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RESEARCH ARTICLE

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Re-Identification of *Aspergillus* Subgenus *Nidulantes* Strains and Description of Three Unrecorded Species From Korea

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ABSTRACT:
Aspergillus subgenus *Nidulantes* with nine section forms the second largest subgenus of the fungi that comes under the genus *Aspergillus*. Species in this group of fungi are important as they are reported to play several important roles in the environment including influencing air quality in confined spaces, food spoilage, production of mycotoxins as well as in human pathogenicity. In the present study, 53 strains of *Aspergillus* subgenus *Nidulantes* (section: *Nidulantes* & *Usti*) isolated from Korea and preserved at the Korean Agricultural Culture Collection (KACC) were subjected to re-identification by using a combined dataset of partial β-tubulin (*BenA*), Calmodulin (*CaM*) gene sequences as well as their morphological data. We confirmed 14 species from 53 isolates in Korea. Of them, eleven species were reported in Korea previously (*A. amoenus*, *A. baeticus*, *A. calidoustus*, *A. creber*, *A. insuetus*, *A. jensenii*, *A. nidulans*, *A. protuberus*, *A. sydowii*, *A. tabacinus* and *A. unguis*), and three species (*A. griseoaurantiacus*, *A. puulaauensis* and *A. sublatus*) were previously unreported from Korea. We detailed the characteristic features of these three species, that remain unexplored in Korea.

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1. Introduction

Aspergillus is a genus of cosmopolitan fungi and belongs to the family *Aspergillaceae*. It currently contains approximately 446 species, with several species drawing human interest in fields such as biotechnology, human health and the food industry [1,2]. The continuous emergence of new species within this genus reveals its high biodiversity among other fungi. Owing to their varied characteristics, the strains of this genus can grow on diverse substrates, and occur in nature as endophytes, saprophytes, parasites, food contaminants, and human pathogens [2–5].

The subgenus *Nidulantes* is found to be the second largest subgenus of *Aspergillus* next to *Circumdati* and comprises of nine sections, twenty-three series with around 130 species [6]. Most of the species in this subgenus exhibit several characteristic traits including having biseriate conidial heads, conidiophores to be brown-pigmented harboring globose and echinulate type conidia. Initially, in the subgenus *Nidulantes* five sections namely *Versicolores*, *Nidulantes*, *Terrei*, *Usti* and *Flavipedes*, were established based on morphological structures [7]. Later, members of this subgenus underwent phylogenetic re-analysis using DNA sequences of their internal transcribed spacer (ITS) region, β-tubulin gene (*BenA*), Calmodulin gene (*CaM*), RNA polymerase II gene (*RPB2*), and the large subunit 28S ribosomal DNA sequences (LSU), to determine infrageneric relationship and were updated to include several new species [8,9]. Subsequently, section *Aenei* was also introduced in the subgenus *Nidulantes* based on emergent phylogenetic analyses which led to inclusion of several additional species [10]. A polyphasic phylogenetic approach led to defining nine sections in *Nidulantes*, which resulted in addition of a new section, *Cavernicolarum* [6]. Houbraken et al. provided an extensive overview of families and genera of the *Eurotiales* which led to the introduction of an updated subgeneric, section as well as series classification [1]. In the recent times, a broad species concept which includes only four *Aspergillus* species, namely *A. creber*, *A. versicolor*, *A. sydowii*, and *A. subversicolor*, has been introduced in the series *Versicolores* [11]. Moreover, in recent times, many

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species were updated in the subgenus *Nidulantes* which include, *A. qilianyuensis* in the section *Nidulantes* [5]; *A. sigarelli* in the section *Usti* [12]; *A. sichuanensis* and *A. tibetensis* in the section *Aenei* [13]; *A. hainanicus*, *A. guangdongensis* and *A. guangxiensis* were described in the sections *Cavernicolarum, Ochraceorosei* and *Sparsi* respectively [5, 13]. A new series *Hainanici* was proposed in the section *Cavernicolarum* to accommodate *A. hainanicus* [5].

In the Korean Agricultural Culture Collection (KACC), *Aspergillus* strains have been deposited since the 1990s and were formerly identified mainly based on their morphological features. Presence of cryptic species has been a significant obstacle as morphology-based identification was frequently found to be ambiguous in the sections of *Aspergillus* over the last two decades [14]. Therefore, multi-locus sequence analysis is currently in use as a reliable approach, for the identification and phylogeny of *Aspergillus* [2]. To accurately identify *Aspergillus*, a polyphasic approach which includes morphological, molecular, as well as ecological analysis, and extrolite profiling has been proposed [15].

In this study, a part of *Aspergillus* strains preserved at the KACC since 1995–2022 were analyzed using their DNA sequence data as well as their morphological characteristics. The identification of strains was based on the partial β-tubulin (*BenA*) as well as Calmodulin (*CaM*) gene sequences of the selected fungal strains. To date, 84 different species of *Aspergillus* have been identified and described in Korea [16–18] and of them, 14 species are included in subgenus *Nidulantes*. This study aimed to re-identify fungal strains belonging to *Aspergillus* subgenus *Nidulantes* from Korea preserved at KACC and provide a detailed description of the unrecorded species from Korea based on their morphological as well as molecular characteristics in addition to supplementing the existing information on the diversity of *Aspergillus* species from Korea.

2. Materials and methods

2.1. Strains

A total of 53 Korean strains belonging to the genus *Aspergillus* subgenus *Nidulantes* in KACC were studied. The strains studied were isolated from all across Korea. The strains were retrieved from storage in Malt extract broth (BD Difco, Sparks, MD, USA), and later moved to Malt Extract Agar (MEA) [Oxoid, Basingstoke, UK]. Details of the strains examined are listed in the Table 1.

2.2. DNA extraction, amplification, and sequencing

Cultures were grown on MEA plates and DNeasy® plant mini kit (Qiagen, Hilden, Germany) was used for DNA isolation. The DNA templates were quantified using a NanoDrop Spectrophotometer (Thermo Scientific, Waltham, MA, USA). Partial *BenA* and *CaM* genes were amplified following the protocols reported earlier by Glass et al. [19] and Hong et al. [20]. The PCR products were bidirectionally sequenced at Macrogen Inc., South Korea, with the same PCR primers and the raw sequences were assembled using DNA STAR Lasergene SeqMan Pro v.10.0.1. (DNASTAR, Inc. Madison, WI).

2.3. Phylogenetic analyses

The obtained gene sequences were combined with reference (preferably ex-type) sequences that were retrieved from earlier studies [1]. Multiple sequence alignment of respective locus was separately performed using CLUSTAL W program in MEGA 11. The alignments were improved following visual inspection and concatenated later in the same software (MEGA 11). Maximum likelihood (ML) phylogenetic trees were generated based on separate and combined datasets of the gene sequences (*BenA* and *CaM*). The trees with 1,000 ultrafast bootstrap replications and aBayes support were generated using IQ-TREE and the substitution model options were auto-evaluated as per the provided alignment files [21]. The sequence of *A. cibarius* KACC 46346 was used as an outgroup. Details of the reference sequences used have been provided in Supplementary Table 1. Sequences obtained during this study were deposited to RDA-GeneBank (http://genebank.rda.go.kr).

2.4. Phenotypic analysis

The fungal strains were inoculated on four different media namely Malt Extract agar (MEA), Czapek Yeast extract agar (CYA), Dichloran 18% Glycerol agar (DG18), and Yeast extract sucrose agar (YES) [22] and incubated at 25 °C for 7 days. At the end of the incubation period, colony diameters and characteristics were recorded. Fungal slides were then prepared with lactic acid and observed under a Zeiss AXIO Imager A1 microscope with differential interference contrast (DIC) illumination, and a digital AxioCam ICc3 camera (Carl Zeiss, Göttingen, Germany). Characteristics including the size, shape, conidial pigmentation and conidiophores were recorded.

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

Section	Re-identified scientific name	Scientific name by depositor	KACC number	Deposited year	Substrate	Location	RDA Genebank accession no.	
							CaM	*BenA*
Nidulantes	*A. amoenus*	*A. amoenus*	49738	2020	Soil	Buan-gun	RDA0066082	RDA0066138
	A. creber	*A. versicolor*	42601	2007	Air	Seoul	RDA0066034	RDA0066091
		A. versicolor	43834	2008	Pepper tillage soil	Nonsan-si	RDA0066040	RDA0066097
		A. versicolor	43837	2008	Soil near by beach	Hwaseong-si	RDA0066044	RDA0066100
		A. versicolor	46503	2011	Meju	Suwon-si	RDA0066056	RDA0066112
		A. versicolor	46583	2011	Rice straw	Buan-gun	RDA0066061	RDA0066117
		A. creber	47128	2013	Soybean	Sunchang-gun	RDA0066064	RDA0066120
		A. creber	47268	2013	Rice straw	Anseong-si	RDA0066069	RDA0066125
		A. creber	47378	2014	Air	Damyang-gun	RDA0066073	RDA0066129
		A. creber	48863	2019	Freshwater	Seogwipo-si	RDA0066080	RDA0066136
		A. protuberus	48866	2019	Seawater	Taean-gun	RDA0066081	RDA0066137
	A. griseoaurantiacus	*A. versicolor*	47392	2014	Air	Damyang-gun	RDA0066077	RDA0066133
	A. jensenii	*A. jensenii*	47130	2013	Soybean	Sunchang-gun	RDA0066065	RDA0066121
		A. jensenii	47380	2014	Air	Icheon-si	RDA0066074	RDA0066130
	A. nidulans	*A. nidulans*	40304	1997	Soil in muskmelons green house	Jinju-si	RDA0066029	RDA0066086
		A. nidulans	43840	2008	Fermented soybeans	Suwon-si	RDA0066047	RDA0066103
		A. nidulans	44342	2009	Sputum	Seoul	RDA0066052	RDA0066108
		A. nidulans	46505	2011	Meju	Seocheon-gun	RDA0066058	RDA0066114
		A. nidulans	46506	2011	Meju	Anseong-si	RDA0066059	RDA0066115
		A. nidulans	46507	2011	Meju	Anseong-si	RDA0066060	RDA0066116
		A. nidulans	46825	2012	Soybean	Gongju-si	RDA0066063	RDA0066119
		A. nidulans	47140	2013	Soybean	Korea	RDA0066068	RDA0066124
		A. nidulans	47271	2013	Rice straw	Sunchang-gun	RDA0066070	RