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RESEARCH ARTICLE

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Re-identification of Strains from *Aspergillus* Section *Aspergillus* and Description of Three Unrecorded Species from Korea

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ABSTRACT

The section *Aspergillus* includes xerophilic fungi that are economically significant and broadly distributed in natural settings as well as human habitats and are recognized for their sustenance on substrates with low water activity. Accurate identification of fungal species is essential for any reliable advances in mycological research. In this study, 108 strains from the section *Aspergillus*, originating from Korea and conserved at the Korean Agricultural Culture Collection, were subjected to re-identification using a combined dataset that included partial sequences of β-tubulin (*BenA*), Calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB2*) genes, along with their morphological characteristics. We confirmed the presence of 12 species among the 108 strains originally isolated from Korea. Of them, nine species have been formerly reported in Korea (*Aspergillus chevalieri*, *Aspergillus cibarius*, *Aspergillus cumulatus*, *Aspergillus glaucus*, *Aspergillus montevidensis*, *Aspergillus proliferans*, *Aspergillus pseudoglaucus*, *Aspergillus ruber*, and *Aspergillus tonophilus*), and 3 species (*Aspergillus aurantiacoflavus*, *Aspergillus intermedius*, and *Aspergillus niveoglaucus*) were found to be previously unreported to be isolated from Korea. Here, the detailed characteristic features of these three unexplored species are presented, including specific morphological traits, genetic variations, and ecological niches in Korea.

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1. Introduction

Aspergillus remains to be the most common genera of ascomycetes. It comprises of numerous species that have potential biotechnological, industrial relevance, and to cause agricultural losses [1–3]. The species within the *Aspergillus* genus can be isolated from a diverse array of substrates and hosts, including soil, decaying vegetation, food products, and various organic materials. These fungi are known for their adaptability and ability to thrive in different environmental conditions, making them common inhabitants of numerous ecosystems [4–6]. The taxonomy of these genera has been extensively researched, with the number of described species increasing annually. Visagie et al. [6] provided an updated accepted species list of *Aspergillus* and a new subgeneric classification. According to the current update on the taxonomy of genus *Aspergillus*, there exists 6 subgenera, 28 sections, 88 series with 453 species.

Aspergillus subgenus *Aspergillus* consist of two sections, namely *Aspergillus* and *Restricti*. The species in this subgenus are observed to have uniseriate conidiophore heads with characteristic hyaline, brownish, or greenish stipes, globose to subglobose vesicles in addition with green conidia in mass [7]. The subgenus *Aspergillus* has undergone many taxonomic revisions in the past and currently holds 11 series covering 54 species [5, 6]. Species of section *Aspergillus*, typified with "*Aspergillus glaucus*" group, commonly produce yellow cleistothecia (white for *Aspergillus leucocarpus*) that contain lenticular ascospores. This section also hosts species that were conventionally classified in the genus *Eurotium*.

Members of section *Aspergillus* are tolerant against several environmental stress conditions. These species are reported all over the world and often colonize organic materials, undisturbed dust, stowed cereals, as well as other food products [8, 9], while some these aspergilli have also been reported in clinical samples [10]. Many *Eurotium* species have been reported in Korea from meju acts as a major source for the fermented products of doenjang and ganjang [11]. Prior to the availability of sequence data, the taxonomy of section *Aspergillus* was based on morphological characteristics. Critical characteristics for

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delimitation of species are ascospore pattern, shape, and size. Additionally, the conidial apparatus in combination with the mycelial pigmentation offer valuable supplementary information [8, 12]. Raper [13] highlighted that in few strains the sexual state was found to be dominant, whereas in others, the asexual state has a major influence on colony appearance. Accurate identification based only on their morphological attributes is therefore challenging and sequence based assessments can be considered as a reliable method for rapid and robust species-level identifications. Multiple marker loci-based phylogenetic analysis has become critically strategy for taxonomic studies in fungi and currently, a polyphasic approach to species delimitation is the standard practice for taxonomy of *Aspergillus* [14, 15].

In this study, we revisit and review the taxonomic position of Korean Agricultural Culture Collection (KACC) strains that belong to the section *Aspergillus*. Phylogenetic relationships across members of section *Aspergillus* was investigated using a combined data set which included *BenA*, *CaM*, and *RPB2* gene sequences, and comparing the phenotypic characteristics (macro- and micromorphology). So far, 88 distinct species of *Aspergillus* have been identified and documented in Korea [16–18] and among them, 10 species are included under the section *Aspergillus*. This study was aimed at re-identifying fungal strains belonging to *Aspergillus* section *Aspergillus* from Korea conserved at KACC and to describe the previously unrecorded *Aspergillus* species from Korea on the basis of their morphological and molecular characteristics. Additionally, the study seeks to supplement the existing information with regards to the diversity of *Aspergillus* with respect to Korea.

2. Materials and methods

2.1. Strains

In this study, 108 strains of section *Aspergillus* which were originally isolated from geographically diverse ecological niches in Korea and deposited to be preserved at KACC were taken for investigation. Malt extract broth (BD Difco, Sparks, MD, USA) was initially used to retrieve the strains from storage and the cultures were subsequently transferred to malt extract agar (MEA; Oxoid, Basingstoke, UK). Information with regards to the strains including substrate, location, deposited year, and re-identified species name was documented in Table 1.

2.2. DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from cultures after 7 days of incubation at 25 °C in MEA, using DNeasy® plant mini kit (Qiagen, Hilden, Germany). This was followed by amplification of partial *BenA,* and *CaM* sequences according to the protocols described by Glass and Donaldson [19] and Hong et al. [20]. The third marker gene *RPB2* [5] was amplified and sequenced only in cases where the phylogenetic analysis using *BenA* and *CaM* sequences was ambiguous. The resulting amplified PCR products were sequenced bidirectionally at Macrogen Inc., South Korea, with primers used for PCR amplification. DNA STAR Lasergene SeqMan Pro v.10.0.1. was used to assemble the raw sequences.

2.3. Phylogenetic analysis

Raw sequences obtained after sequencing were supplemented with reference sequences (preferably ex-type) reported in earlier studies [5, 6]. Sequence datasets from each locus were individually aligned with the help of the multiple alignment program MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/) with the G-INS-1 option. Any poor terminal alignments were trimmed and further improved through visually inspection using MEGA 11 followed by concatenation. This was followed by construction of a maximum likelihood (ML) phylogenetic tree which was inclusive of *BenA, RPB2*, and *CaM* gene sequence information.

IQ-TREE tool was used to construct the tree where the best-fit model "TNe + G4" with the option "auto-evaluation of substitution model options according to the provided alignment files" was employed and bootstrap replications set at 1000 [21]. The outgroup remained to be that of the sequence of *Aspergillus candidus* NRRL 303. Information of the used reference sequences are provided in Supplementary Table 1. All the sequences obtained in course of this study were deposited to the RDA-GeneBank (http://genebank.rda.go.kr).

