273
32	*Chaetomium ascotrichoides*	CBS 113.83	-	KC109770
33	*Chaetomium madrasense*	CBS 315.74	-	KC109769
34	*Chaetomium subfimeti*	CBS 370.66	-	KT214739
35	*Chaetomium fimeti*	CBS 139034	-	KT214736
36	*Chaetomium tectifimeti*	DTO 318-G8	-	KX976982
37	*Chaetomium truncatulum*	C77	-	HM365298
38	*Chaetomium iranianum*	C67	-	HM365297
39	*Chaetomium atrobrunneum*	C64	-	HM365294
40	*Chaetomium acropullum*	C68	-	HM365292
41	*Chaetomium anamorphosum*	CBS 137114	-	KP900704
42	*Chaetomium thermophilum var. thermophilum*	CBS 144.50	-	KP336895

ITS, internal transcribed spacer.

method of Kornerup and Wanscher [10]. For microscopic analysis of the isolates, images were acquired with an HK 3.1 CMOS digital camera (KOPTIC Korea Optics, Seoul, Korea) connected to an Olympus BX50F-3 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) and a scanning electron microscope (LEO Model 1450VP Variable Pressure Scanning Electron Microscope; Carl Zeiss, Thornwood, NY, USA).

Genomic DNA extraction, sequencing, and data analysis. Total genomic DNA of the isolates was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The internal transcribed spacer (ITS) region and β-tubulin gene were amplified using primers ITS1 (5′-TCCGTAGGTGAACCTGCG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), and Bt2a (5′-GGTAACCAAATCGGTGCTTTC-3′) and Bt2b (5′-ACCCTCAGTGTAGTGACCCTT-3′), respectively [11]. Amplified PCR products were sequenced with the ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Reference ITS sequences were used to compare with the sequence information in GenBank at the National Center for Biotechnology Information (NCBI) using the local alignment search tool [12]. The nucleotide sequences were deposited at the culture collection site of the National Institute of Biological Resources (NIBR, Incheon, Korea). NIBR numbers; NIBRG0000499342, NIBRG0000499405, and NIBRG0000499424 were given to the isolates KNU16-31, KNU16-137, and KNU16-168, respectively. ITS and the β-tubulin gene sequences of the isolates (KNU16-31, KNU16-137, and KNU16-168) used in this study, along with their strains and GenBank accession

Table 2. ITS and β-tubulin gene sequences of *Phialemonium globosum* and *P. atrogriseum* and allied species used in this study, along with their strains and GenBank accession numbers

No.	Species	Isolate No.	GenBank Accession No.	
			ITS	β-Tubulin
1	*Lecythophora luteoviridis*	CBS 206.38	HE610333	-
2	*Coniochaeta mutabilis*	CBS 157.44	NR111519	-
3	*Coniochaeta lignicola*	CBS 267.33	NR111520	-
4	*Lecythophora fasciculate*	CBS 205.38	HE610336	-
5	*Coniochaeta Africana*	-	NR137725	-
6	*Lecythophora hoffmannii*	DUMC 153.04	AY945807	-
7	*Coniochaeta cateniformis*	UTHSC 01-16	NR111517	-
8	*Coniochaeta canina*	UTHSC 11-2	NR120211	-
9	*Phialemonium globosum*	UTHSC 03-3	HE610358	-
10	*Phialemonium inflatum*	CBS 259.39	NR145146	-
11	*Phialemonium obovatum*	CBS 279.76	AB278187	-
12	*Phialemonium atrogriseum*	CBS 604.67	NR111521	-
13	*Phialemoniopsis ocularis*	FMR 6632	NR111515	-
14	*Phialemoniopsis hongkongensis*	HKU39	KJ573442	-
15	*Phialemoniopsis curvata*	CBS 490.82	NR132076	-
16	*Phialemoniopsis cornearis*	UTHSC 06-18	NR111516	-
17	*Phialemoniopsis pluriloculosa*	UTHSC 04-7	HE599286	-
18	*Lecythophora fasciculate*	CBS 205.38	-	HE610350
19	*Lecythophora lignicola*	CBS 267.33	-	HE610353
21	*Lecythophora luteoviridis*	CBS 206.38	-	HE610351
21	*Lecythophora mutabilis*	CBS 157.44	-	HE610349
22	*Phialemonium inflatum*	CBS 259.39	-	HE599347
23	*Phialemonium globosum*	UTHSC 03-36	-	HE599349
24	*Lecythophora decumbens*	CBS 153.42	-	HE610348
25	*Phialemonium obovatum*	CBS 279.76	-	HE599334
26	*Phialemonium atrogriseum*	CBS 604.67	-	HE599346
27	*Chaetomidium leptoderma*	CBS:538.74	-	FJ666369
28	*Chaetomidium arxii*	CBS:104.79	-	FJ666375
29	*Chaetomidium pilosum*	CBS:335.67	-	FJ666372
30	*Phialemoniopsis hongkongensis*	HKU39	-	KJ573457
31	*Phialemoniopsis curvata*	CBS 490.82	-	KJ573458
32	*Acremonium citrinum*	CBS 384.96	-	HF680257
33	*Acremonium fusidioides*	CBS 840.68	-	HF680243
34	*Acremonium pilosum*	CBS 124.70	-	HF680249
35	*Acremonium parvum*	CBS 381.70A	-	HF680239

ITS, internal transcribed spacer.

numbers are listed in Table 1 and 2.

