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RESEARCH ARTICLE

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Molecular Identification of a New Species of *Absidia* (Cunninghamellaceae, Mucorales) Isolated from Soil in Korea

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ABSTRACT

The species within the family Cunninghamellaceae during an investigation of soil microfungi in Korea, in which three strains were isolated from Gangwon, Chungbuk, and Gyeongbuk provinces, designated as KNUF-22-121A, KNUF-22-126A, and KNUF-22-316, respectively. Because the morphological and molecular analyses of these three strains were identical, KNUF-22-316 underwent further detailed study. Phylogenetic analyses based on the concatenated nucleotide sequences of the internal transcribed spacer region and the large subunit 28S rRNA gene revealed that the strain belonged to the genus *Absidia*, but occupied a distinct phylogenetic position. The strain KNUF-22-316 was compared with closely related species *Absidia radiata* CGMCC 3.16257^{T} and *Absidia yunnanensis* CGMCC 3.16259^{T}, morphologically different with shorter sporangiophores, smaller sporangia and columellae, and the consistent presence of collars. Here, we provide a detailed description and images of this proposed new species, which we have named *Absidia microsporangia* sp. nov.

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1. Introduction

The number of circumscribed genera in the family Cunninghamellaceae (Mucorales, Mucoromycetes, Mucoromycota) is controversial. Initially, it included three genera: *Cunninghamella*, *Sigmoideomyces*, and *Thamnocephalis* [1]. Currently, six genera are reported in the family Cunninghamellaceae including *Absidia*, *Chlamydoabsidia*, *Cunninghamella*, *Gongronella*, *Halteromyces*, and *Hesseltinella* [2]. *Absidia*, the type of the family Cunninghamellaceae, was first reported by Van Tieghem [3]. To date, genus *Absidia* contains 51 species and members of the genus are characterized by (i) sporangiophores arranged singly or in pairs or groups on stolons, (ii) rhizoids at both ends of stolons and never opposite the sporangiophores, (iii) deliquescent-walled and apophysate sporangia, (iv) columellae bearing one to several projections, and (v) zygospores enclosed by appendages [2,4,5]. The genus *Absidia* includes various fungal species that are found throughout the environment, most of which are ubiquitous in soil, dung, insects, leaf litter, food, air, and particularly in the mycangia of ambrosia beetles as well as the body surface of bats [6]. Furthermore, species within *Absidia* possess important metabolites for industrial and medical applications, such as steroids, α-galactosidase, laccase, fatty acids, and chitosan [7].

This study aimed to expand our knowledge of native fungal species diversity in Korea and the use of fungal resources in various industries. Here, we present the morphological and molecular characteristics of strains KNUF-22-121A, KNUF-22-126A, and KNUF-22-316, belonging to the genus *Absidia*.

2. Materials and methods

2.1. Sample collection and fungal strain isolation

The fungal strains used in this investigation were isolated from soil samples collected from Gangwon (37°07′32.4″N, 129°09′16.3″E), Chungbuk (36°42′37.6″N, 127°39′05.5″E), and Gyeongbuk (35°18′35.83″N, 129°12′10.75″E) provinces, Korea. Isolation was performed using the plate dilution method as previously described [8]. Strains KNUF-22-121A, KNUF-22-126A, and KNUF-22-316 showed similar cultural characteristics and were from different provinces. Thus, they were

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selected for further morphological and molecular phylogenetic analyses.

2.2. Cultural and morphological characterization

Strains KNUF-22-121A, KNUF-22-126A, and KNUF-22-316 were cultured on potato dextrose agar (PDA; Difco, Detroit, MI) and malt extract agar (MEA; Difco, Detroit, MI) for seven days at 25 °C to investigate their cultural and morphological characteristics [9,10]. Cultural characteristics such as color, shape, and size, were observed. Morphological characteristics were examined under light microscopy (BX-50, Olympus, Tokyo, Japan).

2.3. Genomic DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

Total genomic DNA was extracted from fungal mycelia of strains KNUF-22-121A, KNUF-22-126A, and KNUF-22-316 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 and partial sequences of the large subunit (LSU) 28S rRNA gene were amplified using the primer pair NS5M and LR5M [11]. Then, the PCR products were purified using ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA) and sequenced by SolGent (Daejeon, South Korea) using primers ITS1F, ITS4, and LR0R [12–14].