2.4. Phenotypic analysis

For morphological observation, the strains were grown on MEA, Czapek Yeast extract agar (CYA), dichloran 18% glycerol agar (DG18), MEA with 40% sucrose agar (M40Y), and yeast extract sucrose agar (YES) [22] and incubated at 25 °C for 7 days. Additional CYA plates were incubated at 30 and 37 °C. At the end of the incubation, colony diameters and specific characteristics were recorded. Color codes used in description were referred from Rayner [23]. Colonies from MEA and M40Y were taken for microscopic examination which were prepared by mounting with lactic acid. A Zeiss AXIO Imager A1 microscope with differential interference contrast illumination, equipped with a digital AxioCam ICc3 camera (Carl Zeiss, Göttingen, Germany) was used to observe the mounted samples. Recorded microscopic

Table 1. *Aspergillus* section *Aspergillus* strains used in this study.

Species (following re-identification)	Scientific name by depositor	KACC number	Deposited year	Substrate	Location	RDA GeneBank accession no.		
						CaM	*BenA*	*RPB2*
A. aurantiacoflavus[a]	*Eurotium herbariorum*	47311	2013	Rice straw	Damyang-gun	RDA0068878	RDA0068987	–
A. chevalieri	*Eurotium rubrum*	43557	2008	Hay	Daejeon	RDA0068779	RDA0068908	–
	Eurotium chevalieri	45341	2010	Meju	Goryeong-gun	RDA0068788	RDA0068914	–
	E. chevalieri	45342	2010	Meju	Yeoju-si	RDA0068795	RDA0068915	–
	E. chevalieri	45343	2010	Meju	Pocheon-si	RDA0068796	RDA0068916	–
	E. chevalieri	45344	2010	Meju	Pocheon-si	RDA0068797	RDA0068917	–
	E. chevalieri	45345	2010	Meju	Yangpyeong-gun	RDA0068805	RDA0068918	–
	E. chevalieri	45346	2010	Meju	Anseong-si	RDA0068806	RDA0068919	–
	E. chevalieri	45347	2010	Meju	Anseong-si	RDA0068807	RDA0068920	–
	E. chevalieri	46340	2011	Meju	Icheon-si	RDA0068830	RDA0068943	–
	E. chevalieri	46341	2011	Meju	Yongin-si	RDA0068831	RDA0068944	–
	E. chevalieri	46342	2011	Meju	Goesan-gun	RDA0068832	RDA0068945	–
	E. chevalieri	46343	2011	Meju	Sunchang-gun	RDA0068833	RDA0068946	–
	E. chevalieri	46344	2011	Meju	Anseong-si	RDA0068834	RDA0068947	–
	E. chevalieri	46345	2011	Meju	Anseong-si	RDA0068835	RDA0068948	–
	E. chevalieri	47143	2013	Soybean	Anseong-si	RDA0068867	RDA0068976	–
	E. herbariorum	47145	2013	Soybean	Sunchang-gun	RDA0068869	RDA0068978	–
	Eurotium repens	47314	2013	Rice straw	Anseong-si	RDA0068881	RDA0068990	–
	E. chevalieri	47414	2014	Air	Icheon-si	RDA0068884	RDA0068993	–
	E. chevalieri	47800	2014	Meju	Sunchang-gun	RDA0068892	RDA0069001	–
	E. chevalieri	48028	2015	Nuruk	Jinju-si	RDA0068893	RDA0069002	–
	Eurotium sp.	48185	2016	Nuruk	Cheongyang-gun	RDA0068894	RDA0069003	–
	E. chevalieri	48501	2018	Meju	Namyangju-si	RDA0068896	RDA0069005	–
	A. chevalieri	49739	2020	Fresh water	Yeosu-si	RDA0068897	RDA0069006	–
A. cibarius	*A. cibarius*	46346	2011	Meju	Icheon-si	RDA0068836	RDA0068949	–
	A. cibarius	46764	2012	Meju	Yongin-si	RDA0068863	RDA0068972	–
	A. cibarius	46765	2012	Meju	Hoengseong-gun	RDA0068864	RDA0068973	–
	A. cibarius	47141	2013	Soybean	Gongju-si	RDA0068865	RDA0068974	–
	A. cibarius	47683	2020	Fresh water	Yeosu-si	RDA0068891	RDA0069000	–
A. cumulatus	*A. cumulatus*	47316	2013	Rice straw	Anseong-si	RDA0068883	RDA0068992	–
	A. cumulatus	47513	2013	Air	Icheon-si	RDA0068889	RDA0068998	–
	A. cumulatus	47514	2013	Air	Anseong-si	RDA0068890	RDA0068999	–
A. glaucus	*Eurotium echinulatum*	47310	2013	Rice straw	Damyang-gun	RDA0068877	RDA0068986	–
	E. herbariorum	47415	2014	Air	Damyang-gun	RDA0068885	RDA0068994	–
A. intermedius	*E. chevalieri*	43552	2008	Soil	Daejeon	RDA0068773	RDA0068903	–
	E. chevalieri	43553	2008	Hay	Daejeon	RDA0068774	RDA0068904	–
A. montevidensis	*Eurotium amstelodami*	43550	2008	Hay	Daejeon	RDA0068771	RDA0068901	–
	E. amstelodami	43551	2008	Livestock feed	Daejeon	RDA0068772	RDA0068902	–
	E. repens	43554	2008	Soil	Daejeon	RDA0068775	RDA0068905	–
	E. rubrum	43556	2008	Soil	Daejeon	RDA0068778	RDA0068907	–
	E. amstelodami	44958	2010	Garlic	Uiseong-gun	RDA0068781	RDA0068909	–
	E. amstelodami	45337	2010	Meju	Anseong-si	RDA0068782	RDA0068910	–
	E. amstelodami	45338	2010	Meju	Yeoju-si	RDA0068784	RDA0068911	–
	E. amstelodami	45339	2010	Meju	Pocheon-si	RDA0068786	RDA0068912	–
	E. amstelodami	45340	2010	Meju	Anseong-si	RDA0068787	RDA0068913	–
	E. amstelodami	46336	2011	Meju	Yongin-si	RDA0068826	RDA0068939	–
	E. amstelodami	46337	2011	Meju	Anseong-si	RDA0068827	RDA0068940	–
	E. amstelodami	46338	2011	Meju	Yangyang-gun	RDA0068828	RDA0068941	–
	E. amstelodami	46339	2011	Meju	Buan-gun	RDA0068829	RDA0068942	–
	E. repens	46360	2011	Meju	Anseong-si	RDA0068854	RDA0068963	–
	E. amstelodami	46370	2011	Meju	Anseong-si	RDA0068862	RDA0068971	–
	E. amstelodami	47142	2013	Soybean	Gongju-si	RDA0068866	RDA0068975	–
	E. amstelodami	47308	2013	Rice straw	Anseong-si	RDA0068875	RDA0068984	–
	Eurotium heterocaryoticum	47312	2013	Rice straw	Chilgok-gun	RDA0068879	RDA0068988	–
A. niveoglaucus	*E. echinulatum*	46347	2011	Meju	Damyang-gun	RDA0068837	RDA0068950	RDA0069326
	E. echinulatum	46348	2011	Meju	Damyang-gun	RDA0068838	RDA0068951	RDA0069327
	E. echinulatum	46349	2011	Meju	Chilgok-gun	RDA0068841	RDA0068952	RDA0069328
	E. echinulatum	46350	2011	Meju	Damyang-gun	RDA0068842	RDA0068953	RDA0069329
	E. echinulatum	47144	2013	Soybean	Anseong-si	RDA0068868	RDA0068977	RDA0069330
	Eurotium sp.	47147	2013	Soybean	Chilgok-gun	RDA0068871	RDA0068980	RDA0069331
	Eurotium medium	47313	2013	Rice straw	Damyang-gun	RDA0068880	RDA0068989	RDA0069332
	Aspergillus restrictus	47386	2014	Air	Buan-gun	RDA0069008	RDA0069010	RDA0069333
A. proliferans	*E. herbariorum*	45349	2010	Meju	Pocheon-si	RDA0068809	RDA0068922	–
	E. herbariorum	46351	2011	Meju	Pyeongchang-gun	RDA0068843	RDA0068954	–
	E. herbariorum	46352	2011	Meju	Yongin-si	RDA0068846	RDA0068955	–
	E. herbariorum	46353	2011	Meju	Yangpyeong-gun	RDA0068847	RDA0068956	–
	E. herbariorum	46354	2011	Meju	Gyeongsan-si	RDA0068848	RDA0068957	–
	Eurotium mangini	47146	2013	Soybean	Chilgok-gun	RDA0068870	RDA0068979	–
	Aspergillus caesiellus	47377	2014	Air	Icheon-si	RDA0069009	RDA0069011	–
	A. proliferans	48500	2018	Delicacy Squid	Haman-gun	RDA0068895	RDA0069004	–