In addition, the nucleotide sequences were deposited in GenBank and assigned accession numbers KY587781, KY587782, and KY587783 for isolates KNU16-31, KNU16-137, and KNU16-168, respectively. Neighbor joining relationships were analyzed using Molecular Evolutionary Genetic Analysis (MEGA 6) software [13]. A neighbor joining tree was constructed using the Kimura 2-parameter substitution model [14]. Bootstrap analysis was carried out with 1,000 replications to determine the support for each clade.

RESULTS

Morphology of the isolate KNU16-31.

Colony morphology: Morphological features of the isolate KNU16-31 are shown in Fig. 1. The colony grew rapidly on PDA and reached a diameter of 50–55 mm after 10 days at 26°C. The obverse side of the colony was white in color while the reverse side was light yellow in color at the center (Fig. 1A and 1F). The colony had a woolly cotton appearance at the center. Sporulation was moderate to dense, and there were numerous conidia present. The form of the colony was irregular and its surface was smooth. On MEA, colony growth was moderate and it attained a diameter of 45–50 mm after 10 days at 26°C (Fig. 1B and 1G). Obverse and reverse sides of the colony were white in color. Texture of the colony was woolly. The form of the colony was irregular and its surface was smooth. Sporulation was absent. Ample conidia were observed. On YESA, the colony grew rapidly and reached a diameter of 55–56 mm after 10 days at 26°C (Fig. 1C and 1H). Color of the colony was feathery white on the obverse side and creamy white on the reverse side (Fig. 1C and 1H). Sporulation was slightly moderate. The texture of the colony was floccose. On CYA, the colony growth was moderate and it attained a diameter of 45–50 mm after 10 days at 26°C. Obverse and reverse sides of the colony were woolly white and yellow in color (Fig. 1D and 1I). Sporulation was moderate to dense and conidia were abundant. The texture of the colony was woolly. The form of the colony was irregular and its surface was smooth. On OA, the colony grew rapidly and reached a diameter of 55–60 mm after 10 days at 26°C. Obverse and reverse sides of the colony were white in color (Fig. 1E and 1J). Sporulation was moderate to dense, and conidia were abundant. The form of the colony was irregular and its surface was smooth.

Chaetomium acropullum X.Wei Wang, Nova Hedwigia 80 (3-4): 414 (2005).

Micromorphology: Conidiophores were erect or sometimes branched irregularly, and were 2.3–3.3 µm wide. Aleurioconidia arose singly at the tips of hyphae and lateral branches. Single cells of aleurioconidia were individually attached to

