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RESEARCH ARTICLE

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Scytalidium terrigenum sp. nov., a New Species Isolated from Soil in Korea

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ABSTRACT

During an investigation of soil microfungi in Korea, a fungal strain designated KNUF-23-236 was isolated from a soil sample collected in Seocheon-gun, Chungcheongnam-do, Korea. Molecular analyses using the ITS regions and the *LSU*, *SSU*, and *RPB2* genes revealed that *Scytalidium aurantiacum* and *Scytalidium album* are its close phylogenetic relatives, with sequence similarity levels ranging from 93.8% to 100.0%. However, based on cultural and morphological characteristics strain KNUF-23-236 differs from *S. aurantiacum* and *S. album* by having white-to-yellow colonies without reddish pigmentation, smaller hyphae (1.4–3.4 µm vs. 1.6–4.8 µm and 3.2–8.0 µm, respectively), arthrospores that transition from hyaline to brown rather than remaining consistently hyaline, and oval, septate chlamydospores that form singly or in chains, without branching. Furthermore, the phylogenetic trees constructed using the ITS sequence alone, the concatenated ITS and *LSU* sequences, and the combined sequences of three loci (ITS, *SSU*, and *RPB2*) confirmed a distinct phylogenetic position of KNUF-23-236 within the genus *Scytalidium*. Based on a combination of phylogenetic and morphological evidence, strain KNUF-23-236 is identified as a novel species of the genus, for which the name *Scytalidium terrigenum* sp. nov. is proposed.

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1. Introduction

The genus *Scytalidium* was first established in 1956 to accommodate fungal species characterized by unique morphological features, particularly the production of arthroconidia and chlamydospores [1]. These fungi are primarily saprotrophic and are known for their ability to colonize a variety of substrates, including soil, decaying plant material, and wood. Initially, *Scytalidium* species were classified based on morphological traits, including darkly pigmented, septate hyphae and the formation of arthroconidia-small, fragmented spores that develop directly from hyphal cells. The genus also included species producing thick-walled, pigmented chlamydospores, which serve as survival structures. These characteristics helped distinguish *Scytalidium* from other genera within related fungal families. Over time, advances in molecular phylogenetics and DNA sequencing have redefined the taxonomy of *Scytalidium* and related genera. Species previously classified within *Scytalidium* have been reclassified into other genera based on molecular evidence, including *Neoscytalidium* and *Fusicoccum*. For example, *Scytalidium dimidiatum* was transferred to the genus *Neoscytalidium* after molecular studies revealed its phylogenetic distinction [2]. Conversely, *Geotrichum candidum* Link 3C was reclassified as *Scytalidium candidum* based on molecular phylogenetic comparisons [3]. According to the MycoBank (https://www.mycobank.org) and Index Fungorum (https://www.indexfungorum.org) databases, the genus *Scytalidium* currently comprises around 30 recognized species.

Members of the genus *Scytalidium* are found in a variety of environments. Certain species are predominantly associated with blue-stained wood products, including poles and pulpwood of pine and birch, and are known to cause wood decay [4]. Several species are found in soil and on mushrooms [5]. For example, *S. auriculariicola* infects the mycelia of *Auricularia polytricha* [6], and *S. ganodermophthora* infects the fruiting bodies of *Ganoderma lucidum* [7]. Some *Scytalidium* species are recognized as plant pathogens. *Scytalidium lignicola*, the type species of the genus, has been identified as the causal agent of black root rot in cassava in Brazil [8] and sudden wilt of "Star Ruby" grapefruit in Israel [9]. In India,

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S. aeglicola has been reported to infect *Aegle marmelos* (Rutaceae), causing brown necrotic lesions on the leaves [10], and *S. melanoxylicola* was found on *Diospyros melanoxylon*, where it caused distinct infection spots on both the upper and lower surfaces [11]. Additionally, *S. assmuthi* has been discovered in the gut of *Odontotermes assmuthi*, a termite species that feeds on wood logs [12]. *Scytalidium* species are also known to survive in extreme environments. For instance, in a culture experiment, *S. candidum* exhibited maximum growth at pH 4 [3]. These examples highlight the remarkable adaptability and resilience of *Scytalidium* under diverse environmental conditions.

The aim of this study was to introduce and characterize a novel species of *Scytalidium*, designated *Scytalidium terrigenum* sp. nov., isolated from soil in Korea. Conducted as part of ongoing research on indigenous Korean fungal species, this study utilized both morphological and molecular analyses to characterize the species. Taxonomic placement within the genus *Scytalidium* was confirmed using multilocus sequence analysis (MLSA).

2. Materials and methods

2.1. Sample collection and fungal strain isolation

The fungal strains used in this study were obtained from soil samples collected in Maseo-myeon, Seocheon-gun, Chungcheongnam-do, Korea (36°3′12.55″N, 126°39′11.32″E). Isolation was performed using a standard serial dilution method. Subsequently, 100 µL of the resulting suspensions were plated onto potato dextrose agar (PDA; Difco, Detroit, MI) and incubated at 25 °C for two weeks. Single colonies were then transferred to fresh PDA plates and incubated at 25 °C. Several fungal strains were isolated and subjected to DNA extraction, PCR amplification, and ITS regions sequencing for preliminary identification. Among these, strain KNUF-23-236 was identified as a potential novel fungal species and selected for comprehensive analysis. The stock culture of strain KNUF-23-236 (NIBRFGC000510713) was deposited in the National Institute of Biological Resources (NIBR) as a metabolically inactive culture.