(*Continued*)

Table 1. Continued.

Species (following re-identification)	Scientific name by depositor	KACC number	Deposited year	Substrate	Location	RDA GeneBank accession no.		
						CaM	*BenA*	*RPB2*
A. pseudoglaucus	*E. repens*	43555	2008	Hay	Daejeon	RDA0068777	RDA0068906	–
	E. herbariorum	45348	2010	Meju	Yecheon-gun	RDA0068808	RDA0068921	–
	E. repens	45350	2010	Meju	Goryeong-gun	RDA0068810	RDA0068923	–
	E. repens	45351	2010	Meju	Yeoju-si	RDA0068811	RDA0068924	–
	E. repens	45352	2010	Meju	Yeoju-si	RDA0068812	RDA0068925	–
	E. repens	45353	2010	Meju	Pocheon-si	RDA0068813	RDA0068926	–
	E. repens	45354	2010	Meju	Pocheon-si	RDA0068814	RDA0068927	–
	E. repens	45355	2010	Meju	Pocheon-si	RDA0068815	RDA0068928	–
	E. repens	45356	2010	Meju	Yangpyeong-gun	RDA0068816	RDA0068929	–
	E. repens	45357	2010	Meju	Buan-gun	RDA0068817	RDA0068930	–
	E. repens	45358	2010	Meju	Anseong-si	RDA0068818	RDA0068931	–
	E. repens	45359	2010	Meju	Anseong-si	RDA0068819	RDA0068932	–
	E. repens	46355	2011	Meju	Icheon-si	RDA0068849	RDA0068958	–
	E. repens	46356	2011	Meju	Yongin-si	RDA0068850	RDA0068959	–
	E. repens	46357	2011	Meju	Yongin-si	RDA0068851	RDA0068960	–
	E. repens	46358	2011	Meju	Seocheon-gun	RDA0068852	RDA0068961	–
	E. repens	46359	2011	Meju	Yongin-si	RDA0068853	RDA0068962	–
	E. repens	46361	2011	Meju	Chilgok-gun	RDA0068855	RDA0068964	–
	E. repens	46362	2011	Meju	Yeongju-si	RDA0068856	RDA0068965	–
	E. repens	46363	2011	Meju	Buan-gun	RDA0068857	RDA0068966	–
	A. pseudoglaucus	46364	2011	Meju	Yangpyeong-gun	RDA0068858	RDA0068967	–
	E. repens	47148	2013	Soybean	Yongin-si	RDA0068872	RDA0068981	–
	E. chevalieri	47309	2013	Rice straw	Gongju-si	RDA0068876	RDA0068985	–
	Eurotium sp.	47416	2014	Air	Yongin-si	RDA0068886	RDA0068995	–
	E. repens	47417	2014	Air	Yongin-si	RDA0068887	RDA0068996	–
A. ruber	*E. rubrum*	45360	2010	Meju	Anseong-si	RDA0068820	RDA0068933	–
	E. rubrum	45361	2010	Meju	Goryeong-gun	RDA0068821	RDA0068934	–
	E. rubrum	45362	2010	Meju	Yeoju-si	RDA0068822	RDA0068935	–
	E. chevalieri	45363	2010	Meju	Yangpyeong-gun	RDA0068823	RDA0068936	–
	E. rubrum	45364	2010	Meju	Yangpyeong-gun	RDA0068824	RDA0068937	–
	E. rubrum	46365	2011	Meju	Yongin-si	RDA0068859	RDA0068968	–
	E. rubrum	46366	2011	Meju	Yongin-si	RDA0068860	RDA0068969	–
	E. rubrum	47149	2013	Soybean	Anseong-si	RDA0068873	RDA0068982	–
	E. rubrum	47315	2013	Rice straw	Sunchang-gun	RDA0068882	RDA0068991	–
	E. rubrum	47418	2014	Air	Anseong-si	RDA0068888	RDA0068997	–
	Eurotium tonophilum	45365	2010	Meju	Yecheon-gun	RDA0068825	RDA0068938	–
A. tonophilus	*E. tonophilum*	46367	2011	Meju	Yangpyeong-gun	RDA0068861	RDA0068970	–
	E. tonophilum	47150	2013	Soybean	Gongju-si	RDA0068874	RDA0068983	–

[a]The Korean unrecorded species of *Aspergillus* are represented in bold.

characteristics included the size, shape, conidial pigmentation, and conidiophore properties.

3. Results

3.1. Phylogenetic analysis

Initially, the phylogenetic associations across 108 section *Aspergillus* KACC strains were compared using the concatenated sequence data *BenA*, *CaM*, and *RPB2* loci (Figure 1). The concatenated dataset alignment contained 2187 characters (1478 constant, 646 distinct, and 391 parsimony-informative sites), including gaps. The concatenated alignment was constituted by 750 characters from *CaM*, 462 from *BenA*, and 975 characters derived from *RPB2* loci. The concatenated phylogenetic analysis assigned all the studied KACC strains into 12 different *Aspergillus* species. Among them, nine species were found to be formerly reported in Korea from various studies but three species (red colored bold text) were not found to be previously described from Korea (Figure 1).

Phylogenetic analysis indicated each of the 12 clades to be well clustered with an ex-type strain of the earlier recorded/described species, supported by 92–100% ML bootstrap values. Among the studied strains, 25 strains clustered with *Aspergillus pseudoglaucus*, 23 strains with *Aspergillus chevalieri*, 18 strains with *Aspergillus montevidensis*, 10 strains grouped with *Aspergillus ruber*, 8 strains with *A. proliferans*, 8 strains with *Aspergillus niveoglaucus*, 5 strains with *Aspergillus cibarius*, 3 strains with *Aspergillus cumulatus*, 3 strains with *Aspergillus tonophilus*, 2 strains with *A. glaucus*, 2 strains with *Aspergillus intermedius*, 1 strain with *Aspergillus aurantiacoflavus*.

3.2. Taxonomy

Aspergillus aurantiacoflavus Hubka, A.J. Chen, Jurjević & Samson, Studies in Mycology 88: 82 (2017) [MB#818732] [24].

Colony morphology: Colonies on CYA 5–6 mm diameter in 7 days at 25 °C, entire margin, mycelium sulfur yellow (15), no soluble pigment, reverse buff (45). Colonies were floccose, sulfur yellow (15) to orange (7) mycelium, dense sporulation at center, ocherous (44) in reverse and reaches 32–33 mm diameter after 7 days on DG18 at 25 °C. Colonies attain 48–50 mm

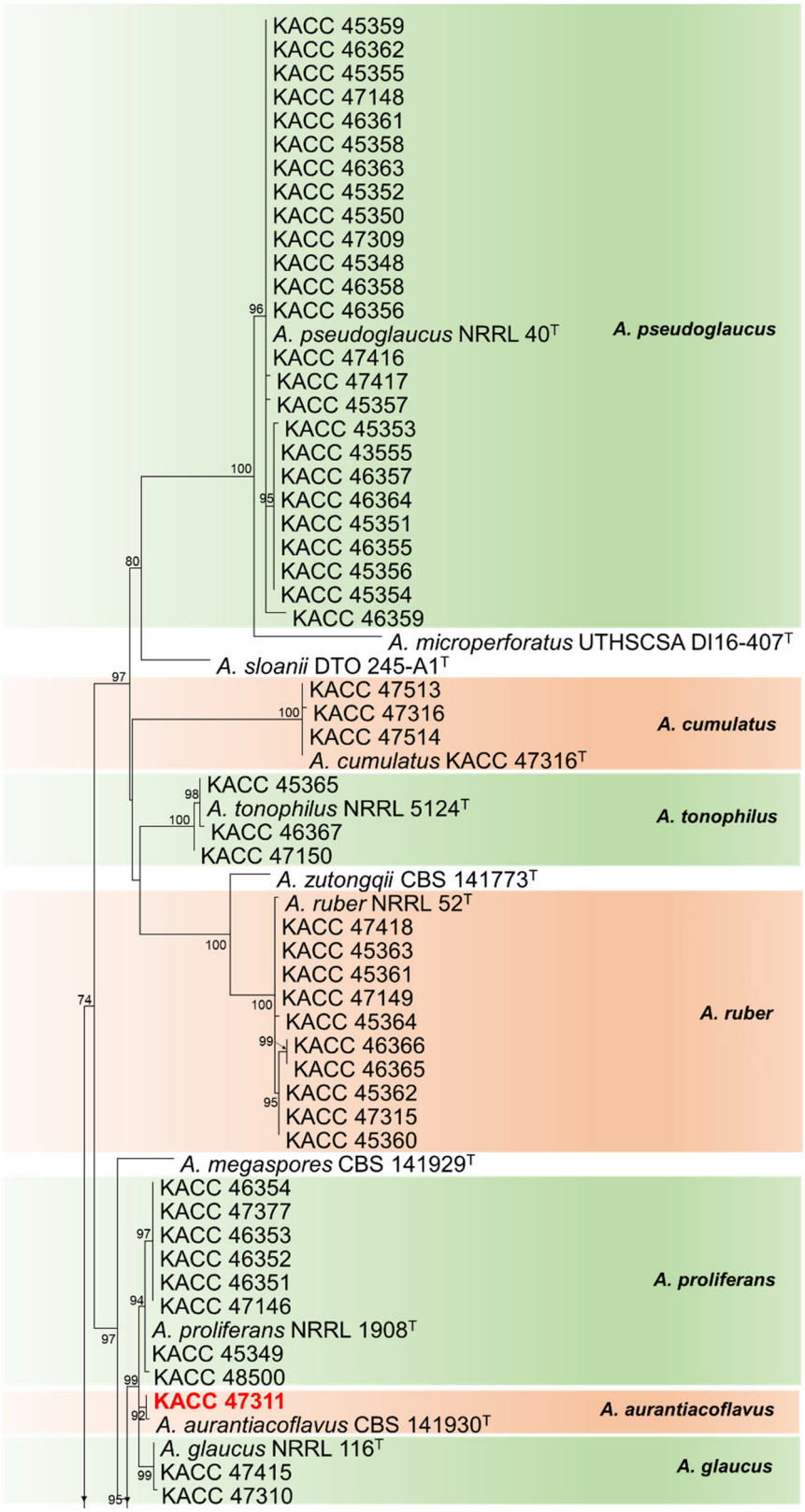

Figure 1. Maximum likelihood tree of *Aspergillus* section *Aspergillus* strains from the KACC based on a combined data set containing partial *BenA, CaM*, and *RPB2* (a few) sequences. Bootstrap values ≥70 are indicated at each node. Scale bar indicates number of substitutions per nucleotide. Unrecorded species are distinguished by bold font and red color. Ex-type strains are designated by ^{T}. The species *A. candidus* was used as the outgroup.

diameter on YES at 25 °C at the end of 7 days; fluffy colonies, luteous (12) at center with white mycelium at margins, reverse pale luteous (11). Colonies growth on MEA was negligible after 7 days at 25 °C. The colonies were floccose, centrally raised dense sporulation, conidia grayish green (50), yellow ascomata, white mycelium at periphery, buff (45) in reverse and reaches 52–53 mm diameter in 7 days on M40Y.

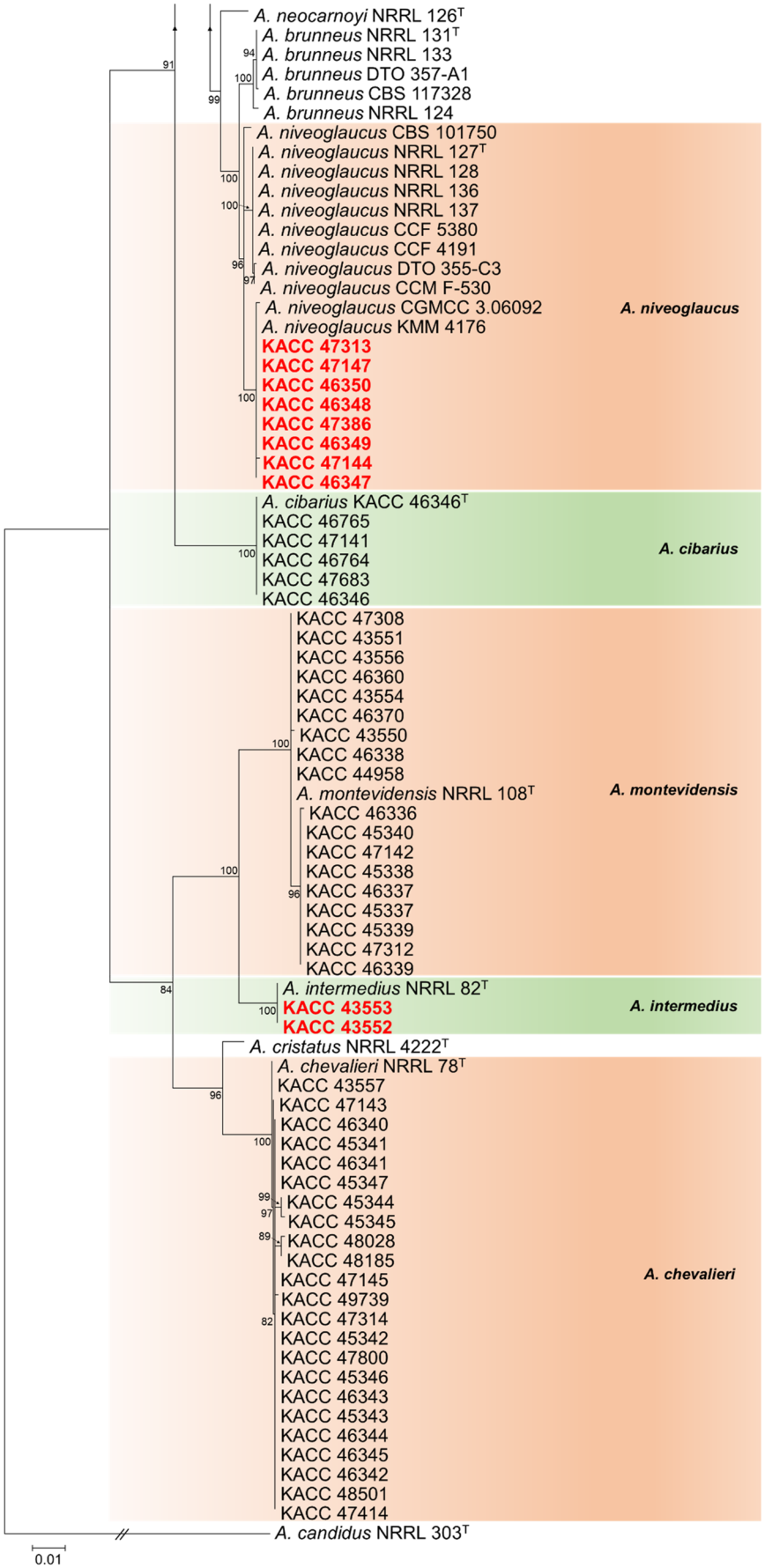

Figure 1. Continued.

Micromorphology: Ascomata eurotium-like, cleistothecial, superficial, yellow, globose to subglobose, 104–210 µm. Ascospores hyaline, in surface view globose to ellipsoidal, (4–) 5–6 × 4–5 µm, in side view lenticular, an equatorial furrow is discernible. Conidiophores are smooth-walled, stipes hyaline,

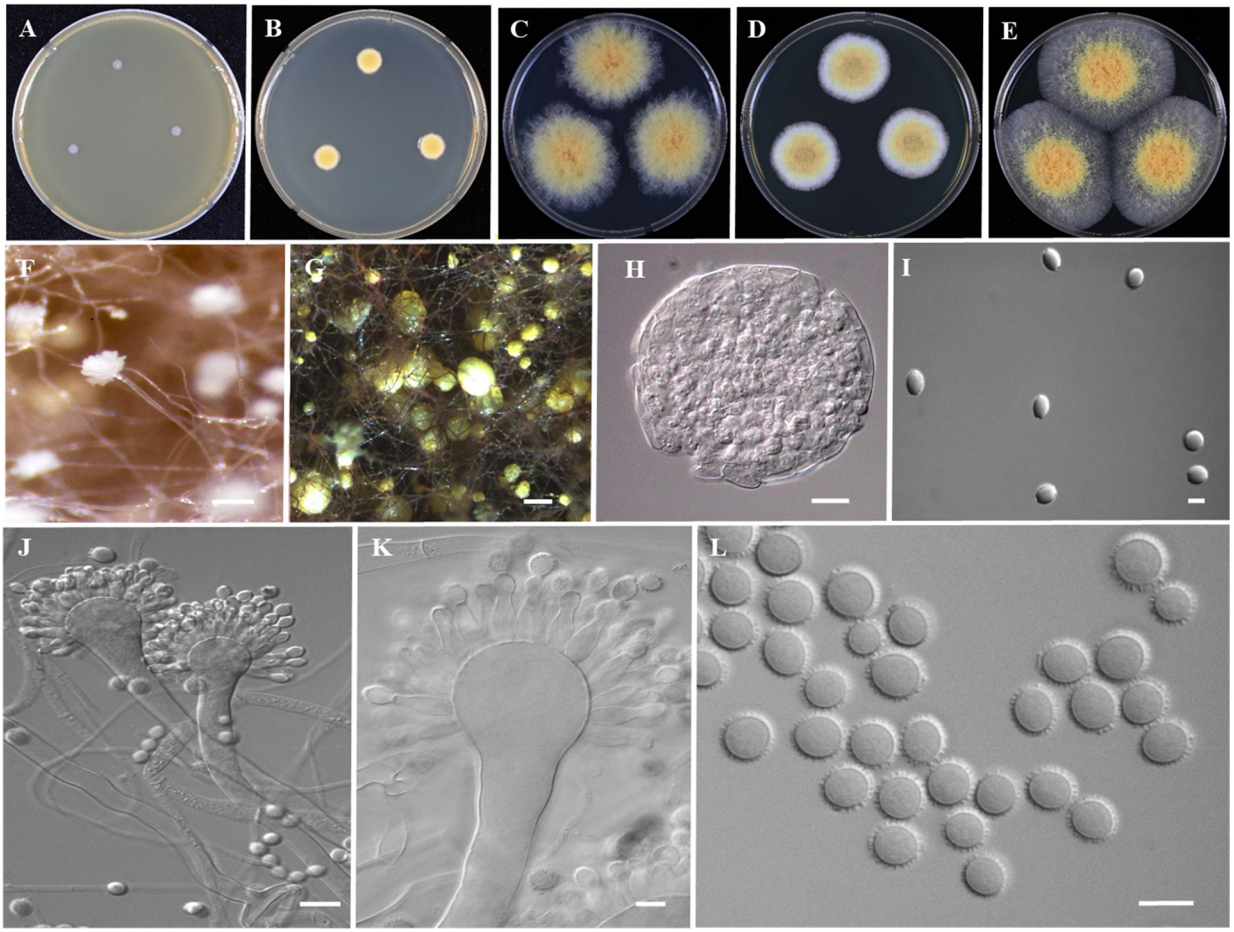

Figure 2. Morphology of *Aspergillus aurantiacoflavus* (KACC 47311). (A–E) Colonies on MEA, CYA, DG18, YES, and M40Y media upon 7 days incubation at 25 °C from left to right; (F) Conidiophores with conidial head on M40Y; (G) Ascomata on M40Y; (H) Ascomata; (I) Ascospores; (J,K) conidiophores with conidial head; (L) Conidia. Scale bars: F,G = 100 µm, H,J = 10 µm, I,K,L = 5 µm.

150–520 × 6–9 µm. globose to subglobose vesicles, 18–27 µm wide, fertile over two thirds to entire surface. Flask-shaped phialides, 7–11 × 3.5–6 µm. Conidia globose to subglobose, few are tuberculate, rough, (4–) 5–6 (–8) × (4–) 4.5–5.5 (–7) µm (Figure 2).

Strain examined: KACC 47311

Remarks: KACC 47311 was similar to *A. aurantiacoflavus* described by Hubka and coauthors [24]. Matching to the description of type strain, colonies of KACC 47311 on DG18 and YES media showed heavy ascomata formation and sulfur yellow (15) color after 14 days at 25 °C.

Aspergillus intermedius Blaser, Sydowia 28: 41 (1975) [MB#309226] [25]

Colony morphology: On CYA, the colonies attained 10–11 mm diameter at the end of 7 days incubation at 25 °C, mycelial areas sulfur yellow (15) with grayish green (50) sporulation, white mycelium at periphery, reverse luteous (12). Colonies on MEA were floccose, slightly sulcate, luteous (12) with fawn (87) sporulation at midpoint, bluish green (23) at margins, minute brown vinaceous (84) exudates, reverse olivaceous (48) and attains 14–15 mm diameter at 25 °C after 7 days. The colonies were pure yellow (14) in appearance with sparse dark green (21) sporulation, reverse luteous (12) and extended 34–35 mm in diameter after 7 days at 25 °C on DG18. In case of YES media, the colonies attain 33–34 mm diameter in 7 days at 25 °C; luteous (12), moderately radially sulcate toward the center, sparse dark green (21) sporulation with white mycelium at margins, reverse olivaceous (48). Colonies on M40Y reached 53–54 mm diameter after 7 days at 25 °C; luteous (12) with sparse grayish green (50) sporulation, reverse pale luteous (11).

Micromorphology: Ascomata eurotium-like, cleistothecial, superficial, yellow, globose to subglobose, 100–200 µm. Ascospores are globose to subglobose, slightly roughened surface, (3–) 4–4.5 × 3–4 µm, in side view lenticular, have a conspicuous equatorial furrow flanked by long, straight, or wavy, crests 0.5–0.8 µm. Conidiophores with smooth stipes, hyaline, 140–400 × 7.0–11 µm. Vesicles globose, 15–40 µm. Ampulliform phialides, 5–9 × 2–3.5 µm. Conidia were found to be globose to subglobose, microtuberculate, finely roughened, 3–4.5 × 2.5–4 µm (Figure 3).

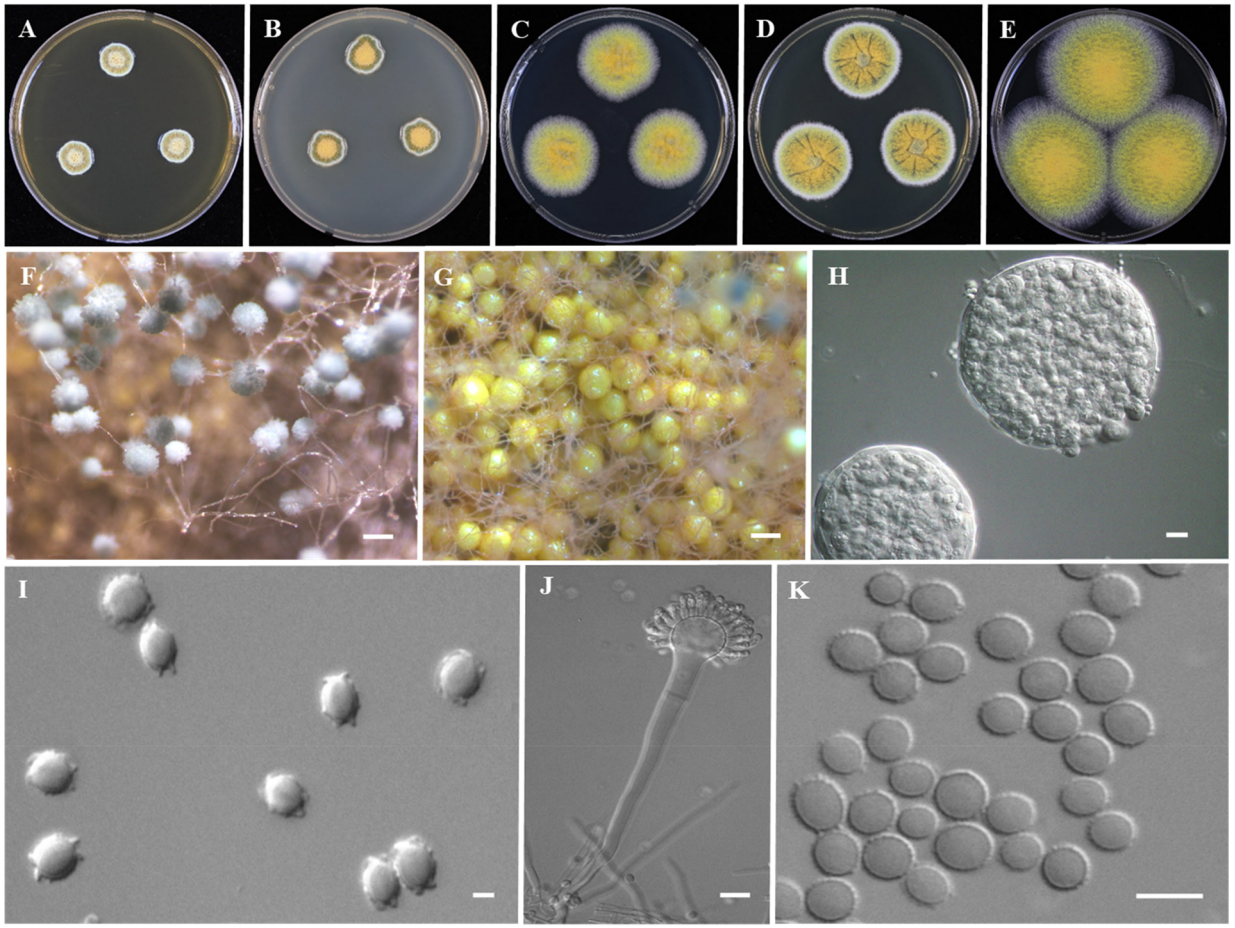

Figure 3. Morphology of *Aspergillus intermedius* (KACC 43522). (A–E) Colonies on MEA, CYA, DG18, YES, and M40Y media upon 7 days incubation at 25 °C from left to right; (F) Conidiophores with conidial head on MEA; (G) Ascomata on M40Y; (H) Ascomata; (I) Ascospores; (J) Conidiophores with conidial head; (K) Conidia. Scale bars: F,G = 100 µm, H,J = 10 µm, I = 2 µm, K = 5 µm.

Strains examined: KACC 43552 and KACC 43553

Remarks: KACC 43552 and KACC 43553 were morphologically close to *A. intermedius* earlier described by Blaser [25]. However, KACC 43552 and KACC 43553 showed finely roughened conidia but *A. intermedius* CBS 523.65 has smooth conidia [24, 25]. Additionally, colonies on MEA indicated brown vinaceous (84) droplets like exudate after 10 days at 25 °C which are not present in the type strain description. At 30 °C on CYA, both strains KACC 43552 and KACC 43553 displayed exceptional growth with dense grayish green (50) sporulation, similar to the type strains.

Aspergillus niveoglaucus Thom & Raper, U.S.D.A. Misc. Pub. 426: 35 (1941) [MB#120985] [12]

Colony morphology: The colonies were white mycelium, no soluble pigment, grayish green (50) sporulation with luteous (12) at center, reverse olivaceous (48) and final diameter of 12–13 mm after 7 days at 25 °C on CYA medium. Colonies growth on MEA was less and reached 5–6 mm diameter after 7 days at 25 °C. The colonies were floccose with grayish green (50) to bluish green (23) sporulation, no soluble pigment, white in reverse, and further reached 49–50 mm in diameter on DG18 at 25 °C after 7 days. On YES, bluish green (23) sporulation encircled by white mycelium fluffy colony and white in reverse with 30–32 mm in diameter at 25 °C after 7 days. On M40Y medium, the colonies were floccose, fluffy, bluish green (23) sporulation with 45–47 mm in diameter after 7 days at 25 °C; conidia grayish green (50), reverse white, white mycelium toward the margins.

Micromorphology: Ascomata eurotium-like, cleistothecial, superficial, yellow, globose to subglobose, 90–200 µm. Asci 8 spored, globose to subglobose. Ascospores hyaline, smooth-walled, globose to subglobose in surface view, 6–7 × 4–7 µm, in side view lenticular, furrow present. Hyaline stipes, smooth-walled, 750–1100 × 7–12 µm. Vesicles were globose to subglobose, measuring 25–50 µm and covered the entire surface of the head. Phialides were flask-shaped, 8–14 × 4–6 µm. Conidia were observed to be subglobose to ellipsoidal, tuberculate, rarely globose, rough, 6–8 (–9) × 5–7 (–8) µm (Figure 4).

Strain examined: KACC 46347, KACC 46348, KACC 46349, KACC 46350, KACC 47313, KACC 47386, KACC 47144, and KACC 47147.

Remarks: Phylogenetic closeness of *A. niveoglaucus* to *Aspergillus brunneus* and *Aspergillus neocarnoyi*