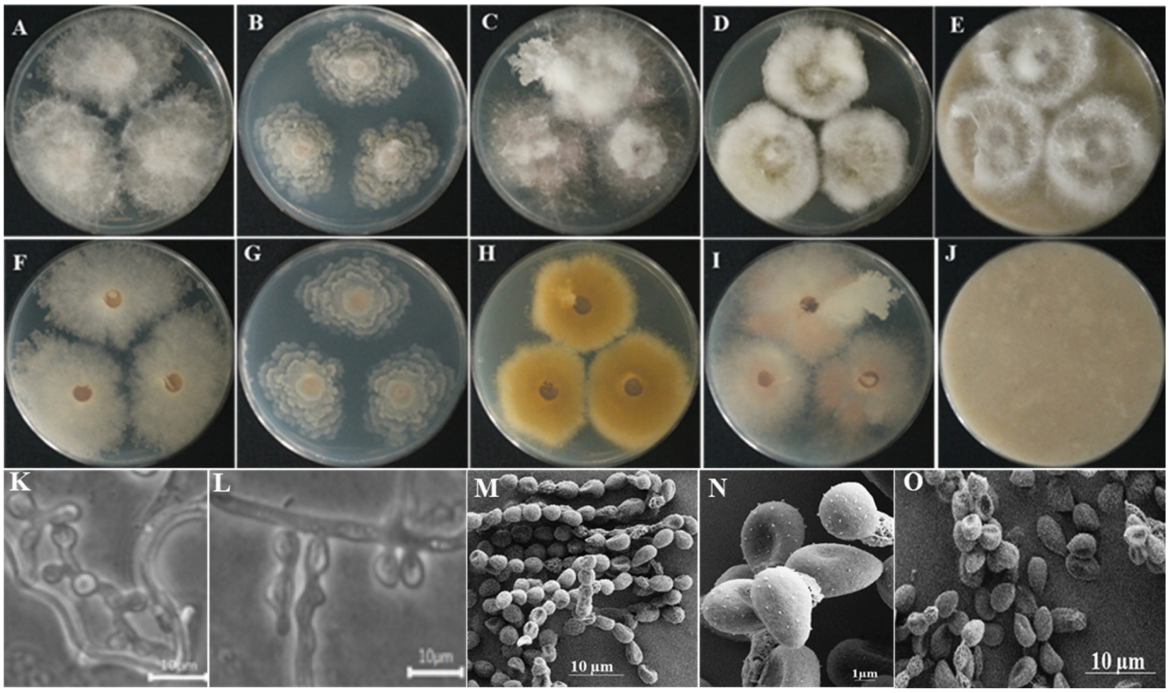

Fig. 1. Morphological characteristics of *Chaetomium acropullum* KNU16-31 grown for 10 days on potato dextrose agar (PDA), malt extract agar (MEA) yeast extract sucrose agar (YESA), Czapek yeast extract agar (CYA), and oatmeal agar (OA) at 26°C. A–J, Obverse side (A–E) and reverse side (F–J) of each colony grown, from left to right, on PDA, MEA, YESA, CYA, and OA; K, L, Image of conidiophores taken with a simple microscope; M–O, Images of conidia and conidiophores taken using a scanning electron microscope (scale bars: K–M, O = 10 µm, N = 1 µm).

the hyphae, pyriform, or clavate, and were 9–11 × 5.3–6.9 μm in size. Individual cells of arthroconidia formed near the segmentation of hyphae. They ranged in size from short to long, were cylindrical in shape with truncate ends, and were 11–28 × 2.4–3.3 μm in size (Fig. 1K–1O).

Morphology of the isolate KNU16-137.

Colony morphology: Detailed morphological features of the fungal isolate KNU16-137 are shown in Fig. 2. On PDA, the colony grew slowly and attained a diameter of 30–35 mm after 10 days at 26°C. Obverse and reverse sides of the colony were white and creamy white, respectively (Fig. 2A and 2F). Sporulation was moderate to dense. The form of the colony was irregular and its surface was smooth. On MEA, the colony grew slowly and attained a diameter of 15–20 mm after 10 days at 26°C (Fig. 2B and 2G). Obverse and reverse sides of the colony were white in color. The texture of the colony was woolly. The form of the colony was irregular and its surface was smooth. Sporulation was moderate. Conidia were abundant. On YESA, the colony grew rapidly and reached a diameter of 30–35 mm after 10 days at 26°C. The obverse side was white in color and the reverse side was creamy (Fig. 2C and 2H). Sporulation was slightly moderate. The texture of the colony was floccose. Conidia were abundant. On CYA, the colony grew moderately and attained a diameter of 35–40 mm after 10 days at 26°C. Obverse and reverse sides of the colony were woolly white and yellow in color, respectively (Fig. 2D and 2I). Sporulation was dense and conidia were abundant. The texture of the colony was woolly. The form of the colony was irregular and its surface was smooth. On OA, the colony grew rapidly and reached a diameter of 30–35 mm after 10 days at 26°C. Obverse and reverse sides of the colony were white in color (Fig. 2E and 2J). Sporulation was moderate to dense. Conidia were abundant. The form of the colony was irregular and its surface was smooth.

Phialemonium globosum D. García, H. Perdomo, Gené, Cano & Guarro, Mycologia 105 (2): 415 (2013).

Micromorphology: Conidiophores were poorly developed. Phialides emerged directly from aerial hyphae, and there were typically 1–2 phialides present. Adelophialides were cylindrical and single-celled. Conidia were globose to subglobose and 3–5 × 4.8–5 μm in diameter (Fig. 2K–2O). Chlamydospores were absent.

Morphology of the isolate KNU16-168.

Colony morphology: Detailed morphological features of the fungal isolate KNU16-168 are shown in Fig. 3. On PDA, the colony grew slowly and attained a diameter of 25–30 mm after 10 days at 26°C. The obverse side was creamy white and the reverse side was light yellow (Fig. 3A and 3F). Sporulation was slightly moderate. The form of