2.4. Molecular phylogenetic analyses

Strains KNUF-22-121A, KNUF-22-126A, and KNUF-22-316 underwent phylogenetic analysis along with sequences retrieved from the National Center for Biotechnology Information GenBank database (Table 1). Ambiguous regions were deleted from alignments, and evolutionary distance matrices for the neighbor-joining algorithm were calculated using the Kimura two-parameter model [15,16]. Phylogenetic relationships were inferred by tree topology using maximum likelihood [17] and maximum parsimony methods with MEGA7.0 software and 1000 bootstrap replications [18,19].

3. Results

3.1. Taxonomy

Strains KNUF-22-121A, KNUF-22-126A, and KNUF-22-316 were found to be morphologically identical, and they clustered together with respect to molecular phylogeny. Thus, these three strains were described as a new species. Since they were identical, only the cultural and morphological characteristics of strain KNUF-22-316 were described in this study.

Absidia microsporangia S.K. Lim, S.Y. Lee and H.Y. Jung sp. nov. (Figure 1)

MycoBank: 853470

Etymology: The specific epithet is derived from Latin "microsporangia", referring to the small size of sporangia.

Typus: The strain was isolated from soil containing plant debris collected in May 2022 from Korea, Gyeongbuk province, Gijang-gun, Daleum mountain (35°18′35.83″N, 129°12′10.75″E). The stock culture of type strain KNUF-22-316^{T} (NIBRFGC000509838) was deposited in the National Institute of Biological Resources as a metabolically inactive culture.

Habitat and distribution: Rhizosphere soil containing plant debris collected from Gangwon (Gagok-myeon, Samcheok-si), Chungbuk (Jeungpyeong-eup, Jeungpyeong-

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

		GenBank accession numbers	
Species name	Strain number	ITS	LSU
Absidia microsporangia sp. nov.	**KNUF-22-121A**	**OQ868364**	**OQ868366**
Absidia microsporangia sp. nov.	**KNUF-22-126A**	**OQ868365**	**OQ868367**
Absidia microsporangia sp. nov.	**KNUF-22-316^{T}**	**PP264375**	**PP264469**
Absidia ampullaceal	CGMCC 3.16054^{T}	MZ354138	MZ350132
Absidia brunnea	CGMCC 3.16055^{T}	MZ354139	MZ350133
Absidia chinensis	CGMCC 3.16057	MZ354141	MZ350135
Absidia digitula	CGMCC 3.16058	MZ354142	MZ350136
Absidia ovalispora	CGMCC 3.16018	MW264071	MW264130
Absidia ovalispora	CGMCC 3.16019^{T}	NR_176748	MW264131
Absidia radiata	CGMCC 3.16257^{T}	ON074698	ON074684
Absidia radiata	XY09330-1	ON074699	ON074685
Absidia saloaensis	URM 8209	MN953781	MN953783
Absidia soli	MFLUCC 20-0089^{T}	NR_172306	NG_075368
Absidia soli	MFLU-20-0414	MT396373	MT393988
Absidia yunnanensis	CGMCC 3.16259^{T}	ON074700	ON074687
Absidia yunnanensis	XY09528	ON074701	ON074686
Cunninghamella elegans	CBS 167.53	JN205882	HM849700

ITS: internal transcribed spacer regions; LSU: partial sequence of large subunit 28S rDNA.
The newly generated sequences are indicated in bold.

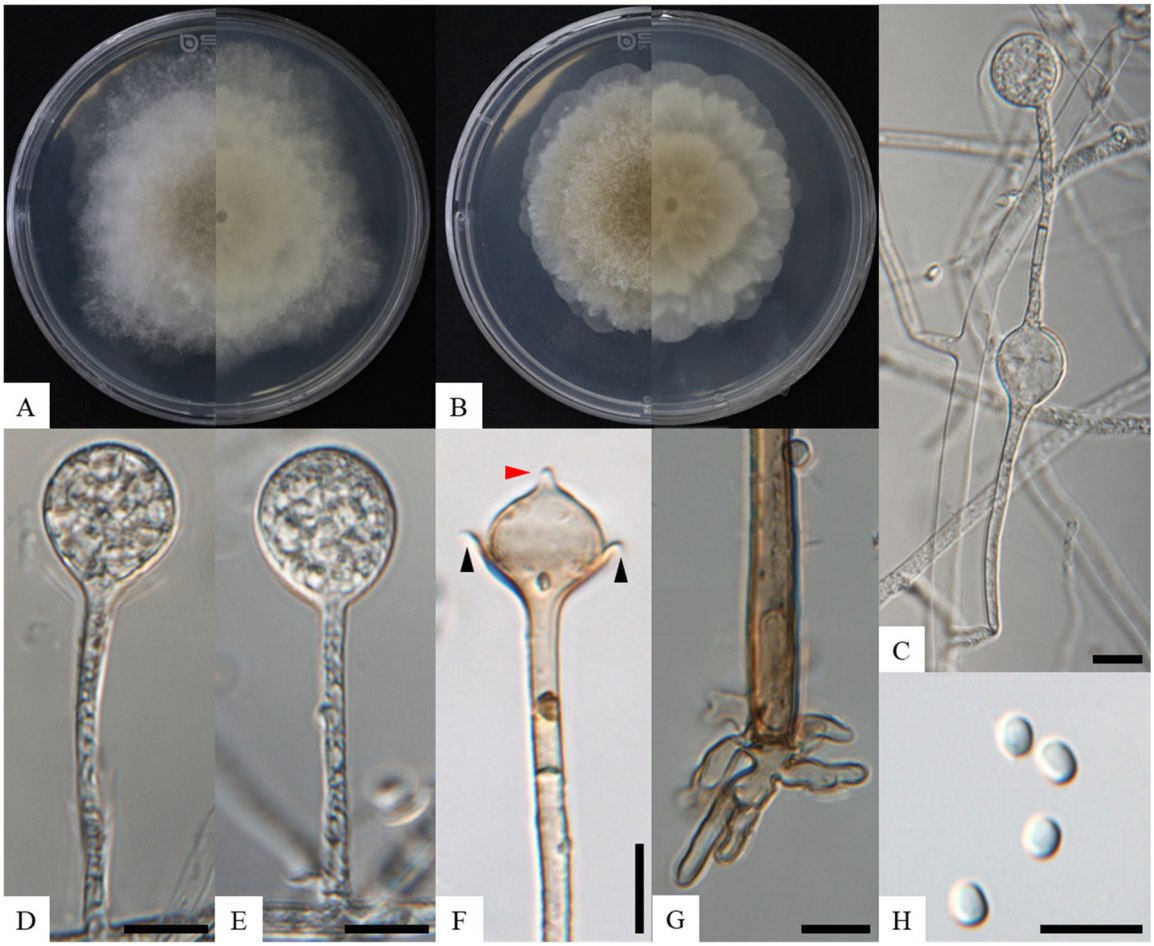

Figure 1. Morphology of *Absidia microsporangia* sp. nov. KNU-22-316[T]. (A, B) Colonies on PDA and MEA at 25 °C for seven days, obverse and reverse, respectively; (C) swelling on sporangiophores; (D, E) sporangium; (F) columellae, collars, and projections; (G) rhizoid; (H) sporangiophores. Scale bars: C–I = 10 µm. Black arrows, collars; a red arrow, projection.

gun), and Gyeongbuk (Jeonggwan-eup, Gijang-gun) provinces, Korea.

Cultural characteristics: Colonies on PDA at 25 °C for seven days moderately fast growth, attaining 73.0–83.2 mm in diameter, floccose, initially white, soon becoming gray to brown, irregular edges; reverse white, irregular zonate (Figure 1(A)). Colonies on MEA at 25 °C for seven days moderately fast growth, attaining 53.6–58.6 mm in diameter, floccose, initially white, soon becoming brown, irregular edges; reverse white, irregular zonate (Figure 1(B)).