2.2. Cultural and morphological characterization

The culture of KNUF-23-236 was cultured on potato dextrose agar (PDA; Difco, Detroit, MI). It was incubated in the dark at 25 °C for two weeks. The morphological characteristics of KNUF-23-236 were examined on PDA, sabouraud dextrose agar (SDA; MBcell, Seoul, Korea), malt extract agar (MEA; Difco, Detroit, MI), Czapek-Dox agar (CDA; MBcell, Seoul, Korea), and nutrient agar (NA; Difco, Detroit, MI). To determine the optimal growth temperature, the culture was incubated in the dark for two weeks at 10, 20, 25, 30, and 37 °C. To assess the effect of pH on growth, the culture was incubated in the dark at 30 °C for 16 d on PDA medium with pH levels ranging from 4 to 9 [3]. Cultural characteristics, such as color and shape, were observed using a Canon EOS 5D Mark III (Canon, Tokyo, Japan). Morphological features, including the size of arthrospores, chlamydospores, and hyphae were examined under a light microscope (BX-50, Olympus, Tokyo, Japan).

2.3. DNA extraction, PCR, sequencing, and phylogenetic analysis

Total genomic DNA was extracted from the fungal mycelia of strain KNUF-23-236 cultured on PDA plates using a HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, South Korea) according to the manufacturer's instructions. The internal transcribed spacer (ITS) regions were amplified with the primers ITS1F and ITS4 [13]. For the small subunit of nuclear ribosomal RNA (*SSU*), amplification was performed with the primers SR1R and SR2, as well as SR9R and SR6 [14]. The RNA polymerase II subunit (*RPB2*) gene was targeted using the primer pairs fRPB2-5F and fRPB2-7cR, along with fRPB2-980F and fRPB2-11a [3]. Amplification of the large subunit of nuclear ribosomal RNA (*LSU*) employed the primers LR0R and LR7 [15]. The PCR products were subsequently purified with the EXOSAP-IT reagent (Thermo Fisher Scientific, Waltham, MA) following the manufacturer's instructions and sequenced by SolGent (Daejeon, South Korea). The ITS regions, *SSU*, *RPB2*, and *LSU* gene sequences have been deposited in GenBank with accession numbers LC859329 (ITS), LC859331 (*SSU*), LC859332 (*RPB2*), and LC859330 (*LSU*), respectively.

The sequences of our isolate were compared with reference sequences from the GenBank database of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). Sequences of closely related strains of *Scytalidium* species were retrieved from the NCBI GenBank database (Table 1). The sequences were initially aligned using ClustalX version 2.0, and phylogenetic analyses were performed using MEGA version 7.0 [16]. Three phylogenetic trees were constructed based on the sequences of the ITS regions, concatenated sequences of the ITS regions and *LSU* gene, and combined sequences of the ITS regions, *SSU* and *RPB2* genes, using the maximum likelihood (ML) method with 1000 bootstrap replicates.

Table 1. GenBank accession numbers of sequences used for the phylogenetic analyses in this study.

Species	Strain number	GenBank accession numbers			
		ITS	*LSU*	*SSU*	*RPB2*
Saccharomyces cerevisiae	CBS 1171^T	OP900088		AY497740	AY497600
Saccharomyces cerevisiae	NRRL Y-12632^T	AY046146	NG_042623		
Scytalidium album	173	MF992676		MF992680	MG021453
Scytalidium album	CBS 372.65^T	MH858617			
Scytalidium album	CBS 373.65	MH858618	MH870258		
Scytalidium assmuthi	MCC 10102^T	OR415883	OR415885		
Scytalidium aurantiacum	CBS 374.65^T	MH858619	MH870259		
Scytalidium candidum	3C^T	MF992675	MG018250	MF992679	MF996858
Scytalidium chinense	HSAUP061091^T	HQ213805	HQ221579		
Scytalidium circinatum	CBS 654.89^T	MH862195			
Scytalidium cuboideum	CBS 241.62^T	MH858144			
Scytalidium cuboideum	KACC 41224	GQ272630		GQ280410	GQ290133
Scytalidium cuboideum	UTHSC 10-2389	HE965762	HE965763		
Scytalidium flavobrunneum	CBS 244.59^T	MH857854			
Scytalidium ganodermophthorum	H55^T	GQ272617		GQ280399	GQ290121
Scytalidium indonesiacum	CBS 259.81^T	MH861338	MH873098		
Scytalidium infestans	CBS 161.91^T	MH862246			
Scytalidium japonicum	CBS 494.88^T	MH873833			
Scytalidium lignicola	KACC 41228^T	GQ272634		GQ280419	GQ428330
Scytalidium multiseptatum	CBS 241.68	MH859124	MH870836		
Scytalidium parasiticum	AAX0113^T	KF925449			
Scytalidium philadelphianum	CBS 148262^T	ON811538			
Scytalidium philadelphianum	UTHSCSA DI24-300	PP812515	PP812514		
Scytalidium sphaerosporum	ATCC 34392^T	GQ272624		GQ280405	GQ290127
Scytalidium synnematicum	CCLAMIC 20713^T	OQ430525	OQ430526		
Scytalidium terminale	CBS 171.40^T	MH856079			
Scytalidium terrigenum	**KNUF-23-236**	**LC859329**	**LC859330**	**LC859331**	**LC859332**
Scytalidium tibetense	HSAUP061127^T	HQ213808	HQ221582		
Scytalidium tuberculatum	HSAUP061195^T	KC466538	HQ221583		
Scytalidium uredinicola	CBS 578.75^T	MH860954			

ITS: internal transcribed spacer regions; *LSU*: partial sequence of large subunit 28S rDNA; *SSU*: partial sequence of small subunit 18S rDNA; *RPB2*: RNA polymerase II subunit

The newly generated sequences are indicated in bold.

Genetic divergence between the species was evaluated using Kimura's two-parameter model [17].