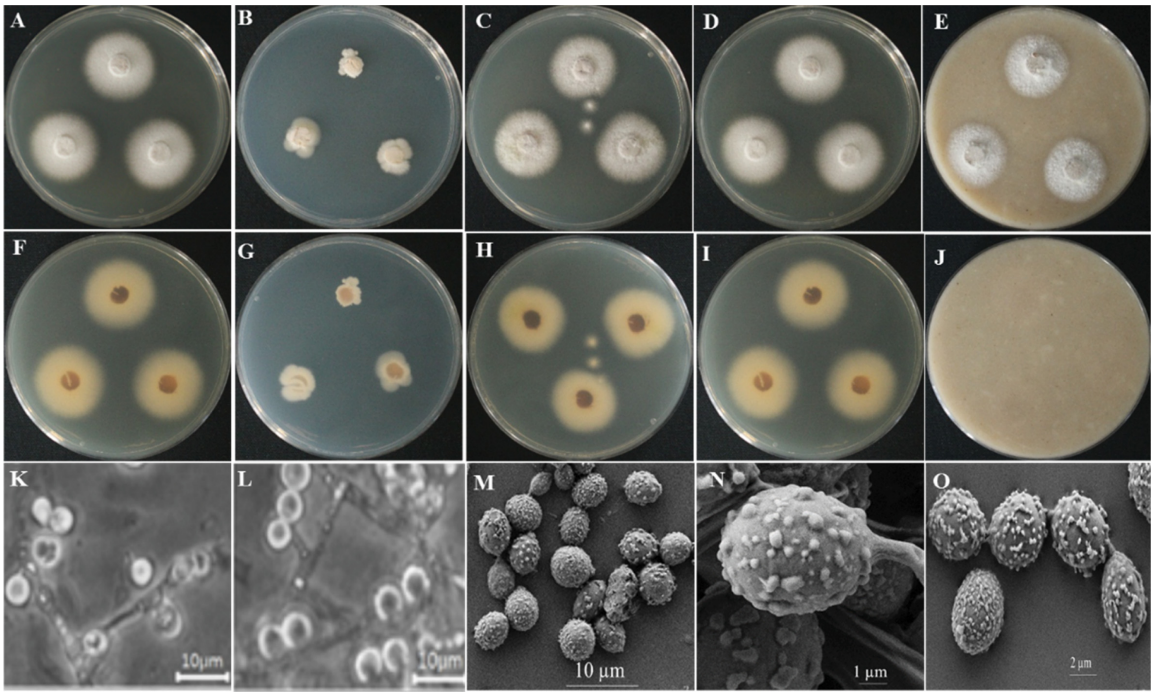

Fig. 2. Morphological characteristics of *Phialemonium globosum* KNU16-137 grown for 10 days on potato dextrose agar (PDA), malt extract agar (MEA) yeast extract sucrose agar (YESA), Czapek yeast extract agar (CYA), and oatmeal agar (OA) at 26°C. A–J, Obverse side (A–E) and reverse side (F–J) of each colony grown, from left to right, on PDA, MEA, YESA, CYA, and OA; K, L, Image of conidiophores taken with a simple microscope; M–O, Images of conidia and conidiophores taken using a scanning electron microscope (scale bars: K–M, O = 10 μm, N = 1 μm).

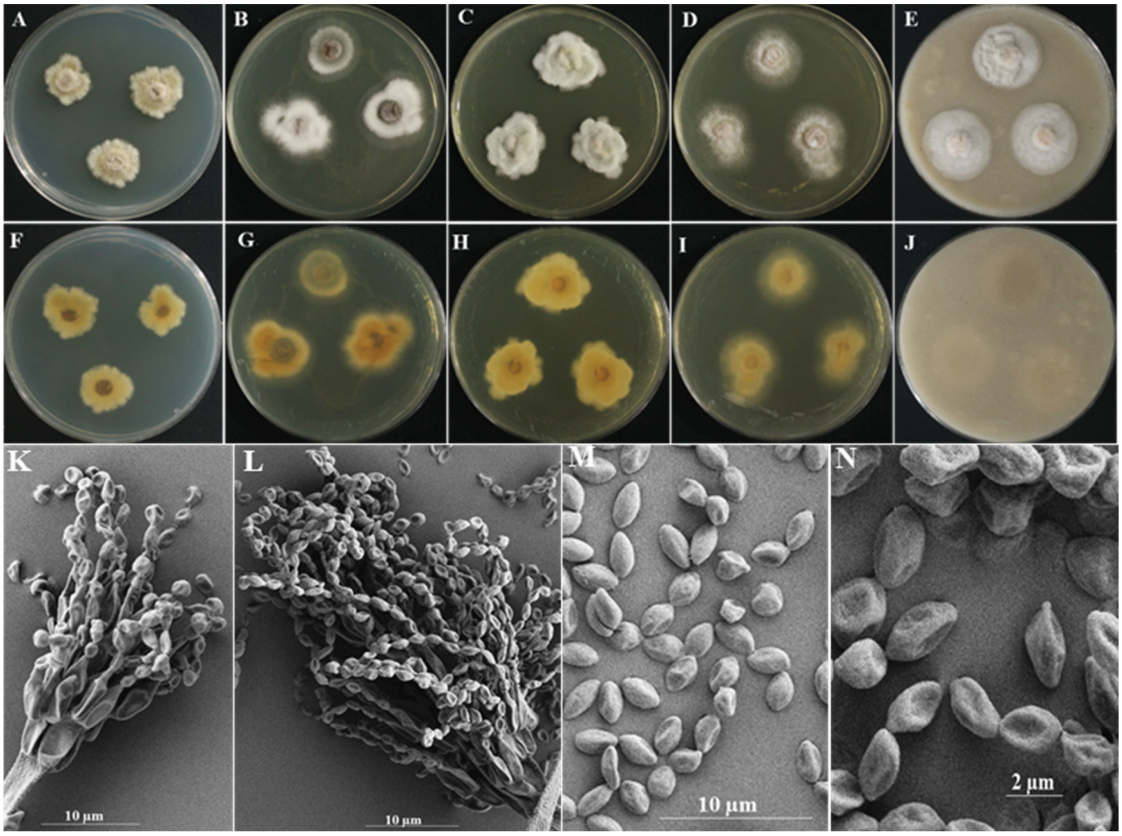

Fig. 3. Morphological characteristics of *Phialemonium atrogriseum* KNU16-168 grown for 10 days on potato dextrose agar (PDA), malt extract agar (MEA) yeast extract sucrose agar (YESA), Czapek yeast extract agar (CYA), and oatmeal agar (OA) at 26°C. A–J, Obverse side (A–E) and reverse side (F–J) of each colony grown, from left to right, on PDA, MEA, YESA, CYA, and OA; K, L, Image of conidiophores taken with a simple microscope; M, N, Images of conidia and conidiophores taken using a scanning electron microscope (scale bars: K–M = 10 μm, N = 2 μm).

