




## Re-Identification of *Aspergillus* Subgenus *Circumdati* Strains in Korea Led to the Discovery of Three Unrecorded Species

Anbzhagan Mageswari<sup>a</sup> , Yunhee Choi<sup>a</sup>, Le Dinh Thao<sup>a,b</sup> , Daseul Lee<sup>a</sup> , Dong-Hyun Kim<sup>a</sup>, Myung Soo Park<sup>c</sup> and Seung-Beom Hong<sup>a</sup>

<sup>a</sup>Korean Agricultural Culture Collection, Agricultural Microbiology Division, National Institute of Agricultural Sciences, RDA, Wanju, Republic of Korea; <sup>b</sup>Plant Protection Research Institute, Hanoi, Vietnam; <sup>c</sup>Department of Crops and Forestry, Korea National University of Agriculture and Fisheries, Jeonju, South Korea

### ABSTRACT

*Aspergillus* is one of the largest and diverse genera of fungi with huge economical, biotechnological, and social significance. Taxonomically, *Aspergillus* is divided into six subgenera comprising 27 sections. In this study, 235 strains of *Aspergillus* subgenus *Circumdati* (section: *Candidi*, *Circumdati*, *Flavi*, *Flavipedes*, *Nigri*, and *Terrei*) preserved at the Korean Agricultural Culture Collection (KACC) were analyzed and re-identified using a combined dataset of partial  $\beta$ -tubulin (*BenA*), Calmodulin (*CaM*) gene sequences and morphological data. We confirmed nineteen species to be priorly reported in Korea (*A. neotritici*, *A. terreus*, *A. floccosus*, *A. allahabadii*, *A. steynii*, *A. westerdijkiae*, *A. ochraceus*, *A. ostianus*, *A. sclerotiorum*, *A. luchuensis*, *A. tubingensis*, *A. niger*, *A. welwitschiae*, *A. japonicus*, *A. nomius*, *A. tamarii*, *A. parasiticus*, *A. flavi*, and *A. oryzae*). Among the studied strains, three species (*A. subalbidus*, *A. iizukae*, and *A. uvarum*), previously unreported or not officially documented, were discovered in Korea, to the best of our knowledge. We have given a detailed description of the characteristic features of the three species, which remain uncharted in Korea.

### ARTICLE HISTORY

Received 3 July 2023  
Revised 7 August 2023  
Accepted 17 August 2023

### KEYWORDS

*Aspergillus* subgenus *circumdati*; unrecorded species; *A. subalbidus*; *A. iizukae*; *A. uvarum*

## 1. Introduction

The genus *Aspergillus* is one of the most ubiquitous and cosmopolitan filamentous fungi of the order *Eurotiales*. Species belonging to this genus are ecologically abundant and can be found in the air, soil, vegetation as well as indoor environments [1,2]. Several of the *Aspergillus* species are economically, biotechnologically, and medically important due to their ability to produce enzymes, organic acids, antibiotics, and other bioactive metabolites [3]. Nevertheless, some of the species are also frequently reported for their detrimental effects such as food spoilage, mycotoxin production, and as causal agents of mycoses [4]. The genus was first introduced in 1729 and has more than one thousand recorded taxa in the database “Index Fungorum.” According to a recent research on the *Aspergillus* taxonomy, the genus comprises – six subgenera (namely, *Aspergillus*, *Circumdati*, *Cremeri*, *Fumigati*, *Nidulantes*, and *Polypaecilum*), 27 sections, 75 series with 446 species [5].

Species delimitation is a vital aspect of taxonomic research and precise identification of strains is essential for targeted applications as well as linking of research taking place across the world. In this

regard, microbial resource centers play an important role in phenetic analysis and conservation of microbial strains of potential value in industry, medicine, environment, agriculture, and other scientific purposes [6]. Among such institutions around the world, Korean Agricultural Culture Collection (KACC) is a major research organization specializing in identification and long-term storage of fungal biodiversity which can serve as a potential source of useful fungal strains. It was established as a part of the Rural Development Administration (RDA) in the year 1995 and the collection currently preserves 14,079 strains of fungi from 3346 species covering all major fungal taxonomic groups, and among these, 9.2% ( $n = 1297$ ) belong to *Aspergillus*.

In earlier days, *Aspergillus* strains were identified based on their morphology and deposited in KACC. In the last two decades, morphology-based identification was often found to be misleading, especially within the *Aspergillus* sections due to the occurrence of cryptic species [7]. Therefore, for accurate identification of *Aspergillus*, a polyphasic approach has been proposed which includes morphological analysis as well as molecular analysis, ecology, and extrolite profiling [8]. Basically, current

CONTACT Seung-Beom Hong  [funguy@korea.kr](mailto:funguy@korea.kr)

identification and phylogeny of *Aspergillus* can be majorly relied on DNA barcodes which include internal transcribed spacer (ITS) region, Calmodulin (*CaM*),  $\beta$ -tubulin (*BenA*), and the RNA polymerase II second largest subunit (*RPB2*) [2].

At present, to improve the quality of the KACC resources, focus has shifted towards re-identification of the conserved strains using molecular techniques mainly based on DNA barcodes mentioned above, in addition to their morphological characteristics. In this context, a subset of *Aspergillus* strains stored in the KACC from 1995 to 2022 was studied using their sequence data as well as morphological characteristics. The identification of all strains was based on partial  $\beta$ -tubulin (*BenA*) and Calmodulin (*CaM*) gene sequences. To date, 81 different *Aspergillus* species have been described from Korea [9,10]. This study aimed to re-identify Korean strains of *Aspergillus* subgenus *Circumdati* preserved in KACC and provide a description of hitherto unrecorded species in Korea based on their morphological and molecular characteristics. This study complements existing knowledge on the diversity of *Aspergillus* species in Korea.

## 2. Materials and methods

### 2.1. Strains

A total of 235 strains belonging to genus *Aspergillus* subgenus *Circumdati* in KACC were studied. All the reagents and media used in the study were procured from Merck, Seoul, South Korea and Oxoid, Basingstoke, UK. The strains were isolated from diverse ecological niches in Korea. All the strains were revived in 4 mL of Malt extract broth, and subsequently transferred to malt extract agar (MEA). The strains examined in the study have been listed in Table 1.

### 2.2. DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from the strains grown on MEA using the DNeasy<sup>®</sup> Plant Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Fragments of the *BenA* (primers Bt2a and Bt2b) and *CaM* (primers CMD5 and CMD6) genes were amplified as outlined by Glass and Donaldson [11], and Hong et al. [12]. The PCR products were sequenced bidirectionally at Macrogen Inc., South Korea, using the same primers used for PCR. Consensus sequences were computed from forward and reverse sequences using DNA STAR Lasergene SeqMan Pro version 10.0.1 (DNASTAR, Inc. Madison, WI).

### 2.3. Phylogenetic analyses

The newly generated sequences were supplemented with reference (preferably ex-type) sequences retrieved from previously published studies [5]. Alignment of the sequences was performed using the CLUSTAL W program [13] and were manually edited with MEGA version 7.0 (University Park, PA) [14]. The maximum likelihood (ML) method was used for the phylogenetic analysis. For ML analysis, the data were first analyzed using the nucleotide substitution model and the best substitution pattern was then used to construct the ML tree with MEGA version 7.0 [14]. To determine the support for each clade, a bootstrap analysis was performed with 1000 replications. The sequence of *Aspergillus calidoustus* CBS 121601<sup>T</sup> was used as an outgroup. The reference sequences used in this analysis have been listed in Table 2. Sequences generated in this study were deposited to KACC-GeneBank (<http://genebank.rda.go.kr>).

### 2.4. Phenotypic analysis

The strains were three-point inoculated on Czapek Yeast extract agar (CYA), Dichloran 18% Glycerol agar (DG18), MEA, and yeast extract sucrose agar (YES) [15]. Media preparation, inoculation, and incubation were performed as described by Samson et al. [2] and all Petri dishes were incubated at 25 °C for 7 d. After 7 d of incubation, colony diameters were measured and colony characteristics were recorded (presence of soluble pigments, exudates, obverse and reverse colony colors, color of conidia). Microscopic examination was performed on colonies grown on MEA using Zeiss Axio imager A1 light microscope equipped with Axio cam ICc3 camera (ZEISS, Seoul, South Korea). Slides were prepared with lactic acid, which was used as the mounting fluid, and ethanol was, at times used to remove excess conidia. The size, shape, and pigmentation of conidia and conidiophores were recorded.

## 3. Results and discussion

### 3.1. Phylogenetic analyses

In this study, phylogenetic position of strains belonging to subgenus *Circumdati* was studied using concatenated data on *BenA* and *CaM* sequences (Figure 1). In the section *Flavi*, most of the strains belonging to *A. flavus* and *A. oryzae* exhibited highly similar *BenA* and *CaM* gene sequences. Therefore, we selected a few representative strains (9 out of 133) of *A. flavus* and *A. oryzae* for further phylogenetic analysis. Information about these strains is given in Table 1. In total, the concatenated

**Table 1.** *Aspergillus* subgenus *Circumdati* strains used in this study.

Section	Re-identified scientific name	Scientific name by depositor	KACC number	Deposited year	Substrate	Region	Location	RDA GeneBank accession no.	
								CaM	BenA
<i>Candidi</i>	<i>A. subalbidus</i> <sup>a</sup>	<i>A. candidus</i>	46481	2011	Meju	Gyeongsangbuk-do	Chilgok-gun	RDA0061631	RDA0061627
		<i>A. candidus</i>	46482	2011	Meju	Gyeonggi-do	Icheon-si	RDA0061632	RDA0061628
	<i>A. neotritici</i>	<i>A. candidus</i>	44245	2005	Rice	Seoul	Seongbuk-gu	RDA0061630	RDA0061629
		<i>A. tritici</i>	46483	2011	Meju	Gyeonggi-do	Yeosu-si	RDA0062637	RDA0062638
<i>Terrei</i>	<i>A. allahabadii</i>	<i>A. tritici</i>	48027	2015	Nuruk	Gyeongsangnam-do	Jinju-si	RDA0062591	RDA0062592
		<i>A. allahabadii</i>	49964	2021	Soil	Jeollanam-do	Damyang-gun	RDA0062655	RDA0062656
	<i>A. floccosus</i>	<i>A. floccosus</i>	49740	2020	Soil	Jeollabuk-do	Buan-gun	RDA0062653	RDA0062654
		<i>A. terreus</i>	47277	2013	Rice straw	Jeollanam-do	Haenam-gun	RDA0062649	RDA0062650
	<i>A. terreus</i>	<i>A. terreus</i>	47390	2014	Air	Korea	Anseong-si	RDA0062647	RDA0062648
		<i>A. terreus</i>	48446	2017	Water	Busan	Saha-gu,	RDA0062651	RDA0062652
<i>Flavipedes</i>	<i>A. iizukae</i>	<i>A. flavipes</i>	43789	2008	Creosote contaminated soil	Seoul	Sangam-dong	RDA0064667	RDA0064670
		<i>A. iizukae</i>	48444	2017	Crab	Busan	Saha-gu	RDA0064668	RDA0064672
<i>Circumdati</i>	<i>A. ochraceus</i>	<i>A. iizukae</i>	48864	2019	Soil	Gyeongsangnam-do	Miryang-si	RDA0064669	RDA0064671
		<i>A. ochraceus</i>	46484	2011	Meju	Jeollanam-do	Haenam-gun	RDA0064675	RDA0064694
		<i>A. ochraceus</i>	46485	2011	Meju	Gyeonggi-do	Icheon-si	RDA0064676	RDA0064695
		<i>A. ochraceus</i>	46486	2011	Meju	Gyeonggi-do	Yeosu-si	RDA0064677	RDA0064696
		<i>A. ochraceus</i>	47132	2013	Soybean	Korea	unknown	RDA0064682	RDA0064701
		<i>A. ochraceus</i>	47273	2013	Rice straw	Jeollabuk-do	Sunchang-gun	RDA0064685	RDA0064704
	<i>A. ostianus</i>	<i>A. ochraceus</i>	47384	2014	Air	Gyeongsangbuk-do	Chilgok-gun	RDA0064687	RDA0064706
		<i>A. ostianus</i>	47134	2013	Soybean	Korea	unknown	RDA0064711	RDA0064702
		<i>A. sclerotiorum</i>	47387	2014	Air	Gyeongsangbuk-do	Chilgok-gun	RDA0064688	RDA0064707
	<i>A. sclerotiorum</i>	<i>A. sclerotiorum</i>	48445	2017	Rice	Jeollanam-do	Gwangju-si	RDA0064691	RDA0064710
		<i>A. steynii</i>	46821	2012	Air	Gyeongsangbuk-do	Chilgok-gun	RDA0064679	RDA0064698
	<i>A. steynii</i>	<i>A. steynii</i>	47388	2014	Air	Gyeonggi-do	Icheon-si	RDA0064689	RDA0064708
		<i>A. westerdijkiae</i>	46822	2012	Wheat straw	Jeollanam-do	Damyang-gun	RDA0064680	RDA0064699
	<i>A. westerdijkiae</i>	<i>A. ochraceus</i>	46823	2012	Soybean	Gyeonggi-do	Yangpyeong-gun	RDA0064681	RDA0064700
		<i>A. westerdijkiae</i>	46487	2011	Meju	Jeollabuk-do	Sunchang-gun	RDA0064678	RDA0064697
		<i>A. westerdijkiae</i>	47139	2013	Soybean	Korea	unknown	RDA0064684	RDA0064703
		<i>A. westerdijkiae</i>	47280	2013	Rice straw	Jeollanam-do	Damyang-gun	RDA0064686	RDA0064705
		<i>A. westerdijkiae</i>	47394	2014	Air	Gyeonggi-do	Yongin-si	RDA0064690	RDA0064709
<i>A. westerdijkiae</i>		42845	2007	Soil	Gyeonggi-do	Suwon-si	RDA0064715	RDA0064763	
<i>Nigri</i>	<i>A. japonicus</i>	<i>A. japonicus</i>	43791	2008	Creosote contaminated soil	Seoul	Sangam-dong	RDA0064716	RDA0064764
		<i>A. aculeatus</i>	47127	2013	Soybean	Chungcheongnam-do	Gongju-si	RDA0065389	RDA0065410
<i>A. luchuensis</i>	<i>A. japonicus</i>	<i>A. japonicus</i>	48322	2017	Unknown	Gyeongsangbuk-do	Sangju-si	RDA0064754	RDA0064798
		<i>A. luchuensis</i>	41731	1995	Nuruk	Chungcheongbuk-do	Boeun-gun	RDA0064713	RDA0064761
		<i>A. luchuensis</i>	46420	2011	Traditional yeast	Gyeongsangbuk-do	Andong-si	RDA0064722	RDA0064770
		<i>A. luchuensis</i>	46490	2011	Meju	Gyeonggi-do	Yeosu-si	RDA0064724	RDA0064772
		<i>A. luchuensis</i>	46491	2011	Meju	Jeollabuk-do	Sunchang-gun	RDA0064725	RDA0064773
		<i>A. welwitschiae</i>	46492	2011	Meju	Jeollanam-do	Haenam-gun	RDA0065375	RDA0065396
		<i>A. niger</i>	46493	2011	Meju	Gyeonggi-do	Icheon-si	RDA0065376	RDA0065397
		<i>A. niger</i>	46494	2011	Meju	Gangwon-do	Hoengseong-gun	RDA0065377	RDA0065398
		<i>A. luchuensis</i>	46516	2011	Nuruk	Korea	unknown	RDA0064727	RDA0064775
		<i>A. luchuensis</i>	46879	2012	Meju	Gyeonggi-do	Yongin-si	RDA0064729	RDA0064777
		<i>A. luchuensis</i>	46958	2012	Nuruk	Gyeonggi-do	Hwaseong-si	RDA0064733	RDA0064781
		<i>A. luchuensis</i>	48309	2017	Meju	Korea	Yangpyeong	RDA0064744	RDA0064788
	<i>A. niger</i>	<i>A. luchuensis</i>	48310	2017	Meju	Korea	Hoengseong	RDA0064745	RDA0064789
		<i>A. luchuensis</i>	48312	2017	Meju	Korea	Yongin-si	RDA0064746	RDA0064790
		<i>A. luchuensis</i>	48313	2017	Meju	Korea	Yongin-si	RDA0064747	RDA0064791
		<i>A. luchuensis</i>	48314	2017	Meju	Korea	Yongin-si	RDA0064748	RDA0064792
		<i>A. luchuensis</i>	48315	2017	Meju	Korea	Yongin-si	RDA0064749	RDA0064793
		<i>A. luchuensis</i>	48316	2017	Meju	Korea	Yongin-si	RDA0064750	RDA0064794
		<i>A. luchuensis</i>	48318	2017	Meju	Korea	Yongin-si	RDA0064751	RDA0064795
		<i>A. luchuensis</i>	48319	2017	Meju	Korea	Anseong-gun	RDA0064752	RDA0064796
		<i>A. luchuensis</i>	48320	2017	Meju	Korea	Yongin-si	RDA0064753	RDA0064797
		<i>A. luchuensis</i>	49930	2021	Soy bean paste	Jeollabuk-do	Wanju-gun	RDA0064758	RDA0064802
		<i>A. luchuensis</i>	49931	2021	Soy bean paste	Jeollabuk-do	Wanju-gun	RDA0064759	RDA0064803
		<i>A. niger</i>	<i>A. niger</i>	40279	1997	Unknown	Korea	unknown	RDA0064712
<i>A. tubingenis</i>	<i>A. niger</i>	42589	2007	Man	Korea	Seoul	RDA0064714	RDA0064762	
	<i>A. niger</i>	44333	2009	Sputum	Korea	Seoul	RDA0064719	RDA0064767	
	<i>A. tubingenis</i>	46498	2011	Meju	Gangwon-do	Hoengseong-gun	RDA0065382	RDA0065403	
	<i>A. luchuensis</i>	46880	2012	Meju	Gyeonggi-do	Yangpyeong-gun	RDA0065380	RDA0065401	
	<i>A. luchuensis</i>	48321	2017	Meju	Gyeonggi-do	Yangpyeong-gun	RDA0065381	RDA0065402	
	<i>A. niger</i>	40278	1997	Unknown	Korea	unknown	RDA0065393	RDA0065414	
	<i>A. niger</i>	40280	1997	Unknown	Korea	unknown	RDA0065394	RDA0065415	
	<i>A. niger</i>	41018	2002	Soil	Korea	unknown	RDA0065392	RDA0065413	
	<i>A. niger</i>	43547	2008	White pine	Korea	Daejeon	RDA0065395	RDA0065416	
	<i>A. tubingenis</i>	43792	2008	Ground soil	Seoul	Sangam-dong	RDA0064717	RDA0064765	
	<i>A. tubingenis</i>	44304	2008	Unknown	Chungcheongnam-do	Gongju-si	RDA0064718	RDA0064766	
	<i>A. tubingenis</i>	44334	2009	Sputum	Korea	Seoul	RDA0064720	RDA0064768	
<i>A. tubingenis</i>	44335	2009	Sputum	Korea	Seoul	RDA0064721	RDA0064769		
<i>A. tubingenis</i>	46446	2011	Campbell early	Chungcheongbuk-do	Yeongdong-gun	RDA0064723	RDA0064771		