3. Results

3.1. Taxonomy

Scytalidium terrigenum Y.S. Jeong, S.Y. Lee, and H.Y. Jung, sp. nov. (Figures 1 and 3)

MycoBank: 857370

Etymology: This refers to the soil, the substrate from which the type strain was isolated.

Typus: KNUF-23-236 was isolated from soil collected in Maseo-myeon, Seocheon-gun, Chungcheongnam-do Province, Korea (36°3′12.55″N, 126°39′11.32″E) in 2023.

Habitat: The genus *Scytalidium* is primarily found in plants, wood, mushroom fruiting bodies, soil, and humans. In this study, a new species was discovered in the soil of a mountain in Korea.

Cultural characteristics: Colonies of the fungal isolate KNUF-23-236 were observed on various media, appearing white to cream-colored on NA (Figure 1(A)), PDA (Figure 1(B)), SDA (Figure 1(D)), CDA (Figure 1(E)), and MEA media (Figure 1(F)). On PDA, the colonies initially appeared white but turned yellow over time due to the production of yellow pigments. Additionally, by the third week, the formation of chlamydospores caused the colonies to turn black (Figure 1(B,C)). The cultural characteristics of KNUF-23-236 were examined under various conditions. The strain was incubated for two weeks on PDA, SDA, MEA, and CDA at different temperatures. Growth was observed at 10 °C, but no growth at 37 °C. The fastest growth was recorded on PDA and SDA media at 25 °C, while MEA showed the most rapid growth at 20 °C, and CDA supported the fastest growth at 30 °C (Figure 2(A)). When cultured on PDA medium at 30 °C for 16 d under varying pH conditions, the strain exhibited distinct growth patterns. During the first 8 d, the fastest growth was observed at pH 5, with colony diameters reaching 47.28 mm by day 8. However, after 8 d, the optimal pH shifted to pH 6, where the colony diameter peaked at 74.98 mm by day 16. At pH 5, the final diameter reached 68.33 mm by day 16, showing slightly slower growth compared to pH 6. In comparison, growth at other pH levels, such as pH 4, pH 7, and pH 9, resulted in final diameters of 53.75 mm, 70.02 mm, and 63.07 mm, respectively (Figure 2(B)).

Morphological characteristics: The hyphae are hyaline, septate, and branched, with a diameter ranging from 1.4 to 3.4 µm ($n = 100$). Arthrospores transition from hyaline to brown in color and exhibit a cylindrical shape. They are septate and can form either singly or in chains. Their size ranges from 2.5–8.9 × 1.7–2.9 µm ($n = 100$) (Figure 3(A,B)).

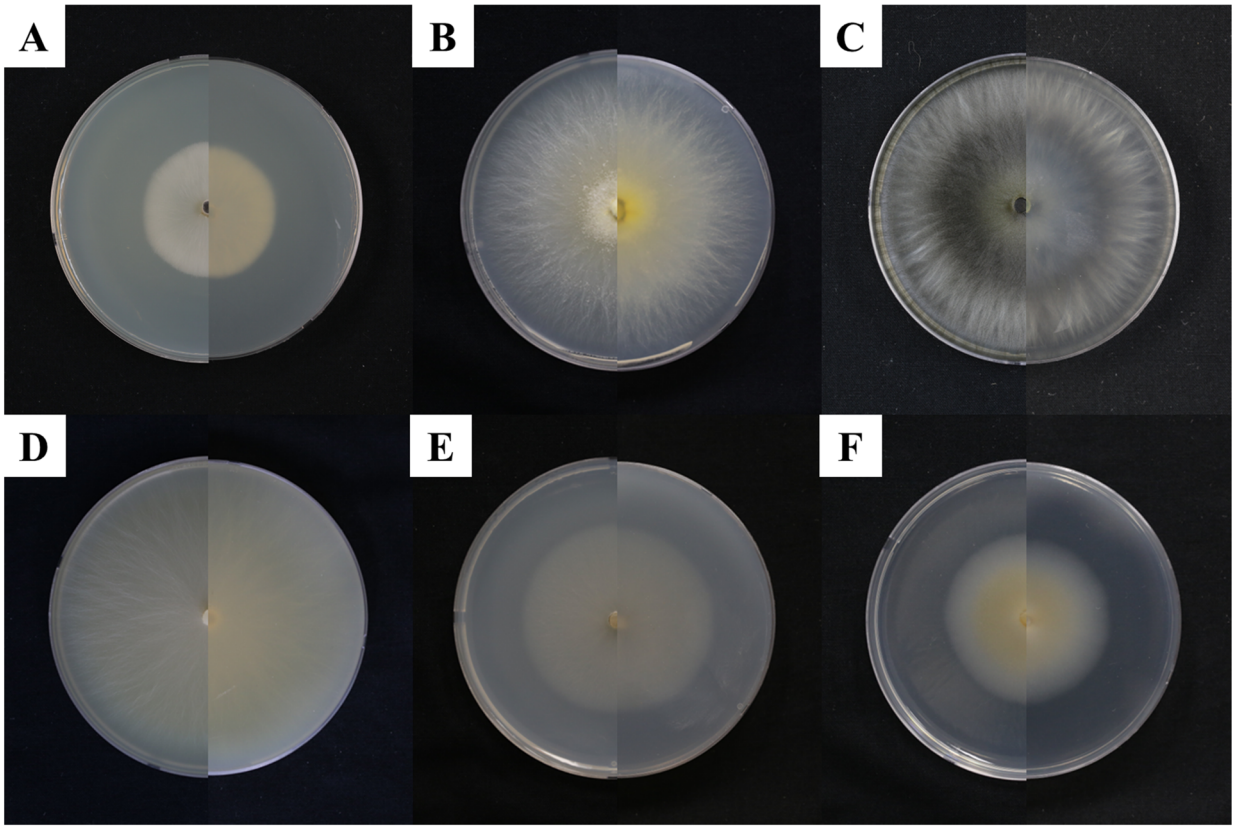

Figure 1. Morphology of KNUF-23-236 grown on the media used: (A) The nutrient agar; (B, C) Potato dextrose agar; (D) Sabouraud dextrose agar; (E) Czapek-dox medium; (F) Malt extract agar. A, B, D, E, F grow for 14 d, C grows for 3 weeks at 25 °C.