the colony was irregular and its surface was rough. On MEA, the colony grew slowly and attained a diameter of 25–30 mm after 10 days at 26°C (Fig. 3B and 3G). The obverse side of the colony was white in color while the reverse side was yellow (Fig. 3B and 3G). The texture of the colony was woolly. The form of the colony was irregular and its surface was smooth. Sporulation was moderate. Conidia were abundant. On YESA, the colony grew moderately and reached a diameter of 25–30 mm after 10 days at 26°C. The obverse side was white in color and the reverse side was yellow (Fig. 3C and 3H). Sporulation was slightly moderate. The texture of the colony was floccose. Conidia were abundant. On CYA, the colony grew moderately and attained a diameter of 30–35 mm after 10 days at 26°C. Obverse and reverse sides of the colony were woolly white and yellow in color (Fig. 3D and 3I). Sporulation was dense and conidia were abundant. The texture of the colony was woolly. The form of the colony was irregular and its surface was smooth. On OA, the colony grew rapidly and reached a diameter of 35–40 mm after 10 days at 26°C. Obverse and reverse sides of the colony were white in color (Fig. 3E and 3J). Sporulation was moderate to dense. Conidia were abundant. The form of the colony was irregular and its surface was smooth.

Phialemonium atrogriseum (Panas.) D. García, Perdomo, Gené, Cano & Guarro, Mycologia 105 (2): 414 (2013).

Micromorphology: Conidiophores were raised irregularly and 2.2–2.3 × 1.8–2.1 μm in size. Lateral phialides emerged directly from aerial hyphae in whorls of 2–4. Phialides were flask-shaped. Conidia were ellipsoidal or ovoid in shape and were 3–4 × 2–3 μm in size (Fig. 3K–3N).

Molecular phylogeny of all studied fungal isolates. Genetic sequences of ITS and β-tubulin were analyzed to construct evolutionary relationships between the isolates KNU16-31, KNU16-137, and KNU16-168 with previously described species of *C. acropullum*, *P. globosum*, and *P. atrogriseum*, respectively. Results obtained from the phylogenetic relationship from the ITS sequence analysis suggested that the isolate KNU16-31 was most closely related to *C. acropullum* (KP994323.1) (Fig. 4). KNU16-31 had a 99% similarity with *C. acropullum* as determined by a bootstrap analysis with 1,000 replicates. Isolates used for the phylogenetic relationships were type strains obtained from GenBank. β-Tubulin gene sequence analysis suggested

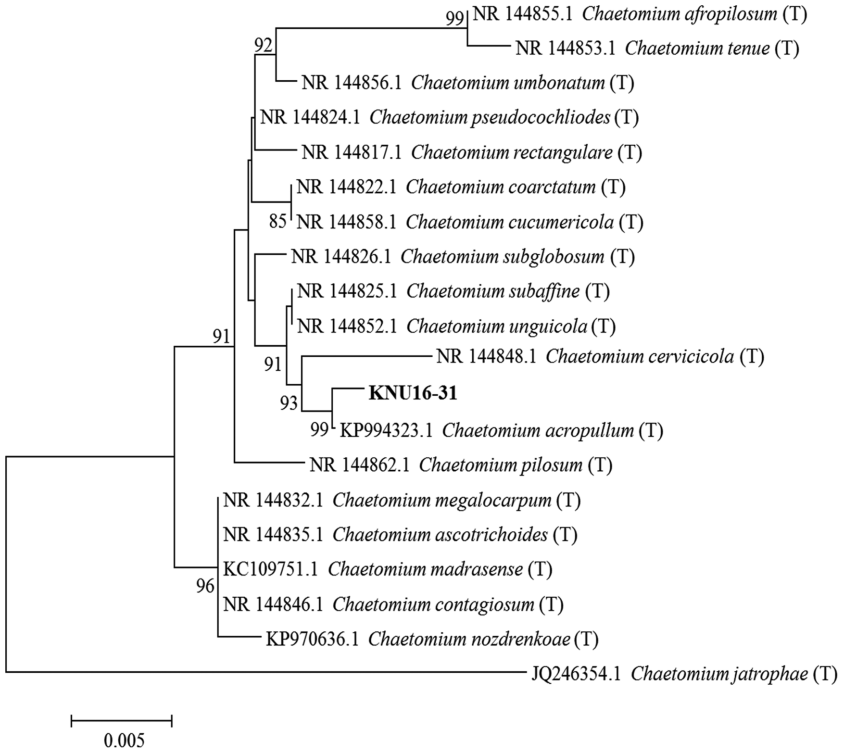

Fig. 4. Neighbor joining phylogenetic analysis of *Chaetomium acropullum* KNU16-31 using the partial 18S-ITS1-5.8S-ITS2-28S rDNA region sequence obtained from field soil in Korea. Numerical values (> 50) on branches represent the bootstrap values shown as the percentage of bootstrap replication from a 1,000-replicate analysis. 'T' indicates the type strain of the isolates.

