

A New Species of *Thelonectria* and a New Record of *Cephalotrichum hinnuleum* from Gunwi and Ulleungdo in Korea

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ABSTRACT

Three fungal strains belonging to the class Sordariomycetes were isolated from soils collected from Gyeongsangbuk-do in Korea. They were identified as *Cephalotrichum hinnuleum* (UD CT 1-3-3 and KNU-19GWF1) and *Thelonectria chlamydospora* sp. nov. (UD ST 1-2-1). *T. chlamydospora* sp. nov. was morphologically identical to *T. truncata*, but its specific macroconidial dimensions, lower number of septations, and chlamydospore diameter render it distinct from the strains of the genus *Thelonectria*. The strains UD CT 1-3-3 and KNU-19GWF1 were developed flat, velvety to felty, and golden gray to brown-gray after 14 days of incubation at 25 °C on PDA. These strains were produced polyblastic conidiogenous cells and conidia were pale brown to brown, smooth, thin-walled, subglobose to ellipsoidal, arranged in chains, and the diameters of 6.7–9.0 × 3.7–5.1 μm. The strains were also confirmed by using the multi-locus genes using internal transcribed spacer (ITS) regions, partial large subunit (LSU), translation elongation factor 1α (*TEF1-α*), β-tubulin (*TUB2*), and actin (*ACT*) genes. This is the discovery of *T. chlamydospora* sp. nov. and *Cephalotrichum hinnuleum*, a new record from Korea.

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1. Introduction

The phylum Ascomycota is comprised of extremely diverse fungal classes that play critical roles in the environment. It consists of 1,331 genera under 105 families, 32 orders, and six subclasses and class Sordariomycetes is the second largest [1]. Morphologically, the heterogeneous group of fungi comprising saprobic and plant pathogenic species are classified under the family Microascaceae [2]. The formation of dry-spored, indeterminate synnema, and enteroblastic percurrent conidiogenesis are general characteristics of the genus *Cephalotrichum*, which belongs to the family Microascaceae, containing powdery conidia chains resembling a ‘bottle brush’ or ‘feather’, and comprised of their asexual states, but their teleomorph state is still unknown [3]. However, for many years, the synonymy of *Cephalotrichum* and *Doratomyces* has been a subject of debate. *Doratomyces* was accepted as a separate genus, but *Doratomyces* is a synonym for *Cephalotrichum* [4]. The genus *Thelonectria* is widespread and omnipresent fungi recently established hosts on woody within the family Nectriaceae; it was previously placed in the genus

Neonectria [5]. The genus *Thelonectria* is characterized by inconspicuous stromata; superficial, globose, subglobose, pyriform to elongated and smooth, or warty perithecia; a peritheal wall of 2 or 3 layers with prominent papilla; and smooth, hyaline, 1-septate ascospores. Members of this genus often cause small cancers and are found mostly on the bark of recently killed, dying, or diseased trees and rotting roots in tropical and subtropical regions [6]. Recently, the presence of the cryptic species *Thelonectria coronata* and *T. veuillotiana* was examined, and it was discovered from temperate, tropical and subtropical regions. And the cryptic species were described using the methods of phylogenetic recognition through genealogical agreement between multiple phylogenies [7]. Recently, *T. veuillotiana* was isolated from roots of a species of orchid (*Oreorchis patens*) from Korea [8]. Ulleungdo is one of a small group of volcanic islands situated off the eastern coast of the Korean peninsula. Ulleungdo inhabits approximately 600 species of vascular plants, of which 39 are endemic to the island [9]. The diversified fungal strains were recovered from agricultural soil in Ulleungdo such as *Penicillium raphiae* [10], and *Metarhizium guizhouense* and

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Mortierella oligospora were also isolated from Dokdo nearest from Ulleungdo [11].

The objective of the current study was to explore newly recorded fungal species from Gunwi and Ulleungdo based on cultural and morphological characteristics along with their molecular phylogeny. The three fungal strains were described and illustrated as a new record and one novel species from Korea.

2. Materials and methods

2.1. Sample collection

The soils were yellowish brown, contain plant debris and limited water efficiency nearby pine tree collected from Gunwi (36°11'45.9"N, 128°34'10.8"E) in 2018. The rhizospheric soils of root area of lantern (*Campanula takesimana*) and Ulleungdo stonecrop (*Sedum takesimense*) were collected from Ulleungdo (37°29'40.8"N, 130°49'39.4"E and 37°30'23.3"N, 130°49'5.9"E) of Gyeongsangbuk-do Province in 2018. Samples were stored in plastic bags at 4°C until analysis. Soil serial dilutions were performed until a concentration of 10⁻³ was achieved, and 100 µL of each sample was spread on potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates and incubated for 2–3 days at 25°C [12]. Then, the pure cultures were transferred to new PDA plates and incubated at 25°C for 5–7 days and cultured on a different media suitable for studying their cultural and morphological characteristics and conducting molecular analyses.

2.2. Morphological studies

Pure cultures were maintained to study the morphology of the three stains. The UD CT 1-3-3 and KNU-19GWF1 strains were grown on PDA and oatmeal agar (OA; Difco) for 14 days at 25°C [13]. According to Nirenberg [14], synthetic nutrient agar (SNA: Agar-14.0 g/L, KH₂PO₄-1.0 g/L, KNO₃-1.0 g/L, MgSO₄·7H₂O-0.25 g/L, KCl-0.5 g/L, Glucose-0.2 g/L, Sucrose-0.2 g/L) and PDA was used to study the strain UD ST 1-2-1 maintaining at 25°C for 14 days [15]. After incubation, the diameters of the colonies were measured, photographs were taken, and colony characteristics for each strain were recorded. A light microscope (BX-50; Olympus, Tokyo, Japan) was used to observe morphological characteristics.