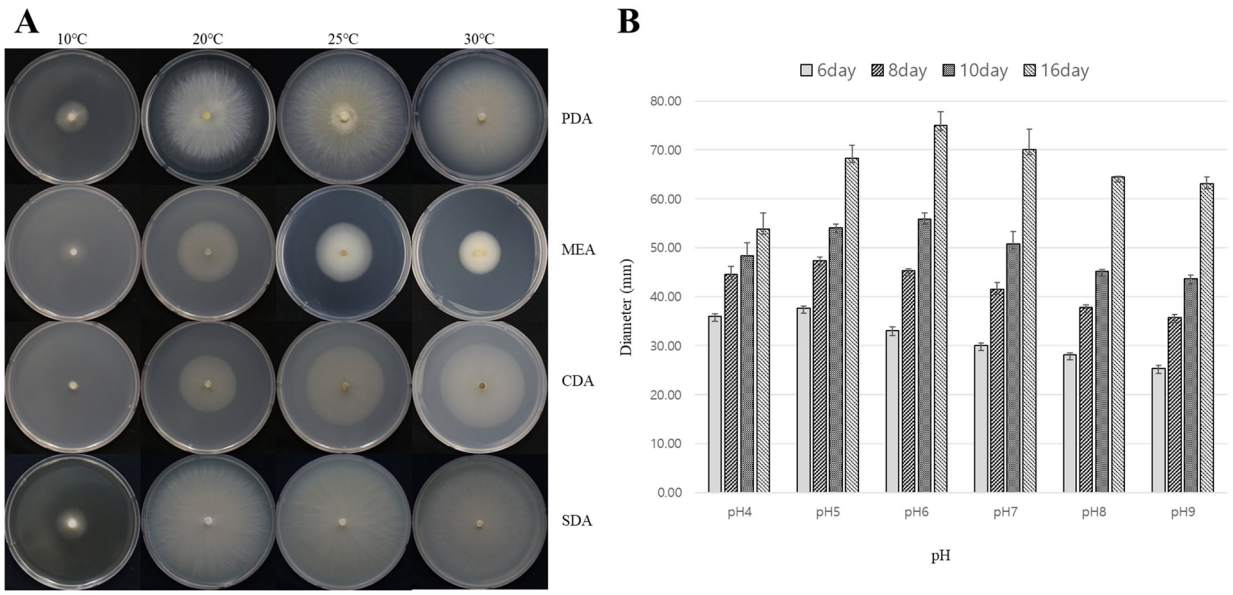

Figure 2. Dependence of colony diameter and density on growth conditions: (A) Influence of temperature and media; (B) Influence of pH of the PDA medium on the growth of culture at 30 °C.

Chlamydospores are pale to brown and exhibit an oval shape. They are septate, can form either singly or in chains, and do not branch. Their size ranges from 5.4–13.0 × 4.2–8.1 µm ($n = 100$) (Figure 3(C,D)).

Note: Colonies of KNUF-23-236 were cultured on PDA medium at 25 °C for 14 d and compared to the phylogenetically related species *Scytalidium aurantiacum* and *S. album* [4]. The colony colors of KNUF-23-236 vary depending on the medium. On NA medium, the colonies appear white, whereas on MEA medium, they range from white to beige and cream. In contrast, *S. aurantiacum* forms yellow colonies with prominent red spots on NA medium and orange-red colonies with discernible reddish spots on MEA medium. Meanwhile, *S. album* produces white colonies on NA medium

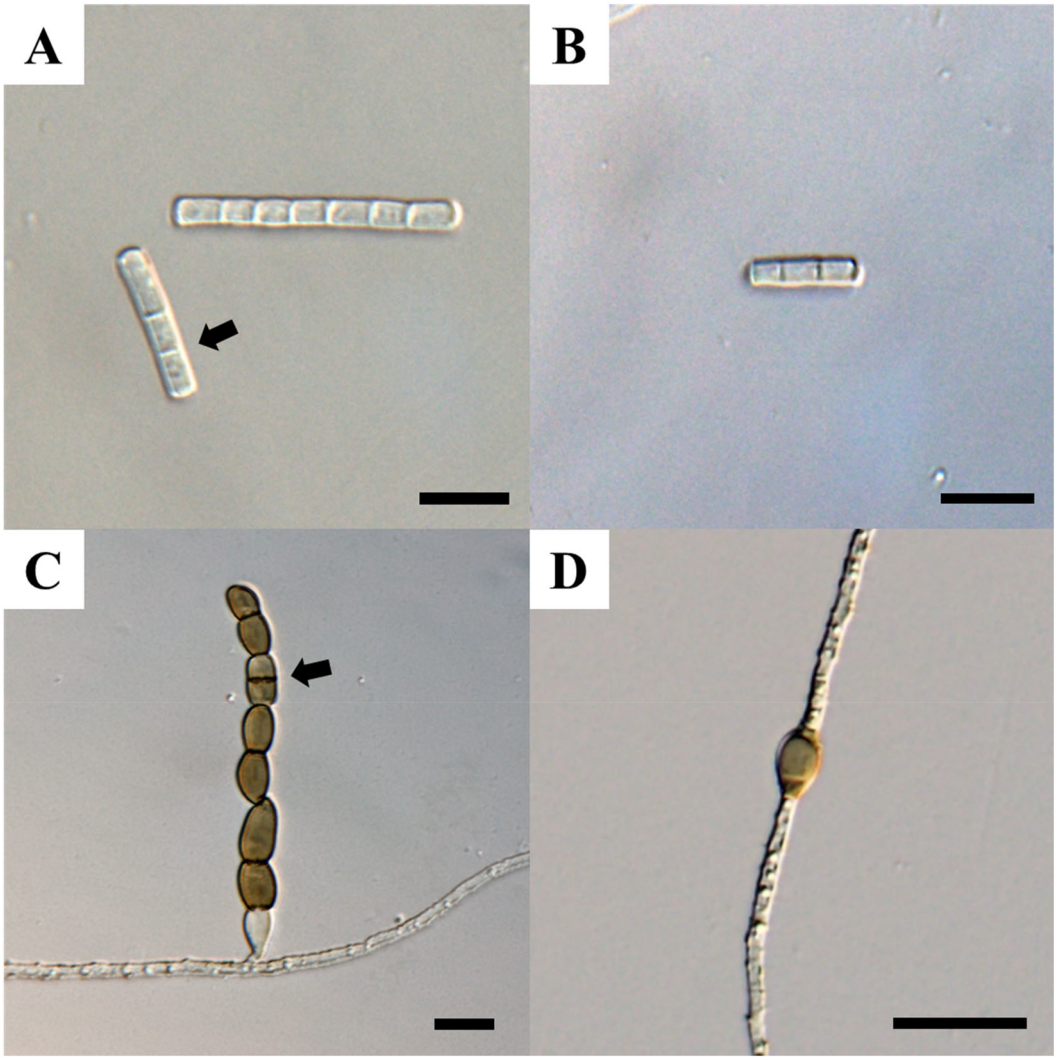

Figure 3. Microscopic characters of KNUF-23-236 grown on the PDA at 25 °C: (A) Arthrospores with arrows indicating septa; (B) Chain-forming arthrospores; (C) Chain-forming chlamydospores with arrows highlighting septa; (D) Single-formed chlamydospores. Scale bars: A-C = 10 µm, D = 20 µm.

and white to yellow colonies on MEA medium [4]. Morphologically, KNUF-23-236 forms arthrospores that transition from hyaline to brown, whereas *S. aurantiacum* and *S. album* consistently form hyaline arthrospores [4]. Additionally, KNUF-23-236 produces smaller arthrospores (2.5–8.9 × 1.7–2.9 µm) compared to *S. aurantiacum* (4.8–9.6 × 1.6–3.2 µm) and *S. album* (4.8–9.6 × 1.6–3.2 µm) [4]. KNUF-23-236 forms pale to brown chlamydospores that are oval in shape and may possess septa. These chlamydospores can be formed singly or in chains. In contrast, the chlamydospores of *S. aurantiacum* and *S. album* are also pale to brown but exhibit globose or ellipsoidal shapes and are formed in chains [4]. The chlamydospores of strain KNUF-23-236 (5.4–13.0 × 4.2–8.1 µm) are slightly narrower than those of *S. aurantiacum* (6.4–12.8 × 4.8–9.6 µm) and *S. album* (6.4–14.4 × 4.8–9.6 µm). The reduced width range in KNUF-23-236 can serve as a distinguishing morphological characteristic from its closely related species. Additionally, the chlamydospores of the isolate did not exhibit branching, which represents another distinct feature of this strain. Overall, KNUF-23-236 differs from *S. aurantiacum* and *S. album* in colony color, arthrospore color and size, the presence of septa in some arthrospores and chlamydospores, and their ability to form singly (Table 2).

3.2. Phylogenetic analysis

The sequence lengths of the ITS regions, *LSU* gene, *SSU* gene, and *RPB2* gene of KNUF-23-236 are 580, 1354, 2042, and 2090 bp, respectively. The ITS regions of KNUF-23-236 showed 97.5% similarity to *Scytalidium aurantiacum* (CBS 374.65; MH858619), 96.9% similarity to *S. album* (isolate 52; MW528599), and 92.8% similarity to *S. circinatum* (CBS 654.89; MH862195). The *LSU* gene of KNUF-23-236 showed 98.5% similarity to *S. aurantiacum* (CBS 374.65, MH870259), 96.9% similarity to *S. album* (strain 173; MF966378), and 96.9% similarity to *S.*

Table 2. Comparison of morphological and cultural characteristics between KNUF-23-236 and the phylogenetically closest species of the genus *Scytalidium*.

Characteristics		KNUF-23-236[Ta]	*Scytalidium aurantiacum*[b,c]	*Scytalidium album*[b,c]
Colony	Color	White on NA; White, beige, cream on MEA; White-to-yellow on PDA	Yellow on NA, prominent red spots; Orange-red on MEA, discernible reddish spots	White on NA; White-to-yellow on MEA
	Pigments	No pigments on MEA Yellow on PDA	Orange-red on MEA	Yellow on MEA
	Growth (mm)	28 on MEA	16–20 on MEA	20–24 on MEA
Hyphae	Size (μm)	1.4–3.4	1.6–4.8	3.2–8.0
Arthrospore	Color	Hyaline to brown	Always hyaline	Always hyaline
	Shape	Cylindrical, septate Forming singly or chains	Bacilliform	Bacilliform
	Size (μm)	2.5–8.9×1.7–2.9	4.8–9.6×1.6–3.2	4.8–9.6×1.6–3.2
Chlamydospore	Color	Pale to brown	Pale to brown	Pale to brown
	Shape	Oval, septum, did not branch Forming singly or chains	Globose or ellipsoidal Forming chains	Globose or ellipsoidal Forming chains
	Size (μm)	5.4–13.0×4.2–8.1	6.4–12.8×4.8–9.6	6.4–14.4×4.8–9.6