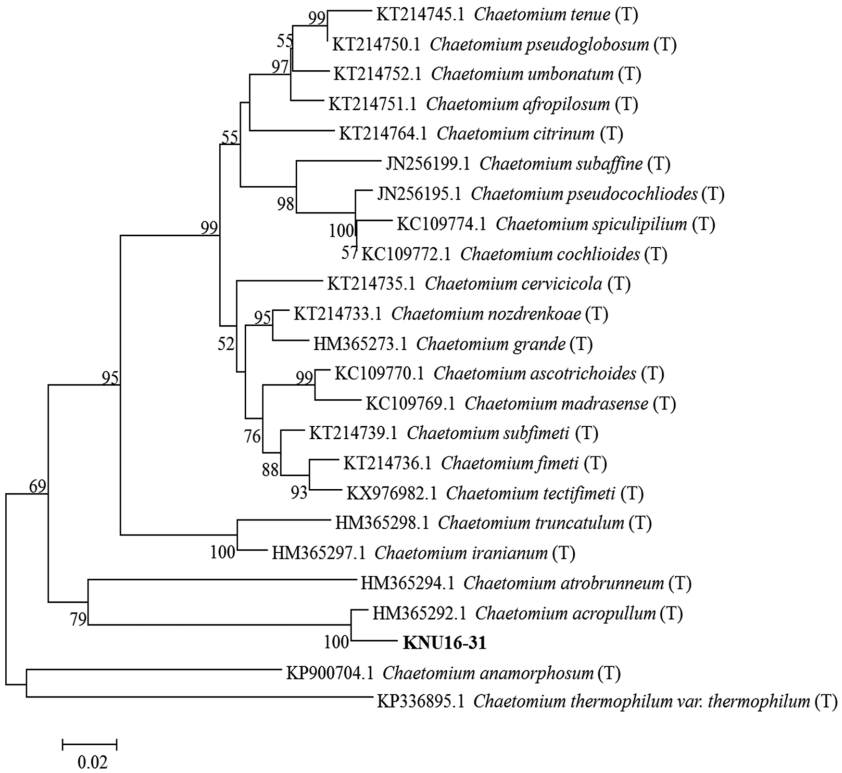

Fig. 5. Neighbor joining phylogenetic analysis of *Chaetomium acropullum* KNU16-31 using the β-tubulin gene sequence obtained from field soil in Korea. Numerical values (> 50) on branches represent the bootstrap values shown as the percentage of bootstrap replication from a 1,000-replicate analysis. 'T' indicates the type strain of the isolates.

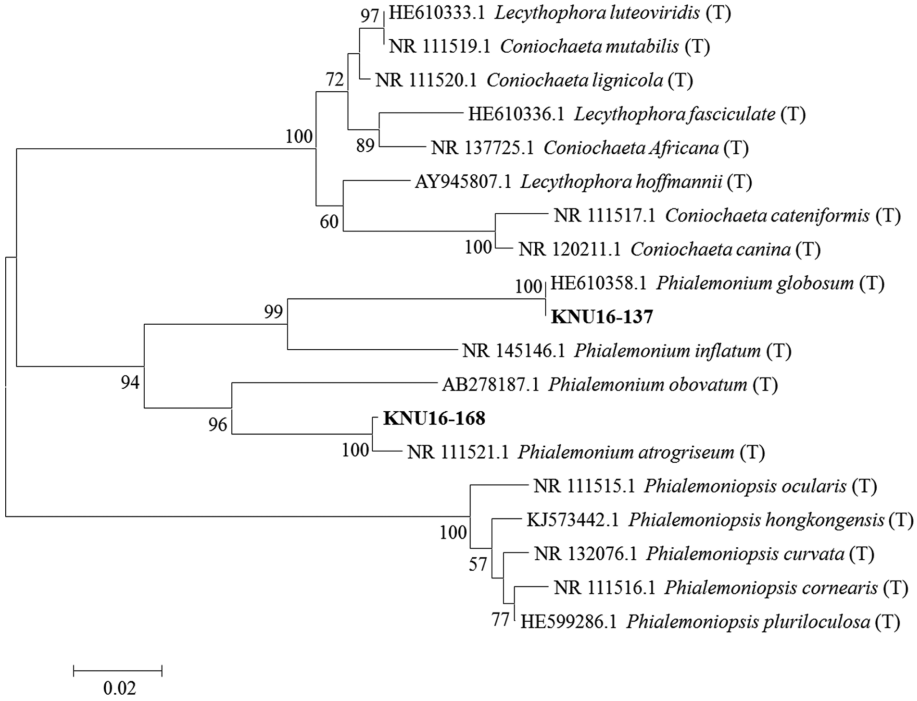

Fig. 6. Neighbor joining phylogenetic analysis of *Phialemonium globosum* KNU16-137 and *P. atrogriseum* KNU16-168 using the partial 18S-ITS1-5.8S-ITS2-28S rDNA region sequence obtained from field soil in Korea. Numerical values (> 50) on branches represent the bootstrap values shown as the percentage of bootstrap replication from a 1,000-replicate analysis. 'T' indicates the type strain of the isolates.

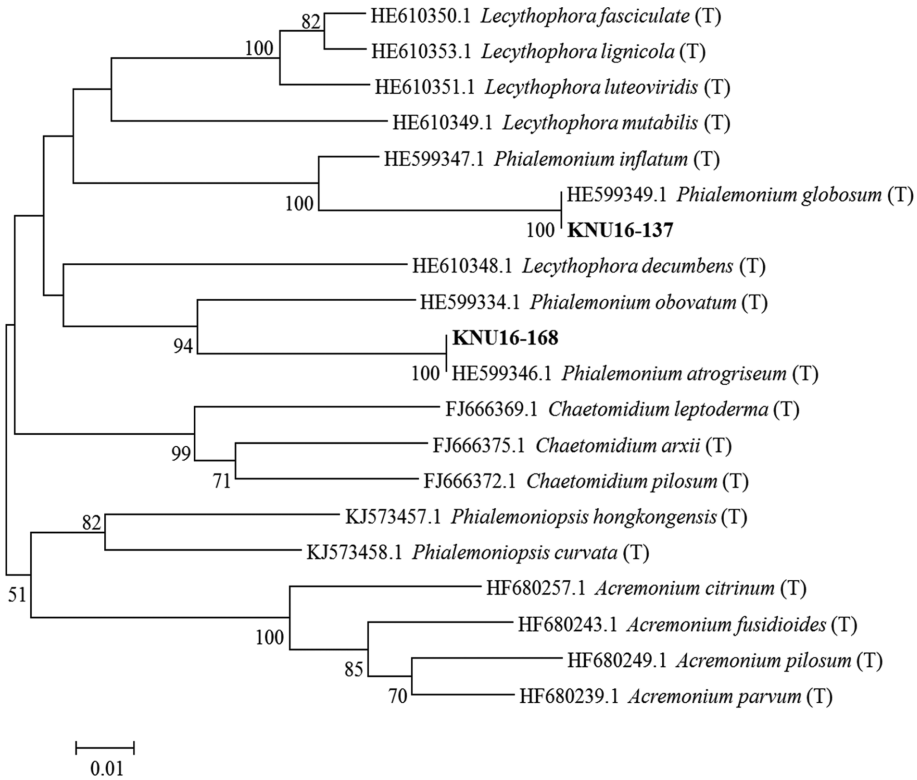

Fig. 7. Neighbor joining phylogenetic analysis of *Phialemonium globosum* KNU16-137 and *P. atrogriseum* KNU16-168 using the β-tubulin gene sequence obtained from field soil in Korea. Numerical values (> 50) on branches represent the bootstrap values shown as the percentage of bootstrap replication from a 1,000-replicate analysis. 'T' indicates the type strain of the isolates.

that the isolate KNU16-31 was most closely related to *C. acropullum* (HM365292.1) with 100% sequence identity (Fig. 5). In addition, ITS sequence analysis of the isolate KNU16-137 was well matched to *P. globosum* (HE610358.1) and formed a monophyletic group with a bootstrap value of 100% (Fig. 6). Type strains were used as references for the construction of a phylogenetic tree using GenBank. β-Tubulin gene sequence analysis suggested that the isolate KNU16-137 was most closely related to *P. globosum* (HE599349.1) (Fig. 5) with 100% sequence identity (Fig. 7). Therefore, molecular phylogeny strongly suggests that the isolate KNU16-137 belongs to the species *P. globosum*. Finally, the ITS sequence analysis of KNU16-168 was to construct evolutionary relationship analysis with the type strains of the species from GenBank, and had 100% similarity with the isolate *P. atrogriseum* (Fig. 6). β-Tubulin gene sequence analysis also revealed 100% sequence identify between KNU16-168 and *P. atrogriseum* (Fig. 7).

DISCUSSION

During a survey of the fungal diversity in soils from various places in Korea, several fungal isolates were collected and identified based on morphological and molecular analysis. Sequence-based identification of fungal isolates is often considered to be the most reliable and accurate identification method. The isolate KNU16-31 acquired from field soil in Gyeongnam, Korea was assumed to be a member of the *Chaetomium* genus based on the shape and size of its conidia, conidiophores, aleuroconidia, and hyphae. KNU16-31 largely matches the morphological description of previously identified *C. acropullum* strains [5] with slight differences in the size of conidia, aleuroconidia, hyphae, and arthroconidia. The basic identifying characteristics of *C. acropullum* are segmentation of hyphae, a solitary nature, hyaline, single-celled, and a short to long cylindrical shape with truncate ends [5]. These morphological features were also observed in KNU16-31. Sporulation, as described for *C. aureum* in Kubatova (2006) [2], was not found in the isolate KNU16-31. In addition, phylogenetic analysis revealed that KNU16-31 was most closely related to *C. acropullum*. Therefore, colony morphology and molecular phylogeny confirm that KNU16-31 belongs to the species *C. acropullum*. This isolate has not officially been reported previously; thus, we report this isolate as newly identified in Korea. Based on the above described taxonomical properties, isolate KNU16-137 derived from crop field soil was classified as an isolate of the species *P. globosum*. This newly identified isolate fits the morphological description as detailed by Perdomo *et al.* [15]. Similar results were obtained from our phylogenetic analysis of the ITS gene of all the type strains used (Fig. 6). According to our analysis, *P. globosum* is monophyletic genera of the Cephalothecaceae family. This isolate is newly discovered from crop field soil in Korea. Our final isolate, KNU16-168 (*P. atrogriseum*), is a synonymy of *Acremonium atrogriseum* [15]. This isolate matches the colony diameter description made by Perdomo *et al.* [15]. In addition, KNU16-168 (*P. atrogriseum*) was found to be monophyletic by phylogenetic analysis. This isolate was not previously reported in Korea. Morphology of the isolates KNU16-31, KNU16-137, and KNU16-168 were compared with their respective reference species in Tables 3, 4, and 5, respectively. ITS and β-tubulin gene sequence analysis strongly suggest that the isolates KNU16-31, KNU16-137, and KNU16-168 belong to the C. *acropullum*, *P. globosum* and *P. atrogriseum* species, respectively (Fig. 4–7).