2.3. Genomic DNA extraction, PCR, and sequencing

Genomic DNA was extracted from mycelia using the HiGene Genomic DNA prep kit (BIOFACT, Daejeon, Korea) according to the manufacturer's

instructions. For the strains UD CT 1-3-3 and KNU-19GWF1, the following target genes were amplified using internal transcribed spacer regions of rDNA including the 5.8S region (ITS1F or ITS5/ITS4) [16,17] and the partial large subunit of rDNA (LROR/LR5) [18] as well as translation elongation factor 1 α (*TEF1- α*) and β -tubulin (*TUB2*) genes using the primer pairs EF1-983F/EF1-2218R [19] and BT2a/BT2b [20], respectively. The ITS regions of rDNA including the 5.8S region and partial large subunit of rDNA (LSU) was also used to amplify the strain UD ST 1-2-1 along with *TEF1- α* (EF1-728F/EF1-986R), and actin (*Tact1/Tact2*) genes for molecular identification [6]. The amplified PCR products were then purified with EXOSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced (Macrogen, Daejeon, Korea).

2.4. Phylogenetic analysis

Strain sequences were compared with the additional sequences retrieved from the GenBank database of the National Center for Biotechnology Information (NCBI). The alignments of each gene were performed, and the sequences were combined to reveal the position of the strains on the phylogenetic tree using MEGA7.0 program. Evolutionary distance matrices were generated using Kimura's neighbor-joining (NJ) algorithm model [21]. To determine the exact taxonomic position of each strain, maximum likelihood (ML) and maximum parsimony (MP) trees were also constructed. In the neighbor-joining phylogenetic tree, nodes with filled circles represent maximum likelihood and maximum parsimony, and open circles indicate nodes corresponding to the maximum parsimony or maximum-likelihood algorithm. Phylogenetic analyses were performed using the MEGA 7.0 program with bootstrap values based on 1,000 replicates [22].

3. Results

3.1. Taxonomy of UD CT 1-3-3

The strains UD CT 1-3-3 (NIBRFGC000505742) and KNU-19GWF1 (NREFFGC000000240) were studied and found same as well as clustered together with respect to molecular phylogeny. Thus, UD CT 1-3-3 and KNU-19GWF1 strains were identified as *Cephalotrichum hinnuleum*. Therefore, the cultural and morphological characteristics of the UD CT 1-3-3 strain were described only in this study.

Cephalotrichum hinnuleum Sand.-Den., Guarro & Gené, *Studies in Mycology* 83: 209 (2016) (Table 1, Figure 1)

Cultural characteristics: The colonies of the strains UD CT 1-3-3 and KNU-19GWF1 were flat,

Table 1. Morphological characteristics of the NIBRFGC000505742 strain in reference to *Cephalotrichum hinnuleum*.

Characteristics	Strain NIBRFGC000505742 ^a	<i>Cephalotrichum hinnuleum</i> ^b
Colony	Colonies were characterized by flat, velvety to felty, initially golden gray later brown-gray with regular white margin on PDA; reverse regular white margin to brown-gray at edge and becoming pale to dark gray. The colonies displayed flat, velvety with regular margin, floccose; reverse dark brown to olivaceous in color on OA. OA: 25–30; PDA: 22–27 mm in 14 days at 25 °C.	Colonies on PDA were velvety to felty, golden gray to brown-gray with regular margin; reverse at first golden gray to brown-gray, turning pale brown to brown with age by the production of a non-diffusible pigment. Colonies on OA and PCA were flat, velvety to floccose with a regular margin, obverse and reverse brown-gray to olivaceous. OA and PCA: 32–38 mm, PDA: 29–30 mm in 14 days at 25 °C.
Synnemata	Compact stipes, dark brown to black, 700–1400 µm high, 10–30 µm wide, light gray to gray heads of conidia, clavate to ellipsoidal; setae absent.	Stipes compact, dark brown to black, 800–1600 µm high, 10–30 µm wide, conidial heads gray, clavate to ellipsoidal; setae absent.
Conidia	Sub-globose to ellipsoidal, shorten base and blunted apex, light brown to brown, smooth and thin-walled, arranged in chains. 6.7–9.0 × 3.7–5.1 µm.	Sub-globose to ellipsoidal, with truncate base and pointed apex, pale brown, smooth- and thin-walled, arranged in long chains. 6–7.5 × 2.5–4 µm.

^aFungal strain studied in this paper. ^bSources of the descriptions [13].