[a]Fungal strain studied in this research.
[b]Source of descriptions [4].
[c]Source of descriptions [1].

candidum (strain 3 C; MG018250). A BLAST search and pairwise comparison with related strains revealed that the *SSU* gene sequence of KNUF-23-236 showed 100.0% similarity to *S. album* (strain 173; MF992680), 99.6% similarity to *S. lignicola* (strain SJ-2010; JQ691626), and 99.4% similarity to *S. candidum* (strain 3 C; MF992679). The *RPB2* gene of KNUF-23-236 showed 93.8% similarity to *S. album* (strain 173; MF996859), 88.1% similarity to *S. candidum* (strain 3 C; MF996858), and 86.1% similarity to *S. sphaerospora* (KACC 41222; GQ290130). The ITS and *LSU* regions of strain KNUF-23-236 exhibited relatively high similarity to several *Scytalidium* species but fell below the threshold for definitive species-level identification, emphasizing the need for additional loci. The *SSU* gene exhibited 100% similarity to *S. album*, but variations in ITS and *RPB2* sequences with other species highlight potential differentiation and support the novel status of KNUF-23-236. The *RPB2* gene displayed significantly lower similarity values across related taxa, further reinforcing the necessity of MLSA for robust taxonomic resolution.

To assess the phylogenetic placement of strain KNUF-23-236, three phylogenetic trees were constructed using the ML method, each incorporating different combinations of gene regions. The first phylogenetic tree was based solely on the ITS regions, a commonly used genetic marker for fungal species identification. In this tree, KNUF-23-236 clustered closely with *S. aurantiacum* and *S. album*, forming a distinct group within the genus *Scytalidium* (Figure 4). This clustering indicated a close genetic relationship, and the tree topology provided preliminary evidence supporting the novelty of KNUF-23-236 as a separate species, as this was mentioned in the Section 2.1. The second phylogenetic tree was constructed using the combined ITS regions and *LSU* gene sequences. This analysis provided greater resolution, revealing that KNUF-23-236 is clearly distinct from other species in the genus, including *S. aurantiacum* and *S. album* (Figure 5). The separation of KNUF-23-236 from these related species underscores its unique phylogenetic position, providing strong evidence for its novelty at the species level. To further corroborate these findings, a third phylogenetic tree was constructed using a more comprehensive dataset that combined the ITS regions, *SSU* and *RPB2* genes. Although sequences of *S. aurantiacum* were unavailable for this analysis, the tree clearly demonstrated the distinct placement of KNUF-23-236 relative to *S. album* and other species for which sequences of all three loci are currently available (Supplementary Figure 1). The inclusion of multiple genetic loci in this analysis provided robust support for the differentiation of KNUF-23-236 within the genus *Scytalidium*. Collectively, these phylogenetic analyses consistently show that strain KNUF-23-236 occupies a distinct position within the genus *Scytalidium*, separate from its closest relatives, *S. aurantiacum* and *S. album*. This strong phylogenetic evidence, combined with morphological differences, confirms KNUF-23-236 as a novel species within the genus *Scytalidium*.

4. Discussion

The genus *Scytalidium* was traditionally classified based on the morphological characteristics of arthroconidia and chlamydospores. With recent advancements in molecular phylogenetics, molecular markers including ITS regions, *LSU*, *SSU*, and *RPB2* genes, alone or in various combinations, have become critical tools for the identification, classification, and reclassification of *Scytalidium* species [2,3,14]. These molecular approaches provide greater resolution and accuracy compared to morphology alone, enabling the differentiation of closely related species and

