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RESEARCH ARTICLE

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Microascus microspora sp. nov. and *Tolypocladium terrae* sp. nov.; Two Novel Fungal Species Isolated from Soil in Korea

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ABSTRACT

During an investigation of microfungi from various ecosystems in South Korea, two novel fungal species (KNUF-22-094^T and KNUF-23-321C^T) were discovered within the genera *Microascus* and *Tolypocladium*. The strain KNUF-22-094^T stands out among other *Microascus* species due to its distinctive conidiogenous cells and conidial size ranges. The conidiogenous cells of KNUF-22-094^T measured 2.3–4.3 × 2.0–3.6 µm, notably smaller than those of *M. rothbergiorum* (7.5–11.5 × 2.5–4.5 µm), *M. sparsimycelialis* (5.0–10.0 × 3.0–5.0 µm), which is considerably larger in both dimensions. For the conidial structures, conidia measured 2.6–4.8 × 2.6–4.4 µm, falling within a distinct range that is smaller than those of *M. rothbergiorum* (4.0–5.5 × 3.5–4.5 µm), *M. verrucosus* (5.0–7.0 × 4.5–6.0 µm), and *M. restrictus* (4.5–6.0 × 4.0–5.5 µm), but comparable in size to *M. murinus*, which tends to be more elongated (4.0–6.0 × 1.5–1.9 µm). The phialides of KNUF-23-321C^T are globose, with a notably broad length range of 3.7–22.5 µm and a width of 0.9–1.5 µm, making it one of the most variable in length compared to species like *T. album* (3.5–10 µm) and *T. endophyticum* (1.3 ± 0.2 µm, 4.1 µm on average). Phylogenetic analyses using a multi-locus sequences supported to confirm their distinctness under the genus *Microascus* and *Tolypocladium* species. Thus, these fungal strains isolated from soil in Korea are proposed as a novel species according to their characteristics and are named *Microascus microspora* sp. nov. and *Tolypocladium terrae* sp. nov. Detailed descriptions, illustrations, and phylogenetic data are provided to support the recognition of these new species.

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Morphology; *Microascus*; phylogeny; *Tolypocladium*; soil

1. Introduction

Fungi represent a vast and largely unexplored component of global biodiversity, playing crucial roles in various ecological processes, from nutrient cycling to symbiotic relationships with plants and animals. Among these diverse fungi, the genus *Microascus* is noteworthy for its ecological versatility and its role in the decomposition of organic matter. Members of *Microascus* are commonly isolated from soil, decaying wood, and other organic substrates [1]. Some species within this genus are also recognized as opportunistic pathogens, capable of causing infections in humans and animals [2]. Recent studies have highlighted the genetic diversity within *Microascus*, revealing numerous cryptic species that traditional morphological methods failed to distinguish [3]. This underscores the importance of molecular techniques in uncovering the true extent of fungal diversity. To identify and classify the species of *Microascus*, the phylogenetic analyses based on ITS region and β-tubulin gene sequences support its classification as a distinct species within the genus [4].

Furthermore, the genus *Tolypocladium* is equally intriguing, primarily known for its entomopathogenic species and their production of bioactive metabolites. *Tolypocladium* species are renowned for their ability to parasitize insects and other arthropods, making them important biological control agents [5]. The certain species within this genus are prolific producers of secondary metabolites, such as cyclosporine, which have significant pharmaceutical applications [6]. Despite the economic and ecological importance of *Tolypocladium*, the genus remains underexplored, with many species likely yet to be discovered.

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The discovery of these two novel fungal species not only expands our understanding of fungal diversity but also highlights the importance of continued exploration in varied ecosystems in South Korea. By characterizing these new species, we contribute to the broader knowledge of fungal taxonomy, ecology, and potential biotechnological applications. Our study presents the cultural, morphological characteristics of the two novel species, providing a comprehensive description and discussion of their significance within their respective genera.

2. Materials and methods

2.1. Collection and isolation of fungal strains

In this study, fungal strains were sourced from soil samples obtained from Gangwon (37°14′04.1″N, 128°56′47.1″E) and Gyeongbuk (35°47′14.1″N, 128°33′19.7″E) provinces, South Korea. These samples, collected at a depth of 10–15 cm, were air-dried and stored at 4 °C in plastic bags. Fungal isolation utilized the traditional dilution plating method, wherein 100 µL of each sample was spread onto potato dextrose agar (PDA; Difco, Detroit, MI) and then incubated at 25 °C for 2–3 days [7]. Single colonies were subsequently transferred to PDA and incubated for 4–5 days at 25 °C. Strains KNUF-22-094^{T} and KNUF-23-321C^{T}, and for long-term storage and additional research, the fungal strains were preserved at −80 °C in 20% glycerol.