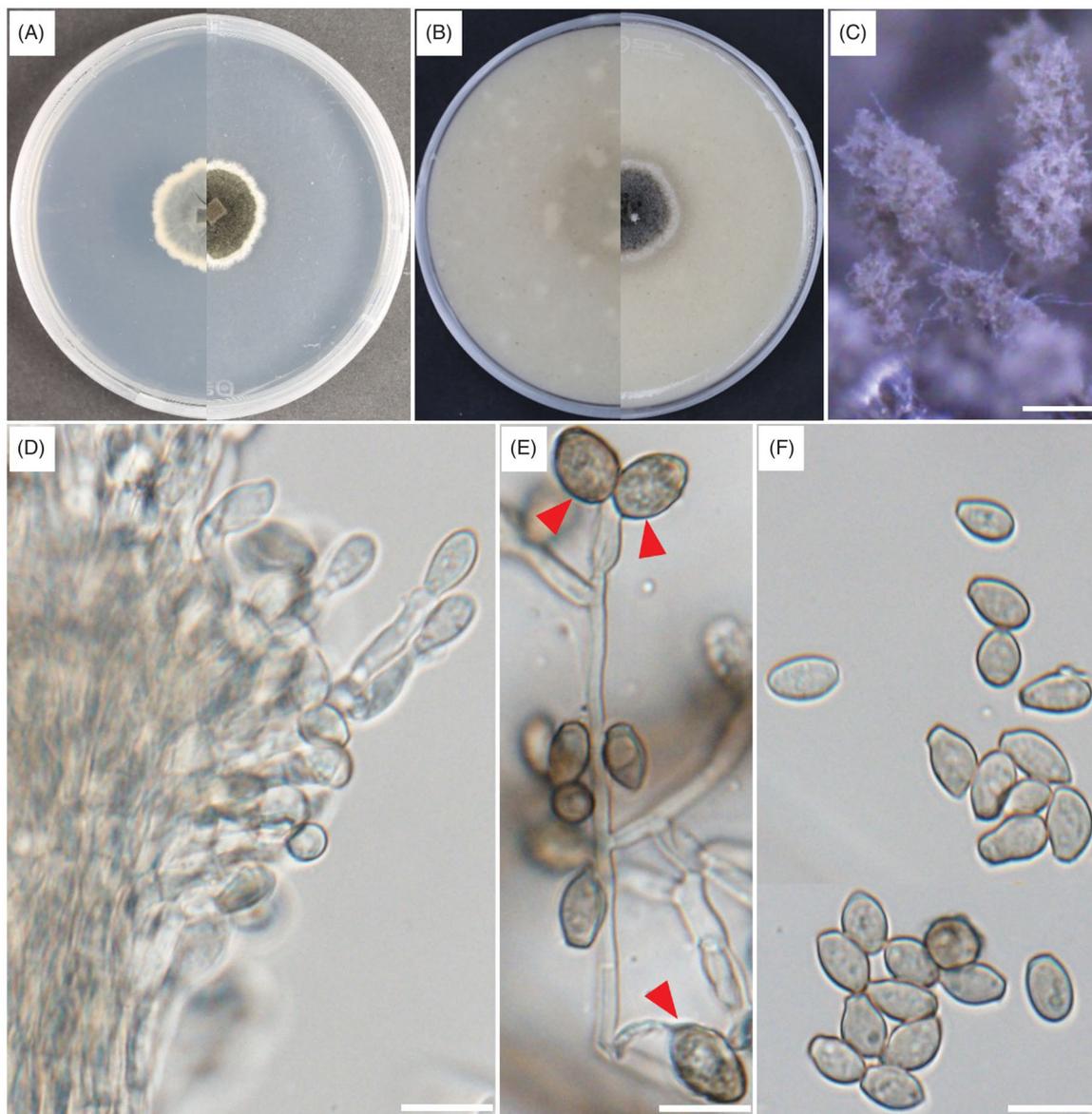


Figure 1. Cultural and morphological characteristics of UD CT 1-3-3. (A) Colonies on potato dextrose agar (PDA); (B) Colonies on oatmeal agar (OA) for 14 days of incubation at 25 °C; (C) Synnemata; (D) Apical portion of a synnema; (E) Polyblastic conidiogenous cells; (F) Conidia. Arrows indicate conidiogenous cells. Scale bars: C = 200 µm; D–F = 10 µm.

velvety to felty, and golden gray to brown-gray on PDA after 14 days of incubation at 25 °C. The white margins of the colonies were regular and reached 22.0–27.0 mm. The reverse side showed a regular

white margin and a brown-gray edge that became pale to dark gray in color (Figure 1(A)). The colonies grown on OA media were flat, velvety, and floccose with regular margins after 14 days of

incubation at 25 °C. The reverse side was dark brown to olivaceous in color, and the colonies grew to 25.0–30.0 mm (Figure 1(B)). The cultural characteristics of both strains UD CT 1-3-3 and KNU-19GWF1 were studied on PDA and OA media and found the same characteristics.

Morphological characteristics: Morphological characteristics were observed using the strain UD CT 1-3-3 grown on PDA for 14 days at 25 °C. The strain UD CT 1-3-3 produced numerous dark-brown to black-colored synnemata, compact stipes, and light gray to gray conidial heads. They were clavate to ellipsoidal, setae-absent, 700–1,400 µm in height, and 10–28 µm wide (Figure 1(C)). The hyphae were septate, hyaline, light brown, smooth, thin-walled, and 2–3 µm wide. The conidiophores were branched, septate, hyaline, pale brown, smooth, and thin-walled and usually aggregated with the dense synnemata (Figure 1(D)). This strain also produced polyblastic conidiogenous cells (Figure 1(E)). The conidia were pale brown to brown, smooth,

thin-walled, and subglobose to ellipsoidal with shortened bases and blunted apices and arranged in chains with diameters of 6.7–9.0 × 3.7–5.1 µm (Figure 1(F)) (Table 1).

3.2. Phylogenetic analysis of UD CT 1-3-3

Four multi-genes were amplified to identify the UD CT 1-3-3 and KNU-19GWF1 strains. The sequences from ITS regions (569 bp, 585 bp), LSU (858 bp, 820 bp), *TEF1-α* (940 bp, 893 bp), and *TUB2* (541 bp, 518 bp) were obtained from both strains. The obtained sequences were submitted in NCBI GenBank and accession numbers were for ITS (LC509451, LC519564), LSU (LC509453, LC519565), *TEF1-α* (LC509454, LC519562), *TUB2* (LC519561, LC519563) from UD CT 1-3-3 and KNU-19GWF1 strains, accordingly (Table 2). BLAST search results revealed that the UD CT 1-3-3 strain showed the highest similarities (100%) with the previously identified *C. hinnuleum* CBS 289.66^T (previously known

Table 2. List of species used in this study and their GenBank accession numbers for phylogenetic analysis.