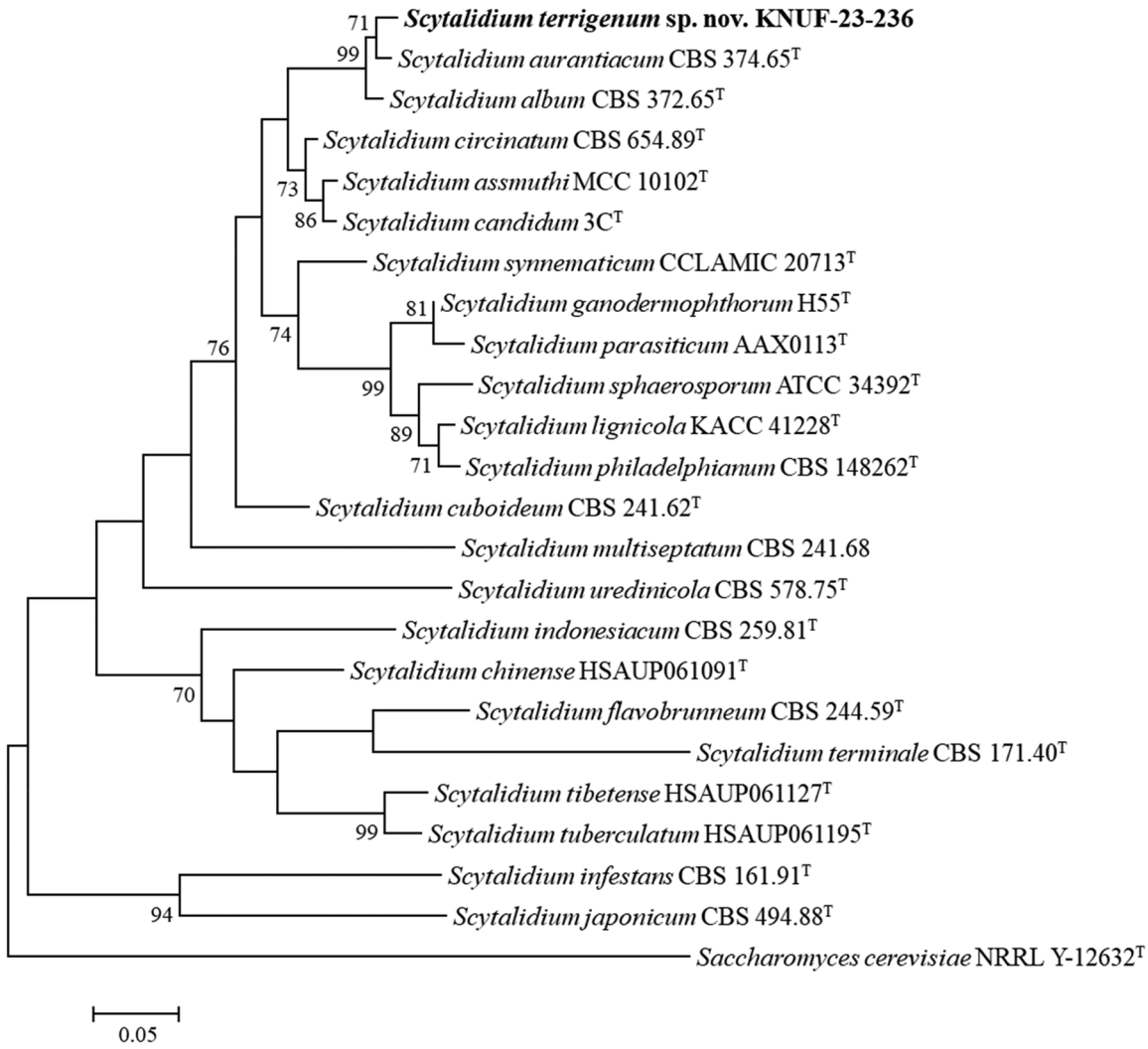

Figure 4. The maximum-likelihood phylogenetic tree based on ITS regions sequences shows the phylogenetic position of KNUF-23-236 within the genus *Scytalidium*. *Saccharomyces cerevisiae* NRRL Y-12632^T was used as the outgroup. The fungal isolate obtained in this study is highlighted in bold. The numbers above the branches indicate bootstrap values (>70%) based on 1000 replicates. Bar = 0.05 substitutions per nucleotide position.

clarifying taxonomic relationships. For example, phylogenetic analysis based on *LSU* sequences was instrumental in establishing *S. chinense*, *S. tibetense*, and *S. tuberculatum* as new species within the genus *Scytalidium* [18]. Similarly, *S. dimidiatum* was reclassified into the genus *Neoscytalidium* based on *LSU* phylogeny [2]. The ITS and *SSU* markers have also been used independently for species classification; for instance, *S. parasiticum* was classified by constructing phylogenetic trees using ITS or *SSU* sequences [19]. More recently, *Scytalidium synnematicum* and *Scytalidium assmuthi* were classified using concatenated ITS and *LSU* sequences for phylogenetic tree construction [12,20]. Furthermore, *S. candidum* was reclassified within the genus *Scytalidium* by employing three phylogenetic trees: one based on ITS alone, another on a combination of *LSU* and *SSU* genes, and a third on the concatenated ITS, *SSU*, and *RPB2* genes [3]. These examples demonstrate the diverse applications of molecular markers, either individually or in combination, in resolving taxonomic questions within the genus *Scytalidium*. In our study, three different phylogenetic approaches were applied to establish the novelty of strain KNUF-23-236. Phylogenetic trees constructed using ITS regions alone, concatenated ITS and *LSU* sequences, and a combination of ITS, *SSU*, and *RPB2* genes all supported the distinct placement of KNUF-23-236 within the genus *Scytalidium*. While ITS-based phylogeny showed close clustering with *S. aurantiacum* and *S. album*, the MLSA provided higher resolution, clearly distinguishing KNUF-23-236 from other species. The absence of sequence data for all four markers (ITS, *LSU*, *SSU*, and *RPB2*) in *Scytalidium* species other than *S. candidum* [3] prevented us from performing phylogenetic analysis simultaneously using all four loci. Despite this limitation, our findings are robust and highlight the value of integrating multiple molecular markers for accurate fungal taxonomy. The distinct phylogenetic