2.2. Cultural and morphological characterization

The culture characteristics and morphological traits of strains KNUF-22-094^{T} and KNUF-23-321C^{T} were assessed using various cultural media including PDA, malt extract agar (MEA; Difco, Detroit, MI), oatmeal agar (OA; Difco, Detroit, MI), and synthetic low nutrient agar (SNA) [8]. Incubation was carried out for 14 days at 25 °C, during which fungal growth and colony features such as color, shape, and size were monitored [9–11]. Morphological examinations were conducted using a BX-50 light microscope (Olympus, Tokyo, Japan).

2.3. Genomic DNA extraction, PCR amplification, and sequencing

Genomic DNA extraction from strains KNUF-22-094^{T} and KNUF-23-321C^{T} were performed using fungal mycelia grown on PDA plates, employing the HiGene™ Genomic DNA Prep Kit (BIOFACT, Daejeon, South Korea) according to the manufacturer's instructions. Molecular identification was conducted by analyzing the sequences of the internal transcribed spacer (ITS) regions and a partial sequence of the large subunit (LSU) 28S rRNA gene, β-tubulin (TUB2), and translation elongation factor 1-alpha (TEF) genes, which were amplified separately using the primer pairs ITS1F/ITS4, LROR/LR5, Bt2a/Bt2b, and EF1-983F/EF1-2218R, respectively [12–16]. The quality of PCR products was confirmed by 0.7% agarose gel electrophoresis stained with ethidium bromide. Purification of products was done using ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA) prior to sequencing, which was performed by SolGent (Daejeon, South Korea).

2.4. Molecular phylogenetic analyses

Phylogenetic analyses were conducted utilizing sequences obtained from the National Center for Biotechnology Information (NCBI) (Table 1). Ambiguous regions were excluded from alignments, and evolutionary distance matrices were calculated for the maximum-likelihood algorithm using Kimura's two-parameter model [17,18]. Phylogenetic relationships were further inferred by tree topology using MEGA11.0 software, and bootstrap values were based on 1000 replications [18,19] (Tables 2 and 3).

3. Results

3.1. Taxonomical analysis of Microascus microspora sp. nov.

The strain KNUF-22-094^{T} isolated from soil sample, demonstrated remarkable congruence in both morphological and molecular characteristics, clustering distinctly within the molecular phylogenetic analysis. As a result, the strain was conclusively identified as a novel species within the genus *Microascus* and proposed species *Microascus microspora* sp. nov.

Microascus microspora S.Y. Lee and H.Y. Jung, sp. nov. (Figure 1)

MycoBank: MB857010

Etymology: *microspora*, referring to this species producing obviously smaller conidia.

Typus: Geomnyongso Spring, Taebaek-si (37°14′04.1″N, 128°56′47.1″E), Gangwon province, South Korea, isolated from soil, containing plant debris near native plantation. The stock culture (NIBRFGC000509837) was deposited in the National Institute of Biological Resources (NIBR) as a metabolically inactive culture.

Habitat and distribution: The genera *Microascus* comprise species commonly isolated from soil, decaying plant material and indoor environments. A few species are also recognized as opportunistic

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

Fungal species	Strain numbers	GenBank accession numbers			
		ITS	LSU	TEF	TUB2
***Microascus microspora* sp. nov.**	**KNUF-22-094^T**	**PQ773316**	**PQ773317**	**PQ772834**	**PQ772835**
Microascus gracilis	CBS 369.70^T	LM652413	HG380476	HG380399	LM652626
Microascus murinus	CBS 621.70	LN850771	LN850819	LN850917	LN850868
Microascus murinus	CBS 864.71	LN850770	LN850820	LN850916	LN850867
Microascus pyramidus	CBS 668.71	LN850779	LN850828	LN850925	LN850876
Microascus restrictus	CBS 138277^T	LM652440	HG380494	HG380417	LM652653
Microascus rothbergiorum	CBS 148579^T	NR_182573	OM509736	OM470475	OM470474
Microascus sparsimycelialis	LC12478	MK329111	MK329016	MK336046	MK336124
Microascus sparsimycelialis	LC12480	MK329112	MK329017	MK336047	MK336125
Microascus verrucosus	CBS 138278	LM652446	HG380493	HG380416	LM652658
Wardomycopsis litoralis	CBS 119740^T	LN851000	LN851055	LN851107	LN851161
***Tolypocladium terrae* sp. nov.**	**KNUF-23-321C^T**	**PQ773315**	**PQ773314**	**PQ772836**	**–**
Tolypocladium album	MS 460	KF747248	KF747137	KF747102	–
Tolypocladium amazonense	CBS 136895^T	JQ905653	KF747134	KF747314	–
Tolypocladium amazonense	LA 100	HQ022485	KF747129	KF747094	–
Tolypocladium bacillisporum	C23	LC684522	LC684522	LC684525	–
Tolypocladium endophyticum	MX 486	KF747245	KF747152	KF747321	–
Tolypocladium endophyticum	MX 560	KF747246	KF747154	KF747118	–
Tolypocladium pseudoalbum	YFCC 875 T	OP207717	OP207737	OP223151	–
Tolypocladium pseudoalbum	YFCC 876	OP207718	OP207738	OP223152	–
Tolypocladium tropicale	MX 338	KF747259	KF747149	KF747318	–
Tolypocladium tropicale	CBS 136897^T	KF747254	KF747125	KF747090	–
Tolypocladium yunnanense	YFCC 877^T	OP207719	OP207739	OP223153	–
Tolypocladium yunnanense	YFCC 878	OP207720	OP207740	OP223154	–
Cordyceps militaris	OSC 93623	JN049825	AY184966	DQ522332	–