(continued)

Table 1. Continued.

Section	Re-identified scientific name	Scientific name by depositor	KACC number	Deposited year	Substrate	Region	Location	RDA GeneBank accession no.		
								CaM	BenA	
		<i>A. tubingensis</i>	46883	2012	Unknown	Gyeonggi-do	Yeosu-si	RDA0064732	RDA0064780	
		<i>A. tubingensis</i>	47137	2013	Soybean	Korea	unknown	RDA0064734	RDA0064782	
		<i>A. aculeatus</i>	47267	2013	Rice straw	Jeollabuk-do	Sunchang-gun	RDA0065378	RDA0065399	
		<i>A. tubingensis</i>	47278	2013	Rice straw	Gyeonggi-do	Anseong-si	RDA0064735	RDA0064783	
		<i>A. tubingensis</i>	47391	2014	Air	Jeollabuk-do	Sunchang-gun	RDA0064737	RDA0064785	
		<i>A. luchuensis</i>	48311	2017	Meju	Korea	Yeosu-si	RDA0065391	RDA0065412	
		<i>A. welwitschiae</i>	48317	2017	Meju	Jeollabuk-do	Sunchang-gun	RDA0065379	RDA0065400	
		<i>A. tubingensis</i>	49868	2021	Plastic debris	Jeollanam-do	Gwangju-si	RDA0064757	RDA0064801	
	<b><i>A. uvarum</i></b>	<b><i>A. uvarum</i></b>	48630	2018	Decayed tree	Seoul	Gwanak-gu	RDA0064755	RDA0064799	
	<i>A. welwitschiae</i>	<i>A. niger</i>	46495	2011	Meju	Gyeonggi-do	Gimpo-si	RDA0065384	RDA0065405	
		<i>A. welwitschiae</i>	46496	2011	Meju	Chungcheongbuk-do	Goesan-gun	RDA0064726	RDA0064774	
		<i>A. niger</i>	46497	2011	Meju	Gyeonggi-do	Yangpyeong-gun	RDA0065383	RDA0065404	
		<i>A. tubingensis</i>	46499	2011	Meju	Jeollabuk-do	Sunchang-gun	RDA0065385	RDA0065406	
		<i>A. welwitschiae</i>	46881	2012	Meju	Gyeonggi-do	Yeosu-si	RDA0064730	RDA0064778	
		<i>A. welwitschiae</i>	46882	2012	Meju	Jeollanam-do	Haenam-gun	RDA0064731	RDA0064779	
		<i>A. niger</i>	47249	2013	Onion	Jeollanam-do	Muan-gun	RDA0065388	RDA0065409	
		<i>A. niger</i>	47272	2013	Rice straw	Jeollanam-do	Haenam-gun	RDA0065387	RDA0065408	
		<i>A. awamori</i>	47376	2014	Air	Gyeonggi-do	Yongin-si	RDA0065386	RDA0065407	
		<i>A. niger</i>	47383	2014	Air	Jeollabuk-do	Sunchang-gun	RDA0065390	RDA0065411	
		<i>A. welwitschiae</i>	48728	2019	Groundnut	Jeollabuk-do	Wanju-gun	RDA0064756	RDA0064800	
Flavi	<i>A. flavus</i> *	<i>A. flavus</i>	45443	2010	Meju	Chungcheongbuk-do	Cheongju-si	RDA0064859	RDA0064879	
	<i>A. nomiae</i>	<i>A. nomius</i>	49741	2020	Soil	Jeollanam-do	Jangseong-gun	RDA0064848	RDA0064868	
	<i>A. oryzae</i> *	<i>A. oryzae</i>	46469	2011	Meju	Korea	unknown	RDA0064866	RDA0064886	
		<i>A. oryzae</i>	46471	2011	Meju	Gyeonggi-do	Icheon-si	RDA0064865	RDA0064885	
		<i>A. oryzae</i>	46640	2012	Meju	Korea	unknown	RDA0064863	RDA0064883	
		<i>A. oryzae</i>	46641	2012	Meju	Korea	unknown	RDA0064864	RDA0064884	
		<i>A. oryzae</i>	46810	2012	Meju	Jeollabuk-do	Sunchang-gun	RDA0064862	RDA0064882	
		<i>A. oryzae</i>	47133	2013	Soybean	Korea	unknown	RDA0064861	RDA0064881	
		<i>A. oryzae</i>	47488	2013	Unknown	Korea	Seoul	RDA0064860	RDA0064880	
		<i>A. oryzae</i>	48141	2016	Air, Indoor	Gyeonggi-do	Bucheon-si	RDA0064867	RDA0064887	
		<i>A. parasiticus</i>	<i>A. parasiticus</i>	46037	2011	Meju environment	Gyeongsangbuk-do	Andong-si	RDA0064856	RDA0064876
			<i>A. parasiticus</i>	46475	2011	Meju environment	Jeollanam-do	Gangjin-gun	RDA0064857	RDA0064877
			<i>A. parasiticus</i>	46901	2012	Peanut	Gyeonggi-do	Yeosu-si	RDA0064858	RDA0064878
		<i>A. tamarii</i>	<i>A. parasiticus</i>	46034	2011	Silkworm	Gyeonggi-do	Suwon-si	RDA0064855	RDA0064875
			<i>A. tamarii</i>	46476	2011	Meju	Jeollabuk-do	Jeongeup-si	RDA0064849	RDA0064869
			<i>A. tamarii</i>	46477	2011	Meju	Gyeongsangbuk-do	Goryeong-gun	RDA0064850	RDA0064870
		<i>A. tamarii</i>	46478	2011	Meju	Gyeonggi-do	Icheon-si	RDA0064851	RDA0064871	
		<i>A. tamarii</i>	46479	2011	Meju	Gyeonggi-do	Suwon-si	RDA0064852	RDA0064872	
		<i>A. tamarii</i>	46480	2011	Meju	Gyeonggi-do	Icheon-si	RDA0064853	RDA0064873	
		<i>A. tamarii</i>	47276	2013	Rice straw	Gangwon-do	Yangyang-gun	RDA0064854	RDA0064874	

<sup>a</sup>The unrecorded *Aspergillus* species in Korea are represented in bold.

\*Only nine representative strains were taken from section *Flavi*.

alignment included 144 sequences: 111 derived from strains of the KACC and the others from publicly available (ex-) type species. The total length of the aligned data set was 1187 characters. The most optimal substitution model was K2 + G+I for the *BenA* and *CaM* data set.

During our studies, the 235 strains taken were spread across 22 different *Aspergillus* species, of which 19 species have been previously reported from Korea. Three species constituted by six strains (highlighted in red bold text) were not previously described from Korea (Figure 1).

In the section *Flavi*, 40 species have been reported worldwide [5,16–18]. Among them, *A. flavus*, *A. nomiae*, *A. parasiticus*, *A. tamarii*, and *A. oryzae* have been recorded in Korea [9,19]. One hundred and forty-four strains from this study, belonging to – the section *Flavi* in Korea clustered with type strains of *A. flavus*, *A. oryzae*, *A. nomiae*, *A. parasiticus*, and *A. tamarii* and identified according to their closest type strain. In this section, *A. flavus/oryzae* complex contributed to a huge number of strains ( $n = 133$ ). Since their *BenA* and *CaM* gene sequences were highly

similar, only a few ( $n = 9$ ) representative strains were selected and shown in Figure 1. Aflatoxin production was used to differentiate the strains between *A. flavus* and *A. oryzae* (data not shown), as the two species cannot be differentiated based on their *BenA* and *CaM* gene sequences.

In the section *Flavipedes*, all 3 strains used in this study were clustered with *A. iizukae*. Nineteen (19) species have been reported in the section *Flavipedes* [5,20,21]. In Korea, 4 species namely *A. capensis*, *A. flavipes*, *A. polyporicola*, and *A. spelaeus* have been recorded already [9]. We are adding a new record of *A. iizukae* in this section which was not previously reported in Korea.

Among 20 known species from the section *Terrei* [5,6,21], four of them, viz., *A. alabamensis*, *A. allahabadii*, *A. floccosus*, and *A. terreus* have been recorded in Korea [9]. Five strains of the section *Terrei* in Korea clustered along with type strains of *A. allahabadii*, *A. floccosus*, *A. terreus* and are therefore identified as *A. allahabadii*, *A. floccosus*, *A. terreus* respectively.

In the section *Candidi* of subgenus *Circumdati*, 5 strains were found to be clustered into two groups

**Table 2.** Reference sequences of *Aspergillus* species used in the phylogenetic analyses.

Section	Species	Strain no.	Country	GenBank Accession no.		
				CaM	BenA	
<i>Candidi</i>	<i>A. campestris</i>	NRRL 13001 <sup>T</sup>	USA	EF669535	EU014091	
	<i>A. candidus</i>	NRRL 303 <sup>T</sup>	Unknown	EF669550	EU014089	
	<i>A. dobrogensis</i>	CCF 4651 <sup>T</sup>	Romania	LT558722	LT627027	
	<i>A. magnus</i>	UAMH 1324 <sup>T</sup>	Canada	ON164619	ON164570	
	<i>A. neotritici</i>	CCF 3853 <sup>T</sup>	Czech Republic	HE661598	FR775327	
	<i>A. pragensis</i>	CCF 3962 <sup>T</sup>	Czech Republic	FR751452	HE661604	
	<i>A. subalbidus</i>	NRRL 312 <sup>T</sup>	Brazil	EF669551	MN969366	
	<i>A. taichungensis</i>	DTO 031-C6 <sup>T</sup>	Taiwan	EU076310	MN969367	
	<i>A. tenebricus</i>	DTO 337-H7 <sup>T</sup>	South Africa	ON164623	ON164584	
	<i>Terrei</i>	<i>A. allahabadii</i>	NRRL 4539 <sup>T</sup>	India	EF669559	EF669531
		<i>A. floccosus</i>	CBS 116.37 <sup>T</sup>	China	KP987066	FJ491714
<i>A. terreus</i>		NRRL 255 <sup>T</sup>	USA	EF669544	EF669519	
<i>Flavipedes</i>	<i>A. flavipes</i>	NRRL 302 <sup>T</sup>	Unknown	EF669549	EU014085	
	<i>A. iizukae</i>	NRRL 3750 <sup>T</sup>	Japan	EF669555	EU014086	
<i>Circumdati</i>	<i>A. elegans</i>	NRRL 4850 <sup>T</sup>	USA	EF661390	EF661349	
	<i>A. ochraceus</i>	NRRL 398 <sup>T</sup>	Unknown	EF661381	EF661322	
	<i>A. ostianus</i>	NRRL 420 <sup>T</sup>	Unknown	EF661385	EF661324	
	<i>A. sclerotiorum</i>	NRRL 415 <sup>T</sup>	USA	EF661384	EF661337	
	<i>A. steynii</i>	NRRL 35675 <sup>T</sup>	India	EF661378	EF661347	
	<i>A. westerdijkiae</i>	NRRL 3174 <sup>T</sup>	South Africa	EF661360	EF661329	
	<i>Nigri</i>	<i>A. aculeatus</i>	NRRL 5094 <sup>T</sup>	Unknown	EF661148	HE577806
<i>A. japonicus</i>		CBS 114.51 <sup>T</sup>	Unknown	FN594551	HE577804	
<i>A. luchuensis</i>		KACC 46772 <sup>T</sup>	Japan	JX500071	JX500062	
<i>A. niger</i>		NRRL 326 <sup>T</sup>	USA	EF661154	EF661089	
<i>A. tubingensis</i>		NRRL 4875 <sup>T</sup>	Unknown	EF661151	EF661086	
<i>A. uvarum</i>		ITEM 4834 <sup>T</sup>	Italy	AM745755	AM745751	
<i>A. welwitschiae</i>		CBS 139.54 <sup>T</sup>	Namibia	KC480196	MN969369	
<i>Flavi</i>		<i>A. flavus</i>	NRRL 1957 <sup>T</sup>	South Pacific Islands	EF661508	EF661485
		<i>A. nomiae</i>	NRRL 13137 <sup>T</sup>	USA	AY017588	AF255067
		<i>A. oryzae</i>	NRRL 447 <sup>T</sup>	Unknown	EF661506	EF661483
	<i>A. parasiticus</i>	NRRL 502 <sup>T</sup>	USA	AY017584	EF661481	
	<i>A. tamaritii</i>	NRRL 20818 <sup>T</sup>	Unknown	EF661526	EF661474	
<i>Outgroup</i>	<i>A. calidoustus</i>	CBS 121601 <sup>T</sup>	Netherlands	HE616559	FJ624456	

CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CCF: Culture Collection of Fungi at the Department of Botany of Charles University in Prague; ITEM: Microbial Culture Collection, Institute of Sciences of Food Production, Bari, Italy; KACC: Korean Agricultural Culture Collection, Wanju, Republic of Korea; NRRL: ARS culture collection, Peoria, IL, USA; T: ex-type strain.

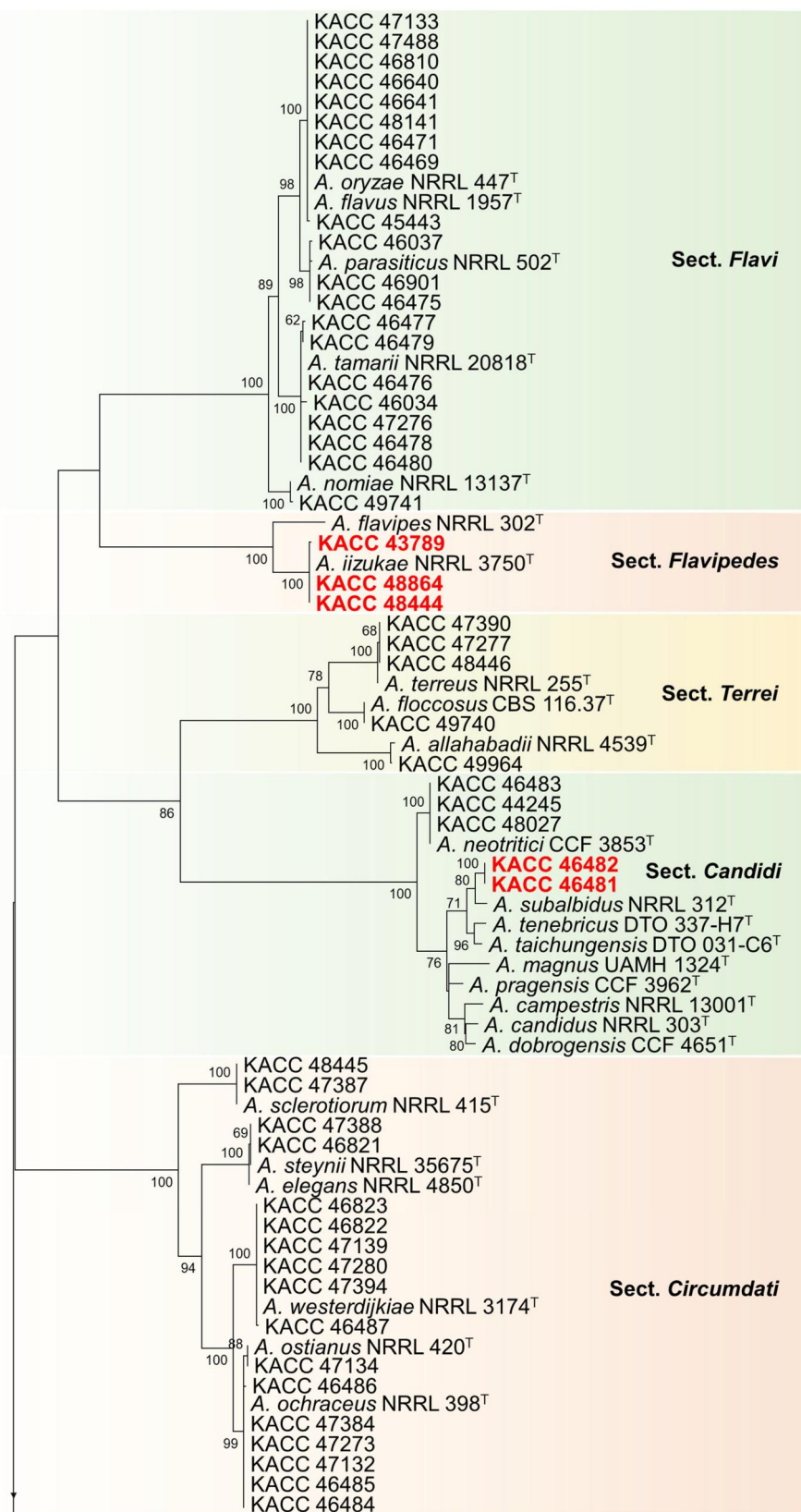
with *A. neotritici* and *A. subalbidus*. Nine species have been recorded in this section [5,22]. Recently, *A. tritici* was synonymized as *A. neotritici* [22]. In *Candidi*, *A. candidus*, *A. pragensis* and *A. neotritici* have been recorded in Korea [9]. *A. subalbidus* has not yet been recorded in Korea, which is included in the current report.

In the section *Circumdati*, 17 strains were found to cluster into five groups, comprising of *A. ochraceus*, *A. ostianus*, *A. sclerotiorum*, *A. steynii*, and *A. westerdijkiae* as nearest type strains. Thirty species have been recorded in the section [5,23,24], of which, *A. ochraceus*, *A. ostianus*, *A. insulicola*, *A. sclerotiorum*, *A. steynii*, and *A. westerdijkiae* are recorded in Korea [9].

In the section *Nigri* of subgenus *Circumdati*, 61 strains grouped into six clusters, with *A. japonicus*, *A. luchuensis*, *A. niger*, *A. tubingensis*, *A. uvarum*, and *A. welwitschiae* as their nearest neighbors. Until now, 32 species have been recorded in this section [5,24,25]. Among them, *A. aculeatus*, *A. brunneoviolaceus*, *A. costaricaensis*, *A. floridensis*, *A. japonicus*, *A. niger*, *A. tubingensis*, *A. luchuensis*, and *A. welwitschiae* are recorded in Korea [9,26]. *A. uvarum* have not been recorded in Korea, and we are reporting new record of *A. uvarum* in Korea in this article.

In the past decade, the classification of *Aspergillus* species has been developed to be based on a combination of molecular data, physiology, morphology, and/or extrolite data [12,27–29]. This approach was used by Houbraeken et al. [5] to clarify the taxonomic position of *Aspergillus*, *Penicillium*, *Talaromyces*, and related genera. In this study, the strains used were documented and preserved at KACC well before the overview paper of Houbraeken et al. [5] and the identification of these strains would have been most likely based on the morphological characters such as growth rate, color of the colony, thermotolerance, and size of conidial heads and conidia. However, currently, morphological features alone are understood to be inadequate to identify species because of morphological characteristics have been found to vary even with respect to change in their ecological habitats [30,31]. In our study, six strains were found to be clustered with unrecorded species of Korea. Till date, there have been few reports on undescribed *Aspergillus* species in Korea despite the genus having a cosmopolitan distribution. On the other hand, there has been a rise of several new *Aspergillus* species worldwide [10].

Among the three hitherto unrecorded species from Korea, the strains KACC 43789, KACC 48444,



**Figure 1.** Phylogenetic position of *Aspergillus* subgenus *Circumdati* strains from the KACC based on a combined data set of partial *BenA* and *CaM* sequences. Bootstrap values >50 are presented at the nodes. The unrecorded species are represented in bold and red in color. Ex-type strains are denoted by the symbol "T." *A. calidoustus* was used as the outgroup.

and KACC 48864 were phylogenetically close to the type strain *A. iizukae* NRRL255<sup>T</sup> belonging to the section *Flavipedes* (Figure 1). Morphological characters of the strains were also consistent with those of *A. iizukae* described by Hubka et al. [32]. The

species was first described by Sugiyama in 1967 [33]. *Aspergillus* section *Flavipedes* endured a re-examination study of species limits using advanced species delimitation methods, and the revised section harbors 19 species with most of the species

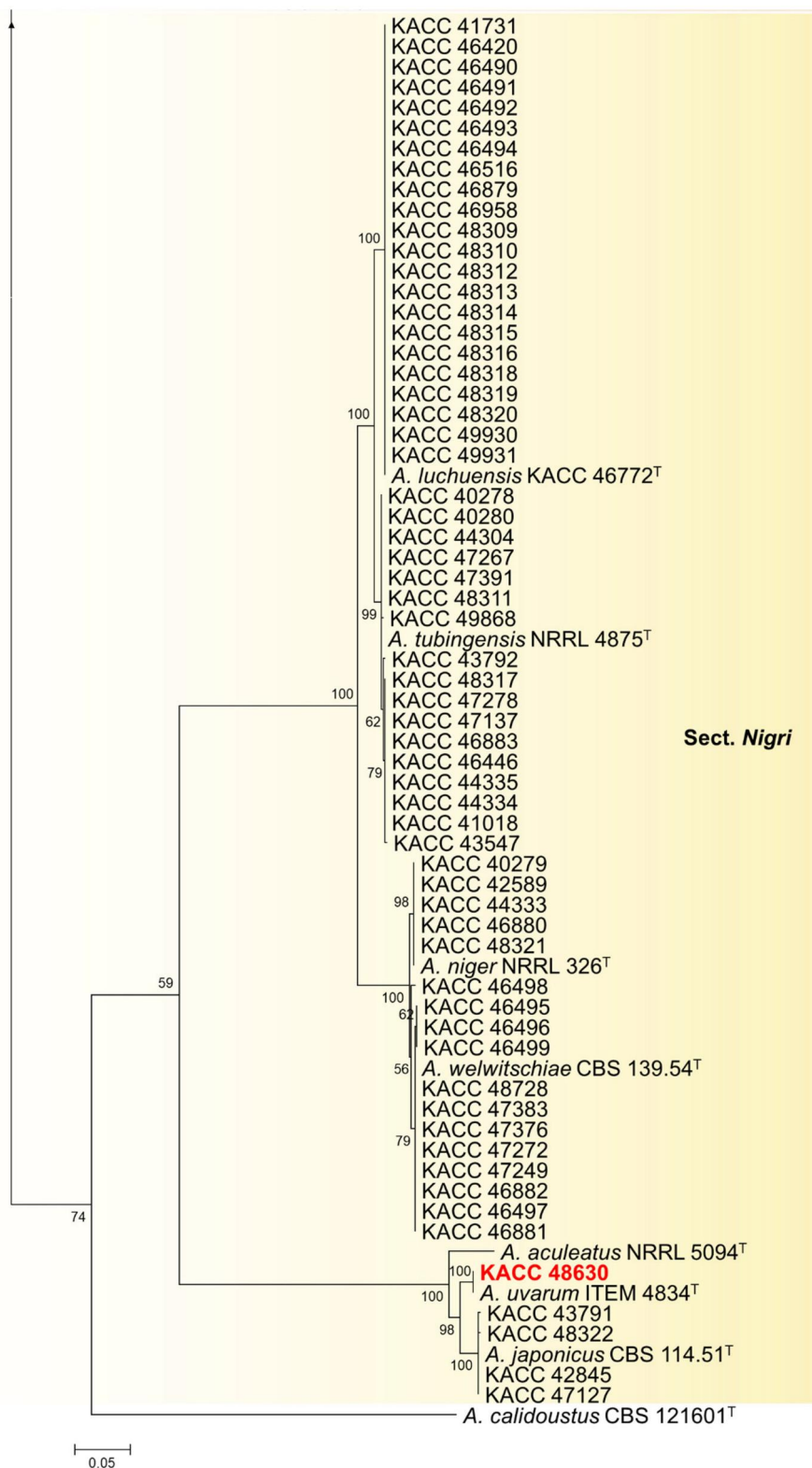


Figure 1. Continued

being reported from soil. Though the most common species from the section are ecologically diverse, occurring in the indoor environment, clinical samples, food and feed, droppings and other less common substrates/environments [20], in this study, the isolates were found to have mainly originated from soil and food. *A. iizukae* have been reported to produce diphenyl derivatives, namely iizukines A (1)

and B (2); flavonolignans, namely Silybin A (1), silybin B (2), isosilybin A (3); oxidative enzymes, such as laccase, manganese peroxidase, and lignin peroxidase [34–36].

Based on their phylogeny, KACC 46481 and KACC 46482 were clustered with *A. subalbidus* NRRL 312<sup>T</sup> (ex-type strain), in section *Candidi* (Figure 1). Strains KACC 46481 and KACC 46482

were morphologically similar to *A. subalbidus*, as described by Visagie et al. [15]. Phylogenetically, *A. subalbidus* forms a separate clade, closely related to *A. tenebricus*, *A. taichungensis*, and *A. neotritici* (Figure 1). Recently, *Aspergillus* section *Candidi* underwent a monographic study, with the revised section *Candidi* hosting nine species. Members in this section have been reported from house dust, soil, herbivore dung, indoor air, and cave environments, and occasionally from clinical specimens [22]. To our best knowledge, this is first study to isolate *A. subalbidus* from meju, thereby revealing its significance as a fungus colonizing food environment.

As shown in Figure 1, strain KACC 48630 aligned with *A. uvarum* ITEM 4834 (ex-type strain) in section *Nigri*. Morphologically, the isolated strain represents similar characters with type strain ITEM 4834 of *A. uvarum* described by Perrone et al. [37]. These include sporulation with dark brown conidia; uniseriate conidiophores; globose to elliptical vesicle, 20–30 µm; and conidia globose to subglobose, spinose, 3–4 µm. Moreover, section *Nigri*, known as black aspergilli includes species with smooth conidiophores and hyaline or pigmentation below the vesicle; globose, subglobose, and pyriform vesicles; typically radiating conidial heads; or divergent columns in certain species [38]. These aspergilli have been isolated from soil samples, air environments, contaminated materials and plants [24,25,39]. In general, 30 species have been accepted in this section [5]. Two additional new species, *A. oxumiae* and *A. hydei*, were reported in soil cultivated with *Agave sisalana*, and from air under the tree *Quercus variabilis* [24,25]. The species *A. uvarum* is a rare member of the group of black aspergilli, which has a high significance in the industry due to its ability to produce secalonic acid, commonly produced by black aspergilli; and geodin, erdin, and dihydrogeodin, which are not produced by any other black aspergilli [37]. Based on macro- and micro-morphological characters and phylogenetic concordance between *BenA* & *CaM* gene phylogenies, we present here three undescribed species of Korea named *A. iizukae*, *A. subalbidus*, and *A. uvarum*.

### 3.2. Taxonomy

***Aspergillus iizukae*** Sugiyama, J. Fac. Sci. Univ. Tokyo, Section 3: 390 (1967) [MB#326636] [33]

Colony characteristics: Colonies on CYA at 25 °C attain 21–22 mm diameter in 7 d, velutinous dull white with granular surface, no soluble pigment, reverse light brown. On MEA, the colonies were velutinous to floccose with granular surface, irregularly or radially wrinkled, light yellowish-brown

sporulation, no soluble pigment, reverse strong yellowish brown and attains 20–21 mm diameter in 7 d. Colonies on YES attain 23–24 mm diameter after 7 d at 25 °C; light yellowish sporulation at center with white mycelium at margins, reverse pale yellow. On DG18, colonies were slow growth, clear white mycelium, reverse white, and reached 8–9 mm in diameter after 7 d at 25 °C.

Micromorphology: Conidial heads biserial, stipes hyaline, smooth-walled, long <1000 µm. Vesicles pyriform, 14–20 µm. Metulae covering one half to entire surface of the vesicle, 3–6 × 2–3 µm. Phialides 5–7 × 2–3.5 µm. Conidia globose, smooth, connectives sometimes remain on free conidia 2–3 µm (Figure 2).

Strains examined: KACC 43789, KACC 48864, and KACC 48444

Remarks: *A. iizukae* is closely related to *A. capensis*. Recent species delimitation study proposed that *A. capensis* is synonymized with *A. iizukae* [20].

***Aspergillus subalbidus*** Visagie, Hirooka & Samson, Studies in Mycology 78: 101 (2014) [MB#809190] [15].

Colony characteristics: On CYA, Colony mycelium and sporulation were found to be white, no soluble pigment present and reverse turned into light brown and eventually reached 17–19 mm in diameter after 7 d at 25 °C. On MEA, the colony surface was floccose, white mycelial areas and sporulation, soluble pigment absent and turned reverse yellow-orange, and further reached 16–22 mm in diameter after 7 d at 25 °C. On DG18, colony mycelium and sporulation were white in color and reverse white with 16–19 mm in diameter after 7 d at 25 °C. Colonies on YES were floccose after 7 d at 25 °C; conidia white, reverse centrally yellowish orange, fading into light yellow toward margin.

Micromorphology: Conidial heads biserial, sometimes reduced Penicillium-like structures present, stipes hyaline, smooth-walled, 100–300 × 4–7 µm. Vesicles globose to subglobose, 6–13 µm. covering 100% of the head; Metulae 4–6 × 2–6 µm; Phialides ampulliform, 6–9 × 2.5–3.5 µm; Conidia globose to subglobose, smooth, 3–4 µm (Figure 3).

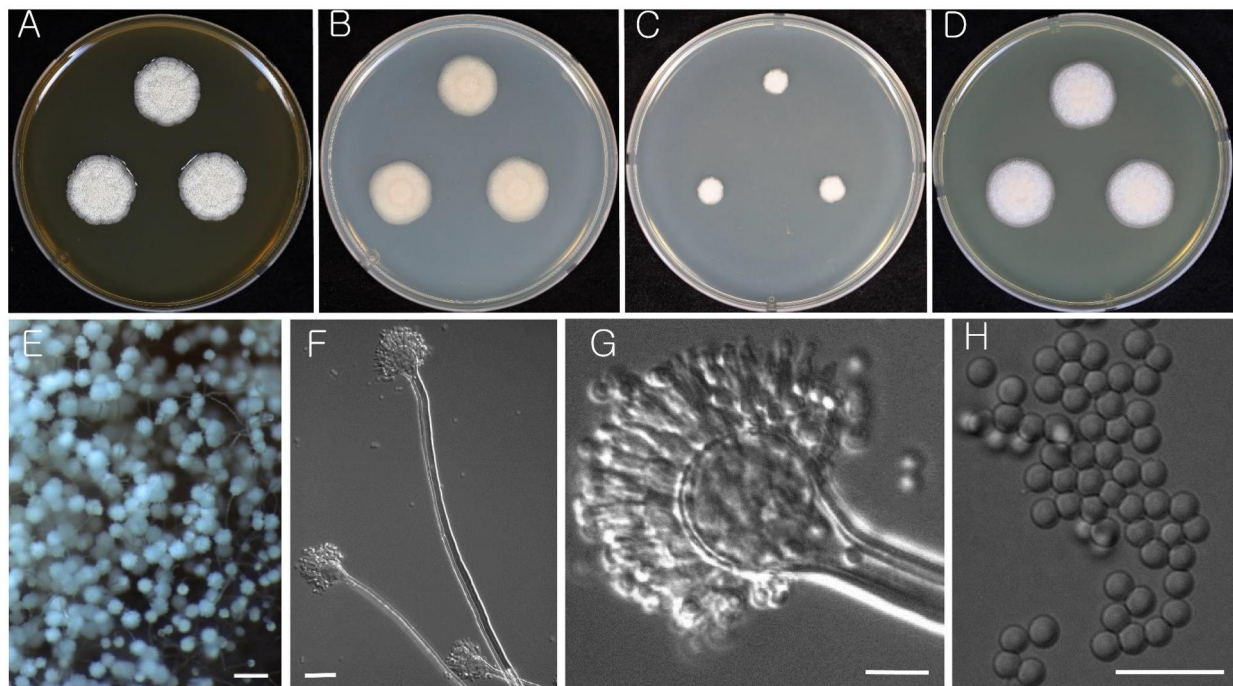
Strains examined: KACC 46481 and KACC 46482

Remarks: *A. subalbidus* is morphologically almost identical to *A. candidus* [15]. Phylogenetically, it forms a separate clade closely related to *A. tenebricus*, *A. taichungensis*, and *A. neotritici* (Figure 1).

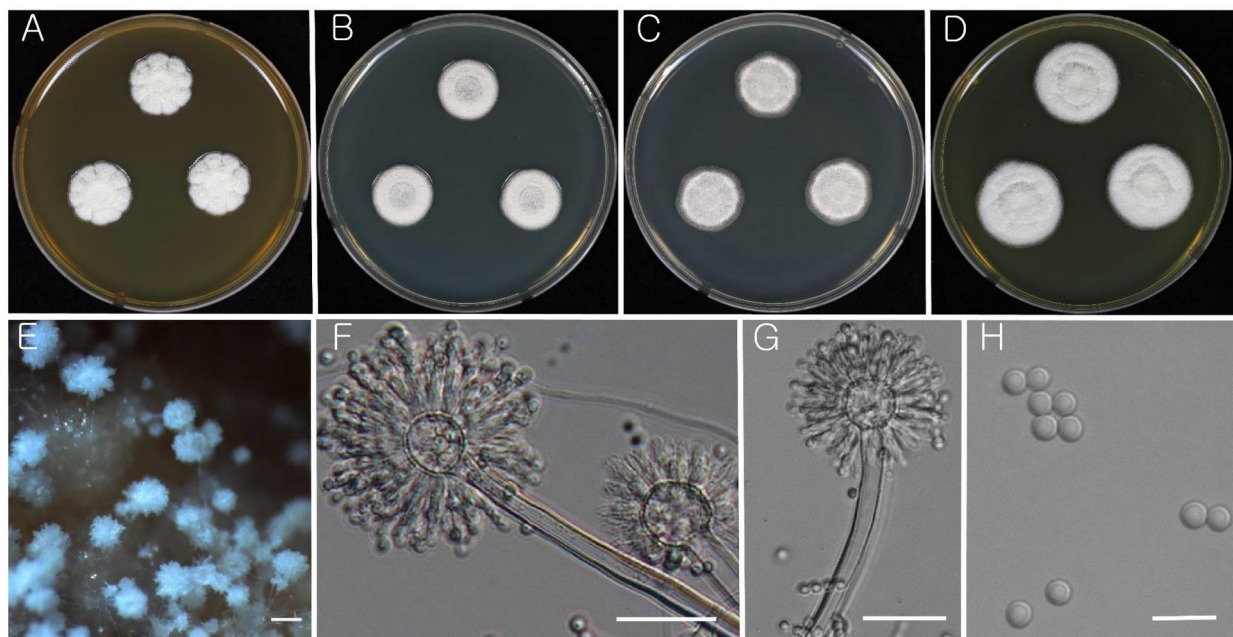
***Aspergillus uvarum*** G. Perrone, Varga & Kozak., International Journal of Systematic and Evolutionary Microbiology 58: 1036 (2008) [MB#510962] [37]

Colony characteristics: On CYA, colonies initially appeared white with flat mycelia and then turned





**Figure 2.** Morphology of *Aspergillus iizukae* (KACC 43789). (A–D) Colonies grown on MEA, CYA, DG18, and YES media after 7 d at 25 °C from left to right. (E) Conidial heads on MEA. (F & G) Conidiophores with conidial head. (H) Conidia. Scale bars: E = 100  $\mu$ m, F = 20  $\mu$ m, G, H = 10  $\mu$ m.

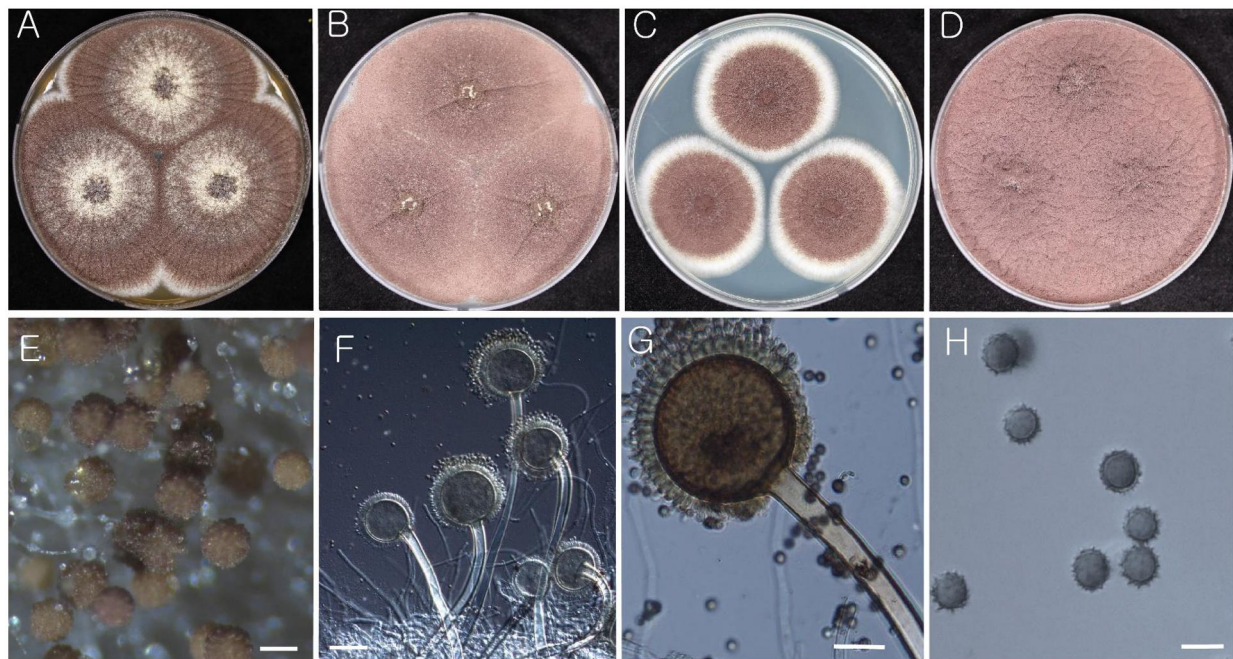


**Figure 3.** Morphology of *Aspergillus subalbidus* (KACC 46482). (A–D) Colonies grown on MEA, CYA, DG18, and YES media after 7 d at 25 °C from left to right. (E) Conidial head on MEA. (F & G) Conidiophores with conidial head. (H) Conidia. Scale bars: E = 100  $\mu$ m, F, G = 20  $\mu$ m, H = 10  $\mu$ m.

brown–black, followed by reverse white, wrinkled, becoming dull yellow with black colony centers by age, and eventually reached 60–62 mm in diameter after 7 d at 25 °C. On MEA, colonies were dark brown–black, with sporulation, widespread, and turned reverse yellow–orange, and further reached 61–63 mm in diameter after 7 d at 25 °C. Colonies on YES were overgrown (90 mm plates) after 7 d at 25 °C; conidia brown–black, Abundant

conidiogenesis, mycelium inconspicuous; reverse light yellow and wrinkled. On DG18, colonies were dark brown with clear white mycelium at margins, reverse white and reached 43–44 mm in diameter after 7 d at 25 °C.

**Micromorphology:** Conidial heads uniseriate, smooth-walled, 500–1000  $\times$  5–10  $\mu$ m. Vesicles globose to elliptical, 50–60  $\mu$ m. Fertile over the entire surface. Phialides 5–7  $\times$  4–5  $\mu$ m. Conidia brown–



**Figure 4.** Morphology of *Aspergillus uvarum* (KACC 48630). (A–D) Colonies grown on MEA, CYA, DG18, and YES media after 7 d at 25 °C from left to right. (E) Conidial head on MEA. (F & G) Conidiophores with conidial head. (H) Conidia. Scale bars: E = 100 μm, F = 50 μm, G = 20 μm, H = 5 μm.

black, globose to sub-globose, 4–5 μm, conspicuously spinose at maturity with spines projecting on the surface with 0.59 μm (Figure 4).

Strain examined: KACC 48630

Remarks: *A. uvarum* is closely related to *A. japonicus* and *A. aculeatus* both morphologically and at a molecular level. Both species have echinulate conidial surface, uniseriate like *A. uvarum* [40]. However, *A. japonicus* has larger vesicle and similar conidial size to *A. uvarum* whereas *A. aculeatus* has a larger vesicle and ellipsoidal conidial shape [37]. KACC 48630 was well differentiated from type strains of *A. japonicus* and *A. aculeatus*. The strain KACC 48630 was deposited as *A. uvarum* in the year 2018. This species was later listed as an unrecorded species within Korea with strain CNUFC YB6 [41], albeit the publication was not effective due to lacking mycological specifics. Therefore, the authors here describe the species officially as unrecorded and publish the species effectively. This paper, therefore, is designed to serve as the official record of *A. uvarum* in Korea.

Microbial resource centers employ several methods to ensure purity of documented strains as any lapses in storage can negate research progress on particular strains [42]. KACC employs a minimum of two different preservation conditions and some of the strains used in this study have been in storage for more than 25 years. All strains in this study successfully revived and showed morphology that is typical of *Aspergillus*. Our study focused on re-identification and describing unrecorded species of *Aspergillus* from various environments, resolving

taxonomic problems, and providing high quality organisms to conserve at KACC. The modern culture collection also has the goal to give access to high quality biological materials with associated information. The data presented in this study reinforces the importance of fungal collections and reassess their strain identification using current techniques.

### Disclosure statement

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

### Funding

This study was financially supported by Ministry of Science and ICT [Grant number M3H9A1081254].

### ORCID

Anbazhagan Mageswari  <http://orcid.org/0000-0002-9278-2614>

Le Dinh Thao  <http://orcid.org/0000-0001-8170-5501>

Daseul Lee  <http://orcid.org/0000-0002-7368-4277>

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