Species	Strain	GenBank Accession Numbers				
		ITS	LSU	<i>TEF1-α</i>	<i>TUB2</i>	<i>ACT</i>
<i>Cephalotrichum asperulum</i>	CBS 582.71 ^T	NR146262	MH872033	KX924043	LN851114	–
<i>C. cylindricum</i>	CBS 448.51	MH856932	MH868459	LN851065	LN851118	–
<i>C. dendrocephalum</i>	CBS 528.85 ^T	NR146265	NG059041	LN851067	LN851120	–
<i>C. hinnuleum</i>	CBS 289.66 ^T	LN850985	LN851032	LN851085	LN851139	–
<i>C. hinnuleum</i>	NIBRFGC000505742	LC509451	LC509453	LC509454	LC519561	–
<i>C. hinnuleum</i>	NREFFGC000000240	LC519564	LC519565	LC519562	LC519563	–
<i>C. inflatum</i>	HHAUF160201	MF002125	MF041796	MF039904	MF511702	–
<i>C. lignatile</i>	CBS 209.63 ^T	NR154842	MH869874	KY249349	KY249309	–
<i>C. nanum</i>	CBS 191.61 ^T	NR146266	MH869582	LN851070	LN851123	–
<i>C. purpureofuscum</i>	CBS 174.68	KY249281	MH870812	KY249361	KY249319	–
<i>C. stemonitis</i>	CBS 103.19 ^T	LN850951	MH866188	LN850953	LN850954	–
<i>C. stemonitis</i>	CBS 180.35	LN850972	LN851019	LN851073	LN851126	–
<i>C. stemonitis</i>	UAMH 1532	LN850973	LN851020	LN851074	LN851127	–
<i>C. telluricum</i>	CBS 336.32 ^T	NR154845	MH866802	KY249367	KY249325	–
<i>C. tenuissimum</i>	CBS 127792 ^T	NR154844	MH876141	KY249366	KY249324	–
<i>C. verrucisporum</i>	HHAUF160178	MH876141	MF041793	MF039900	MF511699	–
<i>Wardomyces inflatus</i>	CBS 367.62 ^T	NR146270	MH869775	LN851099	LN851153	–
<i>Nectria cinnabarina</i>	A.R. 4477	HM484548	HM484562	HM484527	–	HM484503
<i>Thelonectria acrotyla</i>	CBS 123766	JQ403329	JQ403368	JQ394751	–	JQ365047
<i>T. amamiensis</i>	MAFF239820	JQ403338	JQ403376	KJ022348	–	JQ365055
<i>T. brayfordii</i>	CBS 118612	KC153719	KC121445	KC153848	–	KC121381
<i>T. chlamydozpora</i>	NIBRFGC000500679^T	LC509450	LC509452	LC519559	–	LC519560
<i>T. cidaria</i>	CBS 132324 ^T	KJ021972	KJ022027	KJ022351	–	KJ022239
<i>T. coronata</i>	IMI 325844	JQ403315	JQ403355	JQ394741	–	JQ365035
<i>T. diademata</i>	CBS 132331 ^T	JQ403308	JQ403348	JQ394736	–	JQ365029
<i>T. discophora</i>	MAFF 241576	KC153774	KC121500	KC153903	–	KC121436
<i>T. gongyloides</i>	IMI 336160	JQ403336	JQ403374	JQ394756	–	JQ365053
<i>T. pinea</i>	ICMP 5287	KC153759	KC121485	KC153888	–	KC121421
<i>T. rubi</i>	CBS 11312	KC153718	KC121444	KC153847	–	KC121380
<i>T. sinensis</i>	HMAS 183186	FJ560441	FJ560436	JN131813	–	MF669045
<i>T. stemmata</i>	CBS 112468	JQ403312	JQ403352	JQ394739	–	JQ365033
<i>T. torulosa</i>	CBS 132340 ^T	JQ403310	JQ403350	JQ394737	–	JQ365031
<i>T. truncata</i>	CBS 132329 ^T	JQ403319	JQ403359	JQ394745	–	JQ365039
<i>T. truncata</i>	MAFF241521	JQ403339	JQ403377	JQ394757	–	JQ365056
<i>T. veuillotiana</i>	CBS124352	JQ403332	JQ403371	JQ394753	–	JQ365049
<i>T. westlandica</i>	CBS 112.464	HM484559	HM364321	HM364355	–	HM352887

CBS: Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; NIBR: National Institute of Biological Resources, Incheon, South Korea; HHAUF: Herbarium of Henan Agricultural University: Fungi, Henan, China; UAMH: University of Alberta Microfungus Collection and Herbarium, Canada; A.R.: Amy Y. Rossman, USDA-ARS MD USA; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tokyo, Japan; IMI: International Mycological Institute, Bakeham Lane, UK; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; HMAS, Herbarium Mycologicum Academiae Sinicae, Beijing, China.

ITS: Internal transcribed spacer regions of the rDNA; LSU: partial large subunit of 28S rDNA; *TEF1-α*: partial translation elongation factor gene; *TUB2*: partial beta-tubulin gene, *ACT*: actin genes.

The strains identified in this study are indicated in bold.

as *Doratomyces stemonitis*) based on ITS regions. LSU and *TEF1- α* showed maximal similarities (99.64% and 99.78%) with the *C. hinnuleum* CBS 289.66^T, respectively. And *TUB2* also showed maximum similarities (99.82%) with the *C. hinnuleum* CBS 289.66^T.

The KNU-19GWF1 strain also showed maximum similarities with ITS regions (100%), LSU (100%), *TEF1- α* (99.78%), and *TUB2* (99.81%) with the previously identified *C. hinnuleum* CBS 289.66^T strain. The deposited sequences of existing *Cephalotrichum* species in GenBank were used to construct the taxonomic position of KNU-19GWF1 in the phylogenetic tree (Table 2). The neighbor-joining phylogenetic tree revealed through the alignment of their ITS regions including 5.8S of the nuclear ribosomal DNA region, LSU, *TUB2*, and *TEF1- α* gene sequences that the position of UD CT 1-3-3 was closely clustered with the *C. hinnuleum* CBS 289.66^T (Figure 2) showing strong bootstrap values of 100%. These results indicate that UD CT 1-3-3 and KNU-19GWF1 strains are, in fact, *Cephalotrichum hinnuleum*. Neighbor-joining, maximum-likelihood, and maximum parsimony trees were constructed to determine the precise taxonomic location of the strains and are indicated with nodes in neighbor-

joining phylogenetic tree. Filled circles indicate that the corresponding nodes were recovered in trees generated with the maximum-likelihood and maximum parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered from maximum-likelihood or maximum parsimony algorithms. The exact taxonomic position of the strain was determined through the analysis of the maximum parsimony (tree length = 434, consistency index = 0.64, retention index = 0.82, and composite index = 0.63) (Figure 2).

3.3. Taxonomy of UD ST 1-2-1

The strain UD ST 1-2-1 showed different morphological characteristics when compared to other allied species. Therefore, it was defined as a new species. The description includes the cultural and asexual morph features, the sexual morph features were undetermined from the cultural media (PDA and SNA).