ITS: internal transcribed spacer regions of the rDNA; LSU: partial large subunit of 28S rDNA; TEF: translation elongation factor 1-alpha; TUB2: β-tubulin. The newly generated sequences were indicated in bold.

Table 2. Morphological comparison between *Microascus microspora* sp. nov. (KNUF-22-094^T) and the phylogenetically closest species of *Microascus*.

		M. microspora sp. nov. KNUF-22-094[Ta]	*M. rothbergiorum* CBS 148579[b]	*M. verrucosus* CBS 138278[c]	*M. restrictus* CBS 138277[Tc]	*M. sparsimycelialis* LC12478[d]	*M. murinus* CBS 621.70[c]
Cultural characteristics		PDA: whitish-brown on center, white on edge	PDA: N/A	PDA: N/A	PDA: N/A	PDA: cream-white to grey-yellow	PDA: N/A
		SNA: pale-brown	SNA: N/A	SNA: N/A	SNA: N/A	SNA: pale grey to olive grey	SNA: N/A
		OA: olive-green on center, white on edge	OA: dull to pale on center, white on edge	OA: olive-grey	OA: olive grey to brown-grey	OA: dark green	OA: pale grey to olive grey
Hyphae		Subhyaline, septate, 1.2–1.8 µm	Subhyaline, septate, 1.0–2.5 µm	Subhyaline, septate, 1.5–2.5 µm	Subhyaline, septate, 1.5–3.0 µm	Pale brown to brown, septate, 1.5–3.5 µm	Subhyaline, septate, 1.5–2.0 µm
Conidiogenous cells		Annellidic, solitary, 2.3–4.3 × 2.0–3.6 µm	Annellidic, solitary, 7.5–11.5 × 2.5–4.5 µm	Annellidic, solitary, 4.0–5.0 × 2.5–4.0 µm	Annellidic, solitary, 4.0–6.0 × 3.0–5.0 µm	Solitary, 5.0–10.0 × 3.0–5.0 µm	Annellidic, solitary, 6.0–7.5 × 2.0–2.5 µm
Conidia	Shape	Greenish-grey, subglobose	Dull green to greenish grey, subglobose	Dark brown, globose to subglobose	Dark brown, globose to subglobose	Ovoid to globose, pale brown	Cylindrical, brown
	Size	2.6–4.8 × 2.6–4.4 µm	4.0–5.5 × 3.5–4.5 µm	5.0–7.0 × 4.5–6.0 µm	4.5–6.0 × 4.0–5.5 µm	3.5–6.0 × 3.0–5.5 µm	4.0–6.0 × 1.5–1.9 µm

[a]Fungal strain studied in this research.
[b]Sources of descriptions [20].
[c]Sources of descriptions [9].
[d]Sources of descriptions [21].

pathogens of insects and animals, including humans, leaf of *Prunus* sp., roots of *Vitis vinifera*, aquatic sediment, mangrove soil, milled rice, mushroom bed [9], and from *Stylophora pistillata* [20].

Cultural characteristics: Colonies on OA at 25 °C grow slowly, reaching 24–28 mm diameter after 14 days, floccose, olive-green in center, white in edge, with irregular margins; reverse pale brown (Figure 1(A)). On PDA reaching 30–31 mm after 14 days at 25 °C, flat, circular, whitish-brown in center, white on edge, with regular margins in 14 days at 25 °C; reverse beige (Figure 1(B)). On SNA, colonies reaching 31–32 mm diameter in same condition, flat, slightly floccose, pale brown, with regular margin; reverse pale brown (Figure 1(C)).

Morphological characteristics: Vegetative hyphae are septate, subhyaline, smooth-walled, thin-walled, 1.2–1.8 µm wide (n = 30), tapering into a distinct

Table 3. Morphological comparison between *Tolypocladium terrae* sp. nov. (KNUF-23-321C^{T}) and the phylogenetically closest species of *Tolypocladium*.

	T. terrae sp. nov. KNUF-23-321C[Ta]	*T. album* CBS 830.73[Tb]	*T. yunnanense* YFCC877[Tc]	*T. pseudoalbum* YFCC875[Tc]	*T. endophyticum* MX486[d]	*T. amazonense* CBS 136895[Td]
Cultural characteristics	PDA: white, flat	PDA: white, thinly floccose	PDA: white to orange-yellow	PDA: white or pale yellow	PDA: white, floccose	PDA: white
Phialides	Subcylindrical, 3.7–22.5 × 0.9–1.5 µm	Cylindrical, 3.5–10 × 1.0–1.5 µm	Cylindrical, 7.6–62.6 × 0.9–2.3 µm	Cylindrical, 12.3–48.5 × 1.0–2.0 µm	Trichodermoid branching pattern, 3.2–5.0 × 1.4–1.8 µm	Trichodermoid branching pattern, 3.4–5.8 × 1.2–1.8 µm
Conidia	Globose to subglobose, 1.4–2.4 × 1.5–2.2 µm	Globose or obvoid, 1.5–2.0 µm	Ellipsoid or subglobose, 1.2–2.4 × 0.9–1.9 µm	Globose to ellipsoid, 1.8–3.4 × 1.3–1.9 µm	Globose to subglobose, 1.1–1.5 µm	Cylindrical or globose, 2.1–2.2 µm

[a]Fungal strain studied in this research.
[b]Sources of descriptions [22].
[c]Sources of descriptions [23].
[d]Sources of descriptions [24].

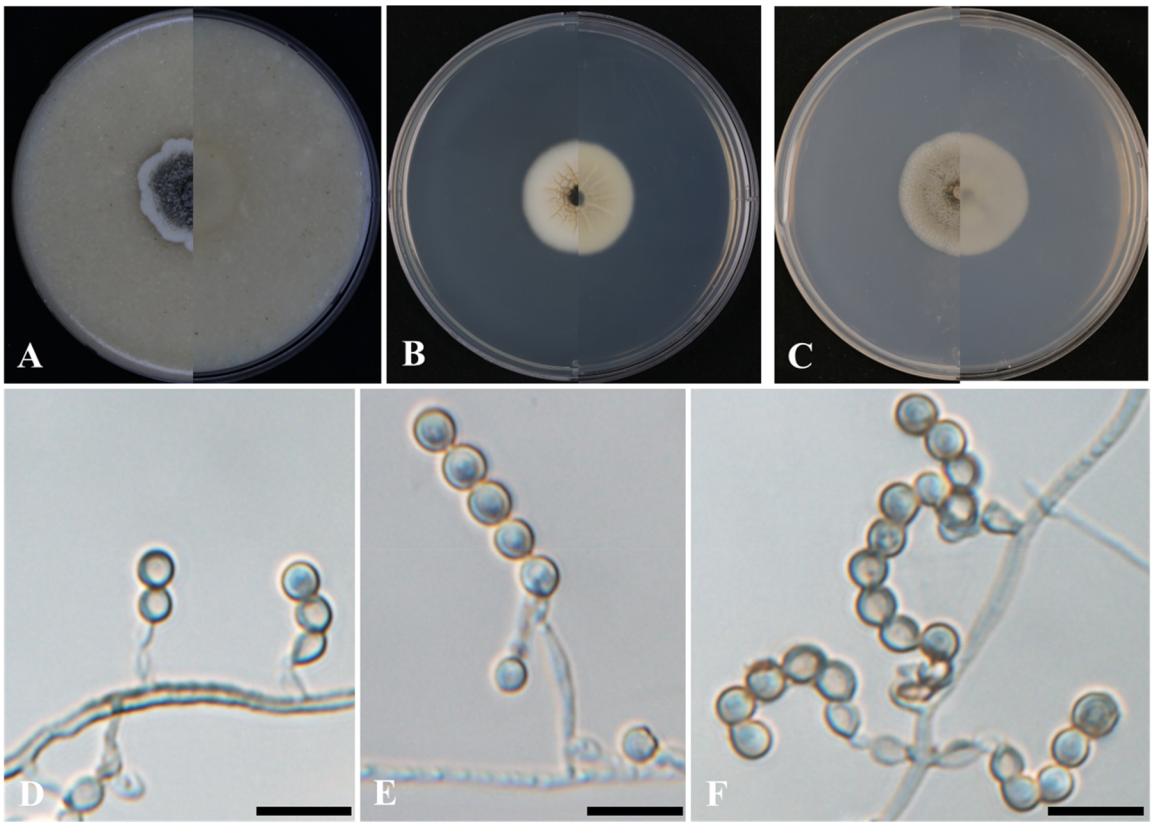

Figure 1. Morphologies of *Microascus microspora* sp. nov. (KNUF-22-094^{T}). Colonies on OA (A); PDA (B); SNA (C) at 25 °C for 14 days, respectively. Conidiophores, annellides, and conidia (D–F). Scale bars = 10 µm.

neck. Conidiogenous cells annellidic, solitary, measured 2.3–4.3 × 2.0–3.6 µm (Avg. 3.3 × 2.6 µm) (n = 15). Conidiophores 4.2–11.8 µm (Avg. 8.68 µm) long and 1.5–3.4 µm wide. The conidia are subglobose to broadly ellipsoidal, with a distinct apical base, prominent reticulation, 2.6–4.8 × 2.6–4.4 µm (Avg. 3.9 × 3.3 µm) (n = 30). In mass, the conidia are greenish-grey and arranged in short chains (Figure 1(D–F)). Sexual morph not observed.