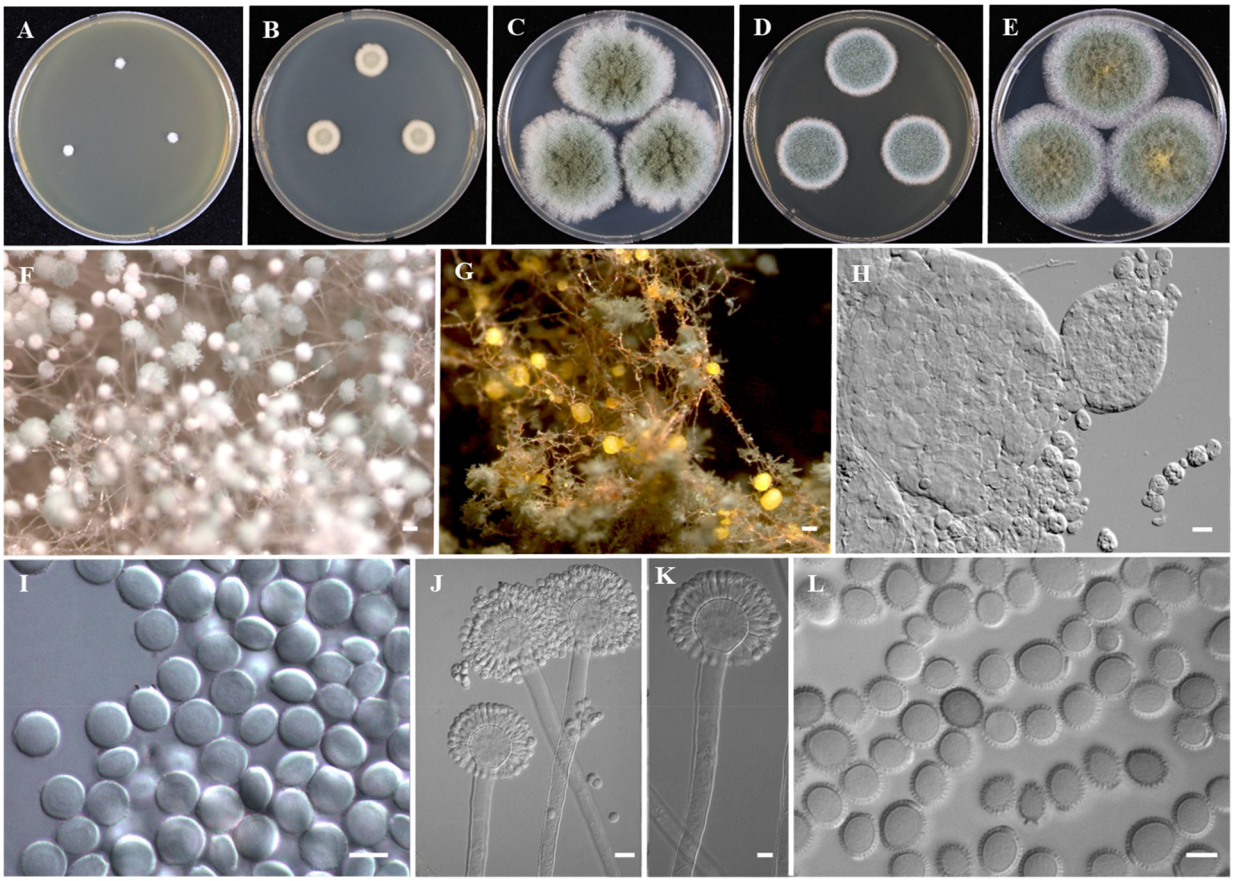

Figure 4. Morphology of *Aspergillus niveoglaucus* (KACC 47386). (A–E) Colonies on MEA, CYA, DG18, YES, and M40Y media upon 7 days incubation at 25 °C from left to right; (F) Conidiophores with conidial head on M40Y; (G) Ascomata on M40Y; (H) Ascomata; (I) Ascospores; (J,K) Conidiophores with conidial head; (L) Conidia. Scale bars: F,G = 100 µm, H,J = 10 µm, I,K,L = 5 µm.

was observed. *BenA* sequences are not sufficient to distinguish between *A. niveoglaucus* and *A. brunneus* but *CaM* and *RPB2* sequences are sufficient for identification. Our KACC strains had morphological similarities to the type strain description, but in CYA medium, Korean strains showed better growth than the type strain description.

4. Discussion

In the present study, we aimed to re-identify the strains submitted to KACC reporting to be either *Aspergillus* or its earlier related sexual state genera (e.g., *Eurotium*), based on a combined analysis of genetic and morphological information. Historically, classification of *Aspergillus* species was conventionally based on their morphology. However, recent findings suggest that supplementation with molecular data and chemical characteristics significantly improves the accuracy of fungal identification [15, 26]. Prior research has demonstrated that relying on only the ITS sequence would be inadequate for the accurate identification of *Aspergillus* species. Instead, they recommend using several markers including *BenA*, *CaM*, and *RPB2* gene sequences for more precise identification of *Aspergillus* [5, 22]. In the current study, phylogenetic studies based on the *BenA*, *CaM*, and *RPB2* genes allowed us to identify three earlier unrecorded species in Korea. Globally, there has been a remarkable increase of new *Aspergillus* species being reported [24, 27].

Lately, section *Aspergillus* underwent an infrageneric classification based on a polyphasic approach which led to introduction of subgenus, section, and series classifications. The revised section hosts 32 species and 7 series [5]. In the currently presented research, three unrecorded species were phylogenetically related with *Aspergillus* (2 species) and *Chevalierorum* (1 species) series.

Section *Aspergillus* species have a worldwide dissemination and commonly observed to be present in indoor atmosphere, house dust, cereals, and food products enriched in sugar, such as sirups, jams, and jellies [8, 11, 24, 28]. They are also prevalent in salted meat products, semi-dry foods and food products, feeds, leather based goods, and more [8, 24]. In our case, *A. aurantiacoflavus*, *A. intermedius*, and *A. niveoglaucus* were isolated from food related environments (meju, rice straw), soil, and air. The association of section *Aspergillus* KACC strains with

meju have been reported in previous publications [11, 28].

In the section *Aspergillus*, thirty-two species have been reported worldwide [5], of which, *A. brunneus*, *A. chevalieri*, *A. cibarius*, *A. cumulatus*, *A. glaucus*, *A. montevidensis*, *Aspergillus proliferans*, *A. pseudoglaucus*, *A. ruber*, and *A. tonophilus* are recorded in Korea [16]. *A. aurantiacoflavus*, *A. intermedius*, and *A. niveoglaucus* have not yet been recorded in Korea, which is included in the current report. The original report describing *A. aurantiacoflavus* indicated that the species to be present in backpack, rubber toy, and cake spread [24]. Moreover, *A. aurantiacoflavus* has also been reported to produce a variety of compounds such as asperflavin, isoechinulins, auroglaucin, questin, dihydroauroglaucin, emodin, epiheveadrides, neoechinulins, erythroglaucin, flavoglaucin, echinulins, bisanthrons, physcion, questinol, tetrahydroauroglaucin, making it relevant to biotechnology industries and in clinical science [24]. To the best of our knowledge, this study remains to be the preliminary study on *A. aurantiacoflavus* from rice straw.

A. intermedius has been isolated from a relatively broader range of environments including food, cotton yarn, soil, clinical samples, and industrial materials [24, 25, 29]. Earlier reports of *A. intermedius* have documented production of numerous extrolites like LL-S491β, asperflavin, auroglaucin, epiheveadrides, dihydroauroglaucin, echinulins, anthraquinones flavoglaucin, questin, neoechinulins, isoechinulins, tetrahydroauroglaucin, physcion [24, 30].

Phylogenetically, *A. niveoglaucus* was observed to share identical *BenA* sequences with *A. brunneus* but it can be distinguished from each other using *CaM* or *RPB2* sequences [24, 29]. In Figure 1, the concatenate dataset clearly distinguished the species of *A. niveoglaucus* and *A. brunneus*. All Korean strains grouped together as a clade with reference strains. In the present study, though phylogenetically *A. niveoglaucus* is closely related with *A. brunneus* in *BenA* sequences but the morphology of the strains was consistent with the type strain of *A. niveoglaucus* CBS 114.27. *A. brunneus* produces mainly globose and tuberculate conidia, while *A. niveoglaucus* produces subglobose to ellipsoidal, tuberculate, rarely globose conidia. Several extrolites, auroglaucin-related neuroprotective compounds and biologically significant echinulin-related indole diketopiperazines alkaloids were derived from *A. niveoglaucus* [31, 32].

In summary, the current study has assessed the *Aspergillus* species diversity including strains originating from various sources like meju, nuruk, soybean, rice straw, soil, air, fresh water, hay, and livestock feed. To date, reports from Korea have only reported on ten species in the section *Aspergillus*. The KACC preserves 10 species from numerous provinces of Korea and we have now, described three unrecorded species which increases it to 13 species in Korea. Such results help to improve our current knowledge on the *Aspergillus* diversity in Korea and supplements the available bioresources in Korea from the section *Aspergillus*.

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Disclosure statement

The authors pronounce that they have no potential conflict of interest.

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