Thelonectria chlamydospora K. Das, S.Y. Lee and H.Y. Jung, sp. nov. (Figure 3)

MycoBank: MB834497

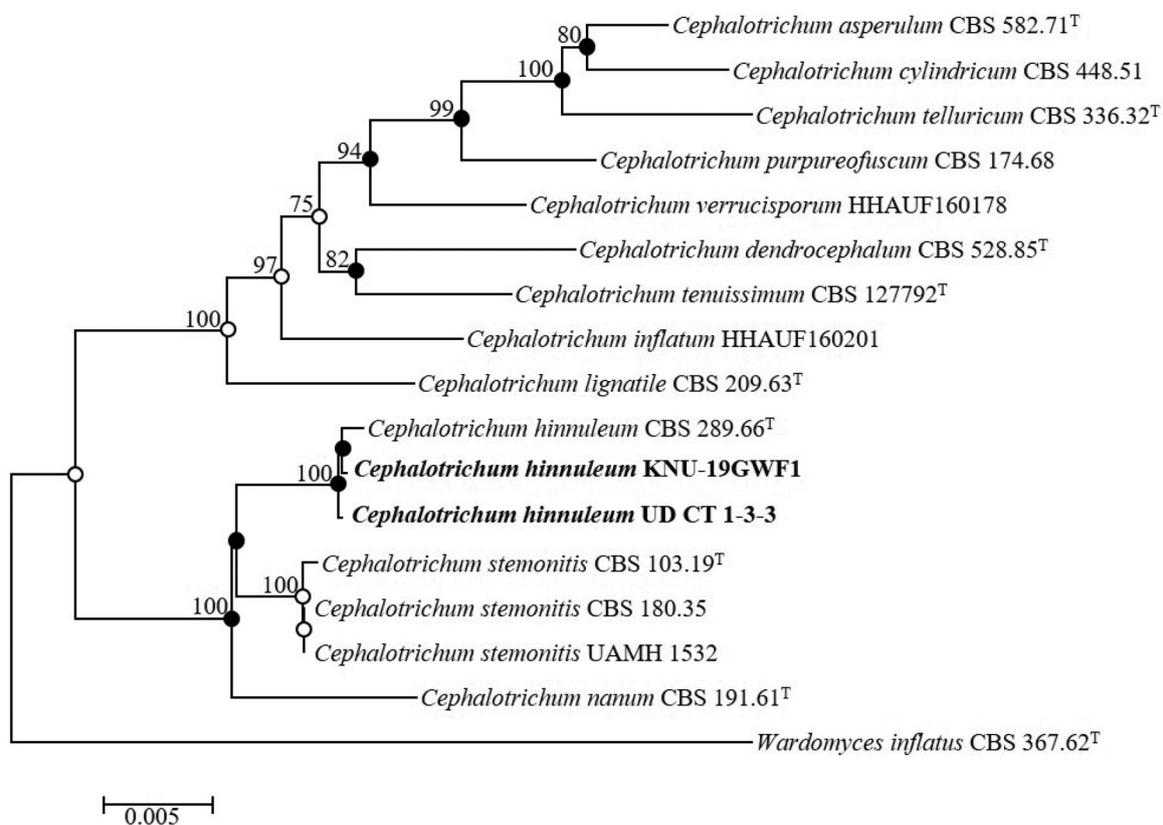


Figure 2. Neighbor-joining phylogenetic tree of UD CT 1-3-3 and KNU-19GWF1 based on the combined sequences (ITS + LSU + *TUB2* + *TEF1- α*), showing the relationships between *Cephalotrichum hinnuleum* and the closest *Cephalotrichum* spp. *Wardomyces inflatus* CBS 367.62^T was used as an outgroup. The numbers above the branches represent the bootstrap values (>70%) obtained for 1,000 replicates. The isolated strains of this study are indicated in bold. Bar, 0.005 substitutions per nucleotide position.

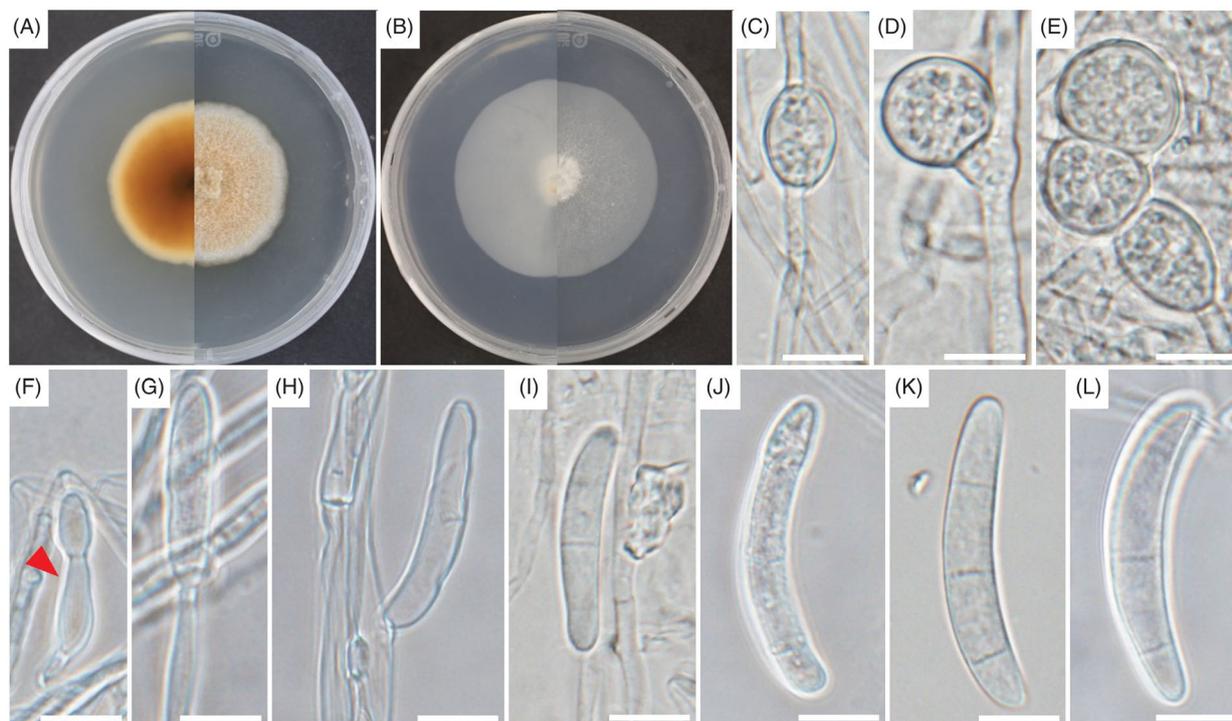


Figure 3. Cultural and morphological characteristics of UD ST 1-2-1. (A) Colonies on potato dextrose agar (PDA); (B) Colonies on synthetic nutrient agar (SNA) for 14 days of incubation at 25 °C; (C–E) Chlamydospores; (F) Phialidic cell; (G) Conidiophore; (H) Conidiogenous cell; (I–L) Macroconidia. Arrows indicate phialidic cells. Scale bars: C–L = 10 μ m.

Etymology: The specific epithet refers to the Greek term *chlamydos*-, cloak, and the Latin term *spora*, spore.

Typus: Ulleungdo, Gyeongsangbuk-do (37°30'23.3"N, 130°49'5.9"E), Korea, isolated from Ulleungdo stonecrop (*Sedum takesimense*) rhizospheric soil. The stock culture (NIBRFGC000500679 = KCTC 56672) was deposited in the National Institute of Biological Resources (NIBR) and Korean Collection for Type Cultures (KCTC), metabolically inactive culture.

Habitat and distribution: Rhizospheric soil regions of Ulleungdo stonecrop (*Sedum takesimense*) in South Korea.

Cultural characteristics: Colonies on PDA formed floccose aerial mycelium that were white to saffron; the colony was saffron to white color in reverse side and reached 42.0–43.0 mm after 14 days of incubation at 25 °C with even margin expansion (Figure 3(A)). Colonies on SNA media were slightly floccose and sparse white to light brown. The colony reverse side was white and grew to 60.0–66.0 mm in diameter after 14 days of incubation at 25 °C (Figure 3(B)).

Morphological characteristics: Sexual morph: undetermined. Asexual morph: micromorphological characteristics were studied by culturing on SNA media maintaining at 25 °C for 14 days. Mycelia were septate branched, hyphae hyaline, light brown, and smooth with a diameter between 1.9–3.9 μ m. Chlamydospores observed in culture were hyaline

and globose to sub-globose with the diameters between 10.8–21.8 \times 5.6–16.7 μ m (Figure 3(C–E)). Phialides were borne apically with clusters of cells in branch irregularly, alone, or developed from hyphae directly; they were curved, cylindrical, or slightly swollen with diameters between 12.8–14.8 \times 3.0–4.3 μ m (Figure 3(F)). The produced conidiophores were branched or unbranched, hyaline, light brown, and gave rise to conidiogenous cells (Figure 3(G)). The conidiogenous cells were septate with diameters of 10.8–30.7 \times 2.4–4.9 μ m (average = 18.1 \times 3.7 μ m) (Figure 3(H)). The UD ST 1-2-1 strain generated a lower quantity of macroconidia on artificial media. On SNA, Macroconidia were hyaline, produced slimy droplets in aerial mycelium or on the agar surface. Macroconidia were cylindrical to slightly fusiform, thick-walled, curved, and rounded on both ends; 1–3-septate formed diameters of 28.6–40.4 \times 4.8–6.8 μ m (average = 37.6 \times 5.9 μ m), one septate (28.6–40.3 \times 4.8–5.7 μ m), and three septate (37.2–40.4 \times 5.5–6.8 μ m) (Figure 3(I–L)). Microconidia were not observed in culture.

Note: The UD ST 1-2-1 strain produced conidiogenous cells with nearly similar diameters (10.8–30.7 \times 2.4–4.9 μ m) with the nearest known species of *T. truncata* (14.0–30.0 \times 2.5–4.5 μ m) [15]; whereas, conidiogenous cells diameter were not mentioned for another strain of *T. truncata* (Table 3) [7]. The UD ST 1-2-1 strain produced smaller, curved, cylindrical, or slightly swollen phialides (12.8–14.8 \times 3.0–4.3 μ m), and *T. truncata* produced

Table 3. Morphological characteristics of *Theλονectria chlamydospora* sp. nov. and a comparison with the closest species from the genus *Theλονectria*.

Sl. No.	Strains Name	Phialides (μm)	Chlamydospores (μm)	Macroconidia (μm)	References
1	<i>T. chlamydospora</i> (NIBRFGC000500679)	12.8–14.8 \times 3.0–4.3	10.8–21.8 \times 5.6–16.7	One septate: 28.6–40.3 \times 4.8–5.7; three septate: 37.2–40.4 \times 5.5–6.8.	This study
2	<i>T. truncata</i> (CBS 132329 ^T)	16.0–20.6 \times 3.5–4.4	not produced	Three-septate: 46.9–58.9 \times 4.9–5.8; four-septate: 55.0–67.5 \times 5.0–6.0; five-septate: 65.4–77.0 \times 5.2–6.3.	[7]
3	<i>T. gongyloides</i> (G.J.S 04–171 ^T)	14.6–19.5 \times 3.5–4.4	not produced	Three-septate: 38.5–53 \times 5.0–6.2; four-septate: 49.0–64.7 \times 5.5–6.5; five-septate: 56.7–69.8 \times 5.8–6.8.	[7]
4	<i>T. veuillotiana</i> (A.R. 1751)	16.0–17.0 \times 3.8–4.3	produced	Three-septate: 40.3–53.4 \times 5.1–6.3; four-septate: 49.9–66.9 \times 5.5–6.8; five-septate: 63.2–69.8 \times 7.0–7.6.	[7]
5	<i>T. torulosa</i> (A.R. 4768A ^T)	15.3–19.7 \times 3.5–4.4	not produced	Three-septate: 51.7–62.7 \times 5.8–6.8; four-septate: 59.8–74.5 \times 5.7–6.7; five-septate: 73.4–86.3 \times 6.3–7.2.	[7]
6	<i>T. blackeriella</i> (CBS 142200 ^T)	8.16–13.39 \times 1.52–5.85	mean, 8.62 \times 4.69	One-septate: 23.36–25.34 \times 3.81–4.48; two-septate: 27.84–29.15 \times 4.11–4.56; three-septate: 27.84–29.15 \times 4.11–4.56; four-septate: 29.54–30.88 \times 4.50–5.19.	[32]
7	<i>T. guangdongensis</i> (HMAS 247233)	20.0–58.0 \times 2.0–4.0	not observed	2–5-septate, 48–70 \times 4.8–5.3.	[33]
8	<i>T. beijingensis</i> (HMAS 188566)	N/A	Absent	0-septate: 41–51 \times 3.2–5.4; one-septate: 32–46 \times 2.7–4; two-septate: 43–51 \times 3.2–4; three-septate: 41–54 \times 3.2–4.9.	[34]
9	<i>T. coronalis</i> (CBS 132337 ^T)	N/A	not produced	Mostly 5–7-septate.	[7]
10	<i>T. discophora</i> (A.R. 4499 ^T)	10.0–25.0 \times 3.0–6.0	chlamydospores rare	(3–)5–7(–9)-septate, 40.0–90.0 \times 4.0–8.0	[6]
11	<i>T. ianthina</i> (CBS 134023)	13.5–20.5 \times 3.0–4.0	not produced	Three-septate: 46–57 \times 5.5–7; four-septate: 55–63 \times 6.5–7.5; five-septate: 59.5–62.5 \times 6.5–8.	[35]
12	<i>T. diademata</i> (A.R. 4765 ^T)	not observed	not produced	not observed	[7]
13	<i>T. coronata</i> (IMI325241)	not observed	not produced	not observed	[7]
14	<i>T. cidaria</i> (G.J.S. 10–136 ^T)	not observed	not produced	not observed	[7]