Notes: The strain KNUF-22-094^{T} stands out among other *Microascus* species due to its distinctive conidiogenous cells and conidial size ranges. The conidiogenous cells of KNUF-22-094^{T} measure 2.3–4.3 × 2.0–3.6 µm, notably smaller than those of *M. rothbergiorum* (7.5–11.5 × 2.5–4.5 µm), *M. sparsimycelialis* (5.0–10.0 × 3.0–5.0 µm), which is considerably larger in both dimensions [20,21]. Additionally, the hyphal width of KNUF-22-094^{T} ranges 1.2–1.8 µm, with an occasional maximum dimension reaching 4.5 µm. In comparison, *M. verrucosus* and *M. restrictus* have slightly wider hyphal ranges, 1.5–2.5 µm and 1.5–3.0 µm, respectively [9]. For the conidial structures, conidia measured 2.6–4.8 × 2.6–4.4 µm, falling within a distinct range that is smaller than those of *M.*

rothbergiorum (4.0–5.5 × 3.5–4.5 µm), *M. verrucosus* (5.0–7.0 × 4.5–6.0 µm), and *M. restrictus* (4.5–6.0 × 4.0–5.5 µm) [9,20,21]. Comparable in size to *M. murinus*, which tends to be more elongated (4.0–6.0 × 1.5–1.9 µm) [9]. This unique combination of smaller conidiogenous cells and conidia sizes distinguishes KNUF-22-094^T within the *Microascus* genus, setting it apart from related species (Table 2).

3.2. Molecular phylogeny of strain KNUF-22-094^T

Based on the sequencing analyses, ITS (527 bp), LSU (733 bp), TUB2 (527 bp), and TEF (850 bp) gene sequences were retrieved from the strain KNUF-22-094^T. For the ITS regions, *M. rothbergiorum* CBS 148579 showed 99.2% similarity, whereas *M. gennadii* WL02353 and *M. sparsimycelialis* FZ2713 showed 97.9% similarity and *M. restrictus* CBS 138277 showed 96.9% similarity with our strain. In the LSU, the isolated strain KNUF-22-094^T showed 100% similarity with *M. rothbergiorum* DTO 454-F5. A similarity of 99.9% was shared between *M. sparsimycelialis* FZ2713, while *M. echinulatus* GUCC 18637, *M. sparsimycelialis* CGMCC 3.19307, and *M. murinus* CBS 864.71 shared a similarity of 99.73%. And *M. verrucosus* CBS: 138278 and *M. restrictus* CBS 138277 showed 98.90% and 98.35%, respectively. For the TEF gene, *M. rothbergiorum* DTO 454-F5 had 97.5% similarity. The *M. sparsimycelialis* LC12480 sequence exhibited 96.1% similarity, while the *M. verrucosus* CGMCC 3.15285 strain showed 96.0% similarity to the *M. sparsimycelialis* LC12480. Strains of *M. restrictus* CBS 138277 and *M. murinus* CBS 864.71 exhibited 96.0% and 95.9% similarities, respectively. In the case of TUB2 gene, *M. sparsimycelialis* LC12478 had a 96.3% similarity while *M. restrictus* CBS 138277 and *M. murinus* CBS 864.71 were 95.8% and 94.7% similar, respectively, and *M. verrucosus* HHAUF170529 presented 94.6% similarity. In the NJ-phylogenetic tree, the KNUF-22-094^T strain was distinctly clustered with various *Microascus* species using ITS, LSU, TEF, and TUB2 gene sequences. Therefore, KNUF-22-094^T represented a distant, novel, and phylogenetically distinct species and thus, it should be placed in the genus as a novel species, for which we proposed the name *Microascus microspora* sp. nov. (Figure 2).

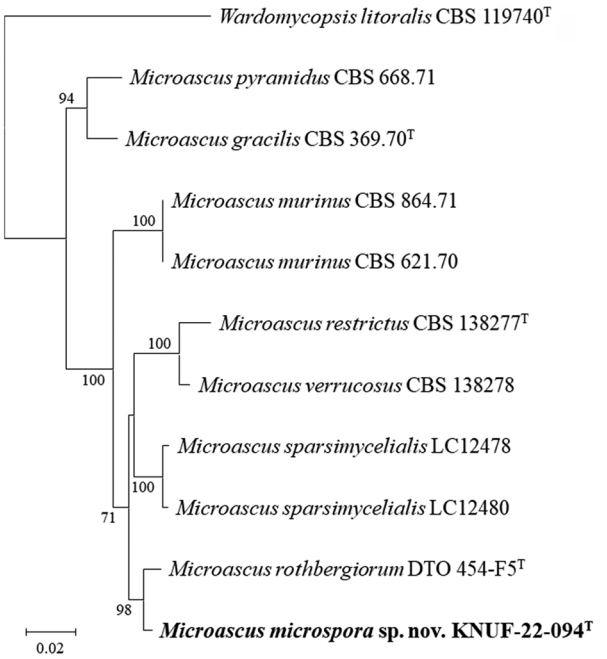

Figure 2. Maximum-likelihood phylogenetic tree based on a combined dataset of the internal transcribed spacer (ITS) regions, partial large-subunit (LSU), translation elongation factor 1-alpha (TEF), and β-tubulin (TUB2) genes sequences showing the phylogenetic position of the strain KNUF-22-094^T among *Microascus* species. Bootstrap values greater than 70% (based on 1000 replications) are shown. The strain isolated in this study is in bold. The tree was rooted using *Wardomycopsis litoralis* CBS 119740^T as an out-group. Bar, 0.01 substitutions per nucleotide position.

3.3. Taxonomical analysis of Tolypocladium terrae sp. nov.

Upon examination, strain KNUF-23-321C^T displayed notable uniqueness in their morphological and molecular characteristics, distantly clustered with the molecular phylogenetic analysis. Given their taxonomic affinity, a comprehensive analysis of cultural and morphological characteristics was exclusively conducted on strain KNUF-23-321C^T, thus representing the proposed species *Tolypocladium terrae* sp. nov.

Tolypocladium terrae S.Y. Lee and H.Y. Jung, sp. nov. (Figure 3).

MycoBank: MB857011

Etymology: *terrae*, ter'.rae. L. gen. n. terrae of the soil, referring to the isolation source of the type strain.

Typus: Chungryoung mountain, Dalseo-gu, Daegu-si, Gyeongbuk (35°47′14.1″N, 128°33′19.7″E) province, South Korea, isolated from soil containing plant debris near native plantation. The stock culture (NIBRFGC000510715) was deposited in the NIBR as a metabolically inactive culture.

Habitat and distribution: Members of the *Tolypocladium* genus have a broad ecological distribution and are found in diverse habitats such as soils, rotifers, lichens, truffle-like fungi, and small dipteran insects [24]. Moreover, some species were isolated from the trunk of *Pinus densa*, indicating that these species have an endophytic lifestyle [22]. Recently, fungal diversity namely *Tolypocladium* explored from mostly soil [7,10]. The novel species proposed in this study was found in soil collected from the rhizosphere of a deciduous tree in the mountains of South Korea.