In conclusion, all the newly discovered fungal isolates from crop field soil in Korea have different morphological and molecular characteristics. Each fungal isolate has its own biotechnological importance; therefore, these fungal isolates should be further studied in the future. Moreover, further studies on the identification of several new fungal species within the Ascomycota phylum are required to

Table 3. Morphological comparison of the newly identified *Chaetomium acropullum* isolate with previously reported *C. acropullum*

Characteristic		Study isolate *C. acropullum*	*C. acropullum*[a]
Colony	Diameter (mm)	PDA 50–55, MEA 45–50, YESA 55–56, CYA 45–50, OA 55–60	NA
Aleuroconidia	Size (μm)	9–11 × 5.3–6.9	10–12 × 5.5–7
	Shape	One-celled, pyriforme or clavate	One-celled, pyriforme or clavate, borne singly at the tips of hyphae
Conidiophores	Size (μm)	2.3–3.3	2.5–3.5
	Shape	Erect or sometimes branched irregularly	Erect or sometimes branched irregularly
Arthroconidia	Structure	Formed by segmentation by hyphae, one-celled	Formed by segmentation by hyphae, solitary, hyaline, one -celled
	Size (μm)	11–28 × 2.4–3.3	12–30 × 2.5–3.5
	Shape	Short to long cylindrical with truncate ends	Short to long cylindrical with truncate ends
Chlamydospores	Structure	Intercalary and hyaline	Intercalary and hyaline

PDA, potato dextrose agar; MEA, malt extract agar; YESA, yeast extract sucrose agar; CYA, Czapek yeast extract agar; OA, oatmeal agar; NA, not available.

[a]Source of original description [16].

Table 4. Morphological comparison of the newly identified *Phialemonium globosum* isolate with previously reported *P. globosum*

Characteristics		Study isolate *P. globosum*	*P. globosum*[a]
Colony	Diameter (mm)	PDA 25–30, MEA 25–30, YESA 25–30, CYA 30–35, OA 35–40	NA
	Color	Creamy white and light yellow on PDA	Velvety to powdery on PDA
Conidia	Size (μm)	3–4 × 4.6–4.9	3–5 × 4.8–5
	Shape	Globose to sub-globose	Globose to sub-globose
Conidiophores	Size (μm)	3–5 × 4.8–5	3–5 × 4.8–5
	Shape	Poorly developed	Poorly developed
Adelophialides	Structure	Cylindrical, one-celled	Cylindrical, one-celled
Phialides	Shape	1–2, Projected directly from aerial hyphae	1–2, Projected directly from aerial hyphae

PDA, potato dextrose agar; MEA, malt extract agar; YESA, yeast extract sucrose agar; CYA, Czapek yeast extract agar; OA, oatmeal agar; NA, not available.
[a]Source of original description [15].

Table 5. Morphological comparison of the newly identified *Phialemonium atrogriseum* isolate with previously reported *P. atrogriseum*

Characteristics		Study isolate *P. atrogriseum*	*P. atrogriseum*[a]
Colony	Diameter (mm)	PDA 25–30, MEA 25–30, YESA 25–30, CYA 30–35, OA 35–40	NA
	Color	White in front and light yellow in back side in MEA	Velvety dark in MEA
Conidia	Size (μm)	3–4 × 2–3	3.5–4.8 × 1.8–2.1
	Shape	Ellipsoidal to obvoid	Ellipsoidal
Conidiophores	Size (μm)	2.2–2.3 × 1.8–2.1	NA
	Shape	Branched irregularly	Absent
Phialides	Structure	Lateral phialides were arised directly from aerial hyphae consisting of whorls of 2–4	Inflated basal part and long tapering neck
	Shape	Flask shaped	Flask shaped
	Size (μm)	1.1–1.4 width	1.0–1.2 width

PDA, potato dextrose agar; MEA, malt extract agar; YESA, yeast extract sucrose agar; CYA, Czapek yeast extract agar; OA, oatmeal agar; NA, not available.
[a]Source of original description [15].

enhance our knowledge of the indigenous fungal species in Korea.

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