N/A: not available in previous references.

phialides that were cylindrical or sometimes slightly swollen 16.0–20.6 \times 3.5–4.4 μm [7]. The UD ST 1-2-1 strain generated fewer, cylindrical to slightly fusiform, thick wall, and curved macroconidia that were rounded on both ends: 1–3-septate: 28.6–40.4 \times 4.8–6.8 μm , one septate 28.6–40.3 \times 4.8–5.7, and three septate 37.2–40.4 \times 5.5–6.8 μm (mean 37.6 \times 5.9 μm). In contrast, the closest species *T. truncata* produced macroconidia that were cylindrical to slightly fusiform with curved or rounded tips, 3–5(–6)-septate: three-septate 46.9–58.9 \times 4.9–5.8 μm (mean 53.3 \times 5.4 μm), four-septate 55–67.5 \times 5.0–6.0 μm (mean 61.2 \times 5.5 μm), and five-septate 65.4–77.0 \times 5.2–6.3 μm (mean 71.2 \times 5.8 μm) [7]. Moreover, another strain of *T. truncata* produced macroconidia that were cylindrical to slightly fusiform and curved with the rounded ends, 3–5(–6)-septate, 42.0–62.0 μm [15]. The UD ST 1-2-1 strain also generated numerous chlamydospores that were observed when cultured on SNA media; they were hyaline, globose to subglobose, and formed diameters of 10.8–21.8 \times 5.6–16.7 μm . On the other hand, the closest known type species of *T. truncata* does not produce chlamydospores on SNA media [7], similar to another strain of *T. truncata* [15]. Also, there was a difference in the number of

septate macroconidia with the closest species. The closest species produced 3–5(–6) septate macroconidia, but the UD ST 1-2-1 strain developed 1–3-septate macroconidia. A comparison between the nearest certain species of the genus showed a lower number of septations in macroconidia, smaller macroconidial size, and chlamydospores. Therefore, the morphology of the UD ST 1-2-1 strain is totally different from previously identified species of *Theλονectria*.

3.4. Phylogenetic analysis of UD ST 1-2-1

Sequences for 563 bp of the ITS region, 843 bp of LSU, 307 bp of *TEF1- α* , and 694 bp of *ACT* were obtained from the UD ST 1-2-1 strain. The obtained sequences from UD CT 1-3-3 was deposited in NCBI GenBank and accession numbers were LC509450, LC509452, LC519559, LC519560 from ITS, LSU, *TEF1- α* , and *ACT*, accordingly. ITS regions showed maximum similarities with the *Theλονectria truncata* CBS 132329^T (98.43%) and *T. truncata* 9386 (98.50%). LSU and *TEF1- α* showed maximum similarities with the strains *T. truncata* CBS 132329^T (99.15%) and *T. veuillotiana* G.J.S.09-407 (86.75%), respectively. And the *ACT* gene also