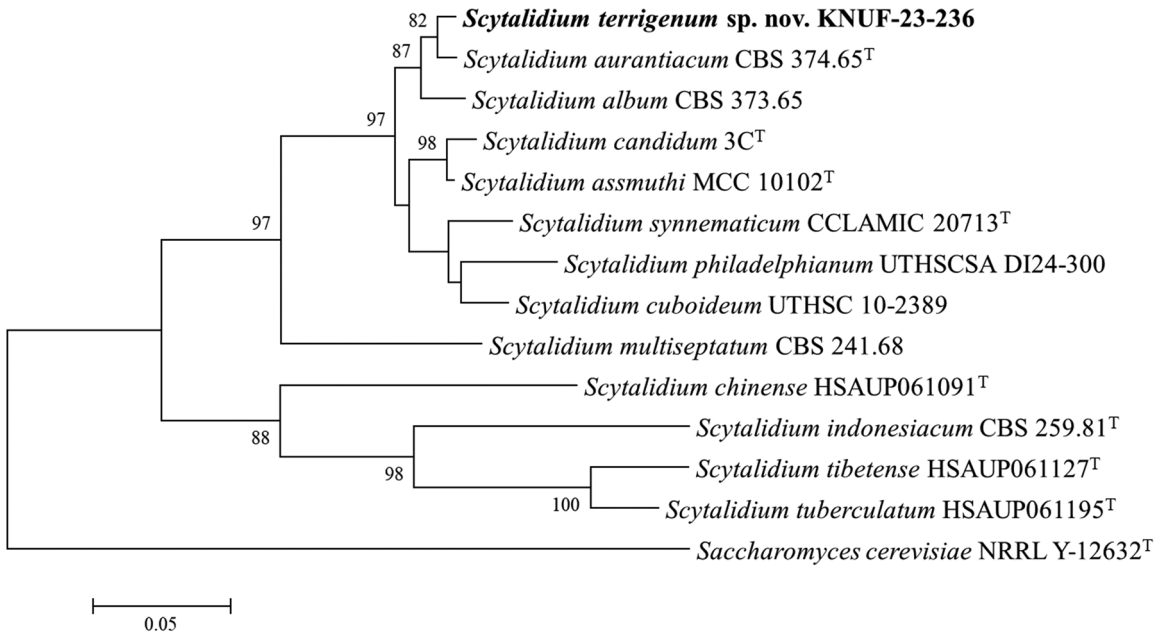

Figure 5. The maximum-likelihood phylogenetic tree based on ITS regions and *LSU* gene sequences shows the phylogenetic position of KNUF-23-236 within the genus *Scytalidium*. *Saccharomyces cerevisiae* NRRL Y-12632^T was used as the outgroup. The fungal isolate obtained in this study is highlighted in bold. The numbers above the branches indicate bootstrap values (>70%) based on 1000 replicates. Bar = 0.05 substitutions per nucleotide position.

clustering of strain KNUF-23-236, supported by MLSA and morphological differences, confirmed its classification as a novel species within the genus *Scytalidium*.

The genus *Scytalidium* exhibits a cosmopolitan distribution and has been isolated from a wide range of ecological niches, highlighting its ecological and functional diversity. Common sources of *Scytalidium* species include soil, wood, mushrooms, and even human infections. For instance, *Scytalidium auriculariicola* was isolated from diseased specimens of the edible mushroom *Auricularia polytricha*, which exhibited slippery scars [6]. Similarly, *S. parasiticum* was recovered from the fruiting bodies of *Ganoderma boninense*, a pathogenic fungus affecting oil palm [19]. Other notable examples include *Scytalidium* species isolated from soil in the Amazon rainforest [5]. Reports of *Scytalidium* species span the globe. For example, *S. ganodermophthora* (former *Xylogone ganodermophthora*) has been documented in Korea [7], *S. auriculariicola* in China [6], *S. parasiticum* in Malaysia [19], and *S. cuboideum* in the United States [7]. Additionally, *S. lignicola* has been recovered from submerged or waterlogged wood, emphasizing its adaptability to aquatic environments [21]. These findings underscore the genus's broad ecological adaptability and significant potential for biotechnological applications. The ability of *Scytalidium* species to thrive in diverse habitats, including extreme environments, such as the Amazon rainforest [5], highlights their evolutionary versatility and ecological importance. Furthermore, their association with economically important crops and human infections demonstrates the need for continued study of their taxonomy, distribution, and potential applications.

The genus *Scytalidium* is a prolific producer of secondary metabolites, including biologically active compounds with diverse chemical structures and pharmacological properties. *Scytalidium lignicola* SC228 produced scytabenzofurans A, B, and C, which exhibit moderate nitric oxide production inhibitory activity in microglial cells, highlighting their potential in anti-inflammatory drug development [22]. Leporin B, produced by *Scytalidium cuboideum* MSX 68345, demonstrated cytotoxicity against human tumor cell lines and antibacterial activity against *Candida albicans* and *Staphylococcus aureus* [23]. Scytalpolyol B, isolated from *Scytalidium* sp. IQ-074, exhibited significant inhibitory activities against hPTP1B1–400, the catalytic domain of human protein tyrosine phosphatase 1B, showcasing its potential in targeting this enzyme for therapeutic purposes [24]. Cytotoxic and antimicrobial sorbicillinoids from *Scytalidium album* have also gained attention for pharmaceutical and agrochemical applications [25]. The antibiotic metabolite scytalidin produced by *Scytalidium album* and *Scytalidium aurantiacum*, has demonstrated antifungal activity, making it a candidate for antifungal agents development [26]. Antimicrobial metabolites such as eudistomin I, naringenin 7-O-beta-D-glucoside, and penipanoid A from *Scytalidium parasiticum* have been identified as promising candidates for controlling basal stem rot

disease caused by *Ganoderma boninense* [27]. In addition to these bioactive compounds, *S. cuboideum* produced xylindein, a natural UV-resistant pigment that enhances the durability of outdoor wood products [28]. This species also produced stable red and orange naphthoquinone crystals, valuable materials for solar cell development [29]. *Scytalidium terrigenum* KNUF-23-236, a novel species identified in this study, exhibits the ability to survive in acidic environments and at low temperatures of 10 °C. This species offers additional opportunities to expand research on bioactive compounds and biotechnological applications, making its further investigation worthwhile. As mentioned earlier, this study is part of our ongoing research on indigenous Korean fungal diversity. Recently, several novel species within the class *Sordariomycetes* [30–34] have been described based on phylogenetic and morphological characteristics. In this context, the establishment of *Scytalidium terrigenum* represents our contribution to the discovery of indigenous fungal species within the class *Leotiomycetes*.

In summary, the discovery of the novel *Scytalidium* species, *S. terrigenum*, highlights the need for further research into its ecological and biological significance. While this study primarily examined its morphological and molecular traits, comprehensive investigations are required to elucidate its ecological interactions, distribution, and potential biotechnological applications.

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