Morphological characteristics: Hyphae are branched, hyaline at first, brownish when mature, aseptate when juvenile, septate with age, and 2.4–12.4 µm (n = 30) in diameter. Sporangiophores arise from stolons, mostly erect, bent, in whorls of 1–3, branched, monopodial or sympodial, hyaline, and 36.1–102.8 µm × 1.8–3.1 µm (n = 30). Swellings are usually present below the sporangia, oval to pyriform, hyaline, often with a septum (Figure 1(C)). Sporangia are oval to subglobose, hyaline when young, dark green when old, smooth, multi-spored, and 9.2–16.6 µm × 8.4–16.6 µm (n = 30) (Figure 1(D,E)). Apophyses are distinct, hyaline or subhyaline, gradually widening upwards (base: 1.8–2.6 µm, top: 3.7–5.0 µm, n = 30). Collars are present. Columellae are subglobose or oval, hyaline, subhyaline, or light brown, and 6.2–11.8 µm × 5.6–11.7 µm (n = 30). Projections are single, hyaline to light brown, and 1.2–2.6 µm long (n = 30) (Figure 1(F)). Sporangiospores are regular in size and shape, mostly oval or subglobose, hyaline, smooth-walled, and 2.9–5.8 µm × 2.0–4.4 µm wide ($\bar{x}$ = 4.0–3.0, n = 70) (Figure 1(H)). Rhizoids are root-like, often unbranched, and well-developed (Figure 1(G)). Chlamydospores are absent. Zygospores are not observed.

Other materials examined: Korea, Samcheok-si, Gagok-myeon, Deokpung valley (37°07′32.4″N, 129°09′16.3″E), on soil containing plant debris, May 2021, KNUF-22-121A; Korea, Jeungpyeong-gun, Jeungpyeong-eup, Jwagu mountain (36°42′37.6″N, 127°39′05.5″E), on soil containing plant debris, May 2021, KNUF-22-126A.

Note: The colonies of the strain KNUF-22-316 grew to 59 mm at 25 °C for seven days, whereas both the phylogenetically closest strains, *Absidia radiata*

CGMCC 3.16257^T and *A. ampullacea* CGMCC 3.16054^T, reached 65 mm in the same conditions. Morphologically, the strain KNUF-22-316 produced shorter sporangiophores (36.1–102.8 μm × 1.8–3.1 μm) than *A. radiata* CGMCC 3.16257^T (45.0–273.0 × 3.0–5.0 μm), *A. ampullacea* CGMCC 3.16054^T (30.0–320.0 × 2.5–5.5 μm), and *Absidia yunnanensis* CGMCC 3.16259^T (29.0–159.0 × 2.0–5.0 μm) [2,10]. Additionally, sporangia produced by KNUF-22-316 (9.2–16.6 × 11.5–16.6 μm) were smaller than those of *A. radiata* CGMCC 3.16257^T (17.5–33.5 × 18.5–30.0 μm), *A. ampullacea* CGMCC 3.16054^T (17.5–37.5 × 17.5–45.0 μm), and *A. yunnanensis* CGMCC 3.16259^T (16.0–27.5 × 16.5–27.0 μm) [2,10]. Collars were absent in *A. radiata* CGMCC 3.16257^T and *A. ampullacea* CGMCC 3.16054^T, sometimes present in *A. yunnanensis* CGMCC 3.16259^T, and consistently present in strain KNUF-22-316 [2,10]. The columellae of strain KNUF-22-316 (6.2–11.8 μm × 5.6–11.7) were smaller compared with those of *A. radiata* CGMCC 3.16257^T (13.5–22.5 × 14.0–24.0 μm), *A. ampullacea* CGMCC 3.16054^T (8.5–22.5 × 10.5–24.0 μm), and *A. yunnanensis* CGMCC 3.16259^T (6.5–18.5 × 7.5–22.0 μm) [2,10]. Thus, strain KNUF-22-316 (representative of the proposed new species) differed from the closest species (*A. radiata* CGMCC 3.16257^T, *A. ampullacea* CGMCC 3.16054^T, and *A. yunnanensis* CGMCC 3.16259^T) in terms of sporangiophores, sporangia, columellae, and the presence or absence of collars (Table 2).