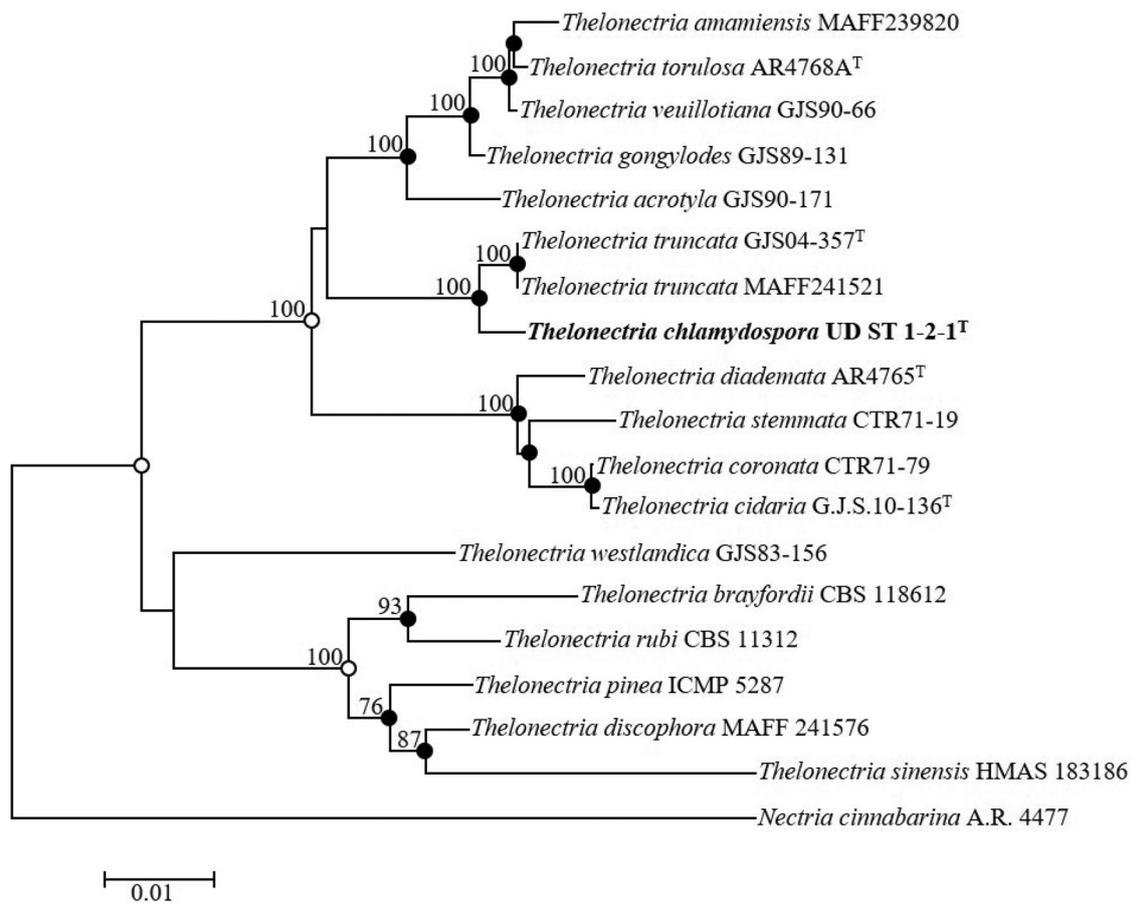


Figure 4. Neighbor-joining phylogenetic tree of UD ST 1-2-1 based on the combined sequences (*ACT+LSU+ITS+TEF1- α*), showing the relationships between *Thelonectria chlamydospora* sp. nov. and the closest *Thelonectria* spp. *Nectria cinnabarina* A.R. 4477 was used as an outgroup. The numbers above the branches represent the bootstrap values (>70%) obtained for 1,000 replicates. The isolated strain of this study is indicated in bold. Bar, 0.01 substitutions per nucleotide position.

showed maximum similarities with the *T. truncata* CBS 132329^T (99.16%). Existing *Thelonectria* species sequences retrieved from GenBank were used to construct the phylogenetic tree (Table 2). The phylogenetic tree showed that the position of UD ST 1-2-1 was distinctly clustered in a different clade with respect to the previously identified strains of *T. truncata* (Figure 4). Thus, the UD ST 1-2-1 strain is phylogenetically distinct from the other *Thelonectria* species. The combination of ITS regions, LSU, *TEF1- α* , and *ACT* gene sequences clarified the boundaries between the 18 strains of *Thelonectria*. The exact taxonomic position of the UD ST 1-2-1 strain was indicated by the node in the neighbor-joining phylogenetic tree along with the filled nodes in the maximum likelihood and maximum parsimony trees. The corresponding nodes were also recovered using the maximum likelihood or maximum parsimony algorithms, as indicated by the open circles. The combination of the sequences was used for the phylogenetic analysis based on maximum parsimony (tree length = 578, consistency index = 0.63, retention index = 0.80, and composite index = 0.58) to determine the taxonomic position of the strain UD ST 1-2-1 (Figure 4).

4. Discussion

In this study, the three strains were collected from rhizospheric soils regions in Gunwi and Ulleungdo, Korea: KNU-19GWF1, UD CT 1-3-3, and UD ST 1-2-1. The strains were identified as *Cephalotrichum hinnuleum* (UD CT 1-3-3 and KNU-19GWF1) and *Thelonectria chlamydospora* sp. nov. (UD ST 1-2-1).

From the previous studies, the most of the members of *Cephalotrichum* found on decaying plant materials, straw, dung, wood, and soil [4]. However, *C. microsporum* has been found on indoor substrates like wood or in man-made environments [23], and *C. purpureofuscum* has been found in indoor air [3,13]. *C. stemonitis* was identified in coyote and rat dung, the indoor air of a honeybee (*Apis mellifera*) overwintering facility, the cones of white spruce, sandy soil, decayed wood of white spruce, soil of elm woods, and agricultural soil [24]. Recently, there are 14 new species of *Cephalotrichum* were explained based on the isolation from soils in China [25]. Although *Cephalotrichum* species are not considered as human pathogens and also not known to produce mycotoxins, but *C. gorgonifer* was collected from human clinical samples and can survive at human body temperatures [13]. The type species *C.*

hinnuleum was isolated from dung of deer in Australia [13], whereas, the strains identified as *C. hinnuleum* (UD CT 1-3-3 and KNU-19GWF1) isolated from rhizospheric soils of root area of lantern (*Campanula takesimana*) and pine tree from Ulleungdo in Korea. Previously, the fungal strains *Penicillium sanguifluum* and *P. pasqualense* were collected under the rhizosphere of a pine tree located Dokdo Island, Korea [26], whereas, *P. piscarium* and *Talaromyces versatilis* reported from freshwater environment [27].

The earliest record of *Thelonectria* (*T. discophora*), previously considered as cosmopolitan, arises from Chile [28]. The *T. discophora* complex occurred with the diversified habitats and plant substrates such as twig of bark and recently dead or dying trees branches [29]. Currently, 9 species within the genus *Thelonectria* have been identified and five of which were previously reported from China [6]. *T. coronata* (as *Nectria coronata*) was recently isolated from Hong Kong [30], *T. jungneri* (Henn.) and *T. lucida* (as *Neonectria lucida*) were later reported from the Taiwan Province [29], and *T. veuillotiana* (as *Neonectria veuillotiana*) was found in the Hubei Province [31]. Also, *T. coronata* and *T. veuillotiana* were sufficient amount in temperate, tropical, and subtropical regions and grow on the bark of dead or dying trees recently and on canker lesions caused with the other causal agents [29,31]. Though, the different fungal strains under the genus *Thelonectria* were isolated from diversified habitats, plant substrates along with plants parts from temperate, tropical, and subtropical regions in different countries, but our strain UD ST 1-2-1 was identified from rhizospheric soil regions of Ulleungdo stonecrop (*Sedum takesimense*) from Ulleungdo in South Korea. In future study, the taxonomy requires further study with the wide ranges of geographical location along with their pathogenicity tests based on the hosts.

Further investigations are necessary to explore the etiology of *C. hinnuleum* and *T. chlamydospora* based on the host(s), environments and agricultural land conditions in Korea. Based on their cultural and morphological characteristics and phylogenetic analyses, the UD CT 1-3-3 and KNU-19GWF1 strains were similar to *C. hinnuleum*. The UD ST 1-2-1 strain was different from other identified species under the genus *Thelonectria*, and it was described as a novel species. Thus, the newly discovered species was proposed as *Thelonectria chlamydospora* sp. nov. and *Cephalotrichum hinnuleum*, a new record from Korea.

Disclosure statement

The authors declare that they have no potential conflicts of interest.

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