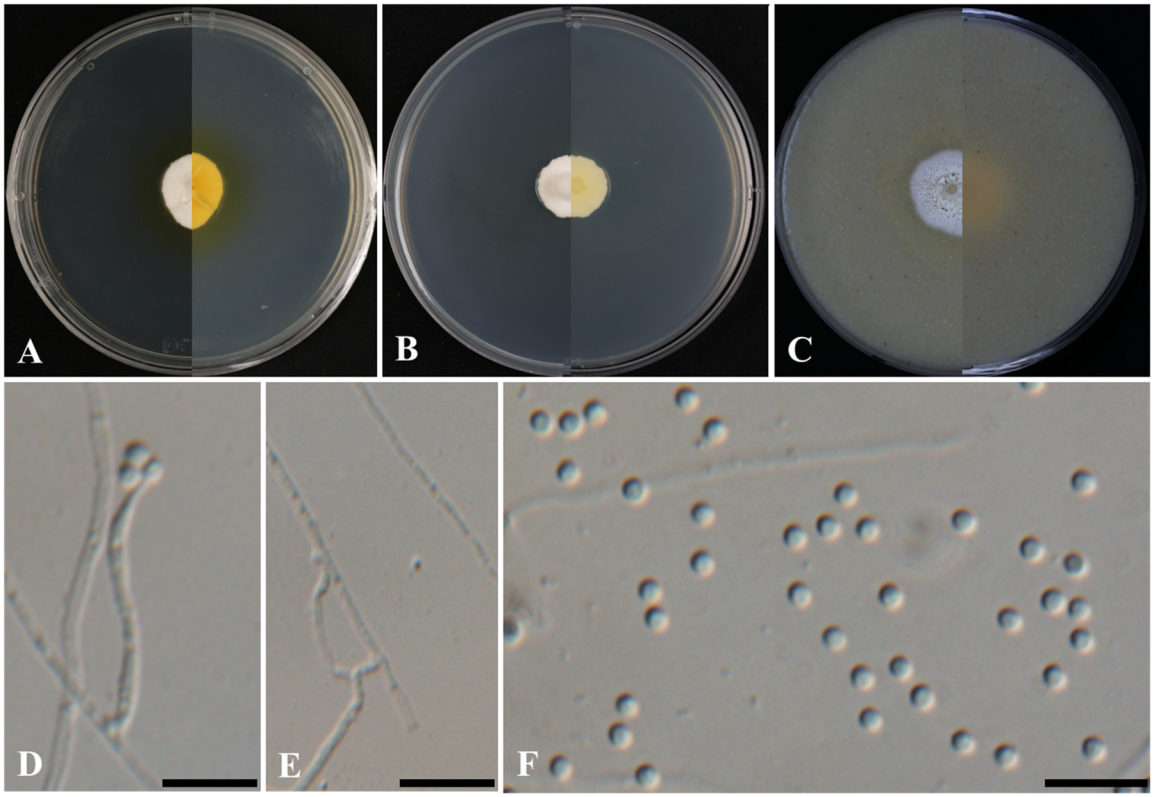

Figure 3. Morphologies of *Tolypocladium terrae* sp. nov. (KNUF-23-321C^T). Colonies on PDA (A); MEA (B); OA (C) at 25 °C for 14 days, obverse and reverse, respectively. Phialides (D, E); conidia (F). Red arrows indicate phialides. Scale bars = 10 µm.

Cultural characteristics: Colonies on PDA, the colonies were flat, white, irregular, wrinkled, reverse colonies were yellow, slow growth with a diameter of 30–31 mm (Figure 3(A)). On MEA, the surface was white, white to light beige, floccose, wavy colonies edge, whereas the reverse surface was pale yellow, with colonies growing to 16–18 mm in diameter (Figure 3(B)). On OA, the colonies grew comparatively faster than PDA and MEA, the surface was floccose and mycelium white, whereas the reverse surface was light yellow, with colonies measuring 21–23 mm in diameter (Figure 3(C)). All media were incubated for 14 days at 25 °C.

Morphological characteristics: The colonies grown on PDA were used to study the morphological structures. Conidiomata were absent, branched hyphae, hyaline, smooth-walled, 1.2–1.7 µm wide (Avg. 1.5) (n = 30). Phialides were 3.7–22.5 × 0.9–1.5 µm, swollen, thick, curved or erect, short phialides were commonly produced attached to conidiophores (Figure 3(D,E)). And the conidiophores were micronematous, erect, conidia were mostly solitary, hyaline, globose, and with a diameter of 1.4–2.4 (–1.9) × 1.5–2.2 µm (Avg. = 1.9 × 1.9 µm, n = 50), L/W ratio of 1.0 (Figure 3(F)).

Note: The strain KNUF-23-321C^T is unique among other *Tolypocladium* species primarily due to its distinctive size range in both conidia and phialides. The phialides KNUF-23-321C^T are subcylindrical, with a notably broad length range of 3.7–22.5 µm and a width of 0.9–1.5 µm, making it one of the most variable in length compared to species like *T. album* (3.5–10 µm, rarely reaching 20 µm) and *T. endophyticum* (4.1 µm on average) [22,24]. Only *T. yunnanense* and *T. pseudoalbum* exhibit similarly broad ranges (up to 62.6 µm and 48.5 µm, respectively) but tend toward cylindrical or ellipsoidal forms rather than subcylindrical [23]. Additionally, KNUF-23-321C^T conidia are mostly globose, measuring 1.4–2.4 × 1.5–2.2 µm, placing them within a compact yet distinct range, larger than the conidia of *T. endophyticum* (1.3 µm) and differing from the cylindrical or broader shapes seen in *T. amazonense* (2.10–2.16 µm) [23,24]. This distinctive size profile in both conidial and phialides dimensions highlights the morphological uniqueness of KNUF-23-321C^T within the genus, thus, the strain was proposed as novel species under the genus of *Tolypocladium* (Table 3).

3.4. Molecular phylogeny of strain KNUF-23-321C^T

Based on the sequencing analyses, the sequence lengths were obtained from strain of 529 bp and

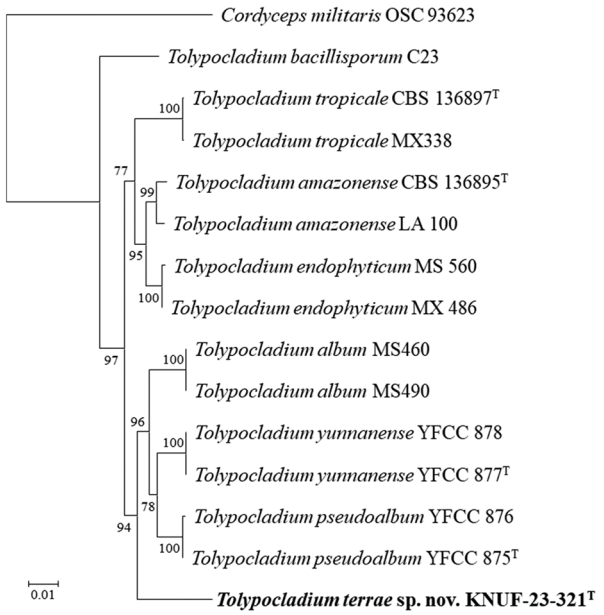

Figure 4. Maximum-likelihood phylogenetic tree based on a combined dataset of the internal transcribed spacer (ITS) regions, partial large-subunit (LSU), and translation elongation factor 1-alpha (TEF) genes sequences showing the phylogenetic position of the strain KNUF-23-321C^T among *Tolypocladium* species. Bootstrap values greater than 70% (based on 1000 replications) are shown. The strain isolated in this study is in bold. The tree was rooted using *Cordyceps militaris* OSC 93623 as an out-group. Bar, 0.01 substitutions per nucleotide position.

804 bp for the ITS regions and 28S rDNA of LSU, whereas TEF gene shows 885 bp. The ITS regions of KNUF-23-321C^T showed 95.6% similarity with *Tolypocladium amazonense* sVPB179, 94.9% with *T. geodes* CBS 126054, 94.3% with *T. album* TR014, and 94.0% with *T. album* CBS 869.73. In LSU gene analyses, *T. paradoxum* YFCC 882 demonstrated 98.4% similarity, followed by *T. globosum* KNUF-22-14A at 98.1% and *T. valdiviae* SGO 171250 at 98.1%. Based on sequence of the TEF gene, *T. amazonense* MS308 showed 94.9% similarity, *T. endophyticum* MX535 demonstrated 94.6%, *T. queenslandicum* BRIP 72623a exhibited 94.3% similarity, and *T. trecense* JMS111 showed 94.3%. The strain KNUF-23-321C was distinctly clustered with other *Tolypocladium* species in the NJ phylogenetic tree based on ITS regions, LSU, and TEF gene sequences, with nodes highlighted in the maximum likelihood. Therefore, KNUF-23-321C^T represented a distant, novel, and phylogenetically distinct species (Figure 4).

4. Discussion

The discovery of two novel fungal species within the genera *Microascus* and *Tolypocladium* underscores the ongoing demand to explore and document fungal biodiversity in South Korea. The novel these two strains were isolated from soil/substrates, adds to the growing body of evidence highlighting the genus's ecological diversity and its role in Korean fungal diversity. Globally, *Microascus* species are recognized for their ecological versatility and occasional pathogenicity. Studies have documented their presence in a variety of environments, from soil and decaying organic matter to clinical settings where they can act as opportunistic pathogens [2,25]. In South Korea, research on fungal biodiversity has highlighted the ecological significance of *Microascus* species. For example, Cho et al. reported the isolation of several *Microascus* species from forest soils, emphasizing their role in nutrient cycling and soil health [26]. Similarly, several novel *Microascus* species identified from tropical forests, illustrating the genus's wide distribution and ecological importance of the genus in Brazil [27].

Furthermore, the novel *Tolypocladium* species are known for their entomopathogenic properties and production of bioactive compounds, *Tolypocladium* species having significant implications for biological control and pharmaceutical applications [5,6]. *Tolypocladium* species have been extensively studied worldwide, particularly for their biocontrol potential and secondary metabolite production. In South Korea, extensive research has been conducted on the genus, particularly regarding its entomopathogenic species. For instance, Wang et al. described the molecular phylogeny and genetic diversity of *Tolypocladium* species, highlighting their complex life cycles and potential for producing valuable bioactive compounds [28]. Additionally, Ranout et al. investigated the use of *Tolypocladium inflatum* in controlling pest populations, demonstrating their practical applications in agriculture [29]. However, the global situation regarding fungal biodiversity research reveals a similar pattern. There is a continuous effort to identify and characterize novel species, driven by advances in molecular techniques and increasing awareness of fungi's ecological roles and biotechnological potential [30,31]. Our findings align with these global efforts, demonstrating the importance of integrating morphological and molecular approaches to uncover fungal diversity. The novel species described in this study not only enhance our taxonomic understanding of *Microascus* and *Tolypocladium* but also highlight their ecological roles and potential applications. Continued exploration and documentation of fungal species are essential for advancing our knowledge of biodiversity and harnessing the biotechnological potential of these organisms. Thus, future research is needed to explore the etiology, pathogenicity, and ecological significance of these novel species in the Korean environment. In addition, various novel and unreported fungal species

continue to be identified from diverse sources, such as marine, plant, and soil samples collected in Korea [32–36]. The report of *M. microspora* sp. nov. and *T. terrae* sp. nov. isolated from soil samples in this study expands our understanding of the distribution of fungi in Korea.

Disclosure statement

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