3.2. Phylogenetic analysis

The sequence lengths of the ITS regions and LSU gene for all strains of KNUF-22-121A, KNUF-22-126A, and KNUF-22-316 were 515 and 907 bp, respectively. The ITS regions of KNUF-22-121A, KNUF-22-126A, and KNUF-22-316 showed a sequence similarity of 94.5%, 89.9%, and 88.6% with *A. radiata* CGMCC 3.16257^T, *A. yunnanensis* CGMCC 3.16259^T, and *A. ampullacea* CGMCC 3.16054^T, respectively. For the LSU gene sequences, the three strains revealed a high similarity of 98.8% with *A. ampullacea* CGMCC 3.16054^T, 97.7% similarity with *A. yunnanensis* CGMCC 3.16259^T, and a 97.5% similarity with *A. radiata* CGMCC 3.16257^T. A multilocus sequence analysis was performed using concatenated ITS regions and partial LSU gene sequences of strains KNUF-22-121A, KNUF-22-126A, and KNUF-22-316 (Table 1). The neighbor-joining phylogenetic tree based on the concatenated sequences revealed that strains KNUF-22-121A, KNUF-22-126A, and KNUF-22-316 occupied a distinct cluster from other *Absidia* species (Figure 2). Based on phylogenetic analysis and morphological observations, strains KNUF-22-121A, KNUF-22-126A, and KNUF-22-316 were distinct from the previously identified *Absidia* species. Thus, they should be considered a novel species in the genus *Absidia*, for which we have proposed the name *Absidia microsporangia* sp. nov.

4. Discussion

Since 2015, 27 out of a total of 51 species of *Absidia* have been reported, outnumbering those described in the last century. The species of *Absidia* are well known as ubiquitous in soil, dung, and decaying plants, as well as insect remains. However, most *Absidia* species including recently reported new strains of the genus *Absidia* such as *A. abundans*, *A. lobata*, *A. radiata*, *A. sichuanensis*, and *A. yunnanensis*, were isolated from soil [10]. Additionally, *A. jindoensis* was isolated from rhizosphere soil of pine trees in Korea [20]. Although, an increasing number of *Absidia* species, their ecological distribution has still not been unraveled [7]. Despite obscure ecological distribution, the genus *Absidia* was well known for its industrial application by secondary metabolites [21]. For example, *A. spinosa* has been reported to decolorize effluents by degrading cresol red, a

Table 2. Morphological comparison between *Absidia microsporangia* sp. nov. (KNUF-22-316A^T) and the phylogenetically closest species of *Absidia*.

Strain name	*A. microsporangia* sp. nov. KNUF-22-316[a]	*A. yunnanensis* CGMCC 3.16259[b]	*A. radiata* CGMCC 3.16257[b]	*A. ampullacea* CGMCC 3.16054[c]
Colonies on MEA	59 mm for 7 d	65 mm for 7 d	65 mm for 7 d	Reaching 90 mm in 10 d
Sporangiophores (μm)	36.1–102.8 × 1.8–3.1	29.0–159.0 × 2.0–5.0	45.0–273.0 × 3.0–5.0	30.0–320.0 × 2.5–5.5
Sporangia (μm)	Oval to subglobose	Subglobose or pyriform	Subglobose or pyriform	Oval to pyriform
	9.2–16.6 × 8.4–16.6	16.0–27.5 × 16.5–27.0	17.5–33.5 × 18.5–30.0	17.5–37.5 × 17.5–45.0
Collars	Present	Absent or present	Absent	Absent
Columellae (μm)	Oval to subglobose, hyaline	Oval, hyaline, or light green	Oval, hyaline	Hemispherical, hyaline
	6.2–11.8 × 5.6–11.7	6.5–18.5 × 7.5–22.0	13.5–22.5 × 14.0–24.0	8.5–22.5 × 10.5–24.0
Projection	Hyaline to light brown	Subhyaline	Subhyaline	Hyaline
	1.2–2.6 μm in length	1.5–3.5 μm in length	2.0–4.5 μm in length	0.5–2.0 μm in length
Sporangiospores (μm)	Oval or subglobose, hyaline	Cylindrical, light green	Oval, subhyaline	Oval or subglobose, hyaline
	2.9–5.8 × 2.0–4.4	3.5–5.0 × 2.0–4.0	3.0–5.0 × 2.0–3.5	3.0–4.5 × 2.5–4.0

[a]Fungal strain studied in this research.
[b]Source of descriptions [10].
[c]Source of description [2].

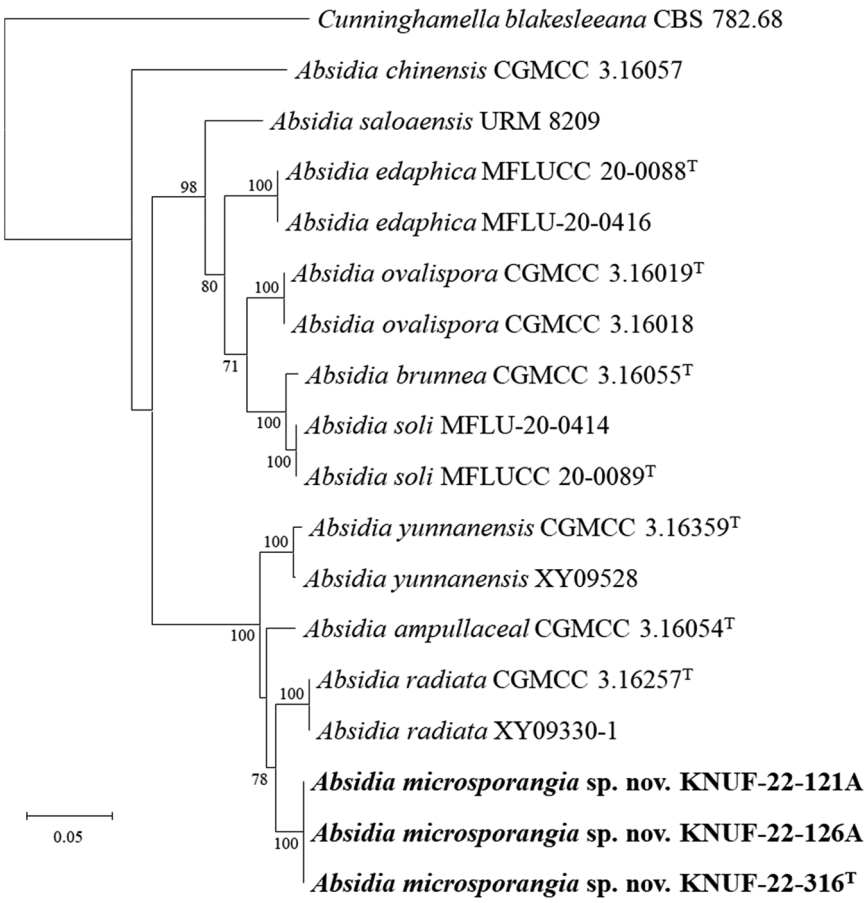

Figure 2. Neighbor-joining phylogenetic tree based on ITS regions and LSU gene sequences showing the phylogenetic position of the three isolated strains among the related strains in the genus *Absidia*. *Cunninghamella elegans* CBS 167.53 was used as the outgroup. The strains isolated in this study are indicated in bold, and the numbers above the branches represent the bootstrap values (>70%) obtained for 1000 replicates. Scale bar = 0.02 substitutions per nucleotide position.

toxic dye used in the textile industry [22], while *A. cylindrospora* was found to remove trace metals, such as Cu, Cd, and Pb, present in soil [23] and produce proteases, which are enzymes of biotechnological interest with high commercial value, using coffee residue as a substrate [24]. Thus, the report of *A. microsporangia* sp. nov. isolated from soil could contribute to enlarging our knowledge of the ecological distribution of the genus *Absidia* and have potential industrial applications by its secondary metabolites.

In conclusion, this research presents our discovery of a new species, *A. microsporangia* sp. nov., isolated from a soil sample in Korea and provides a description according to phylogenetic and morphological evidence. The findings highlight the diversity within the genus *Absidia* and its ecological significance. Additionally, the potential industrial applications of *Absidia* species, particularly through secondary metabolites, underscore the importance of this fungus. Therefore, further research is needed to reveal the ecological distribution of the genus *Absidia* and the industrial potential of secondary metabolites produced by *Absidia microsporangia* sp. nov.

Disclosure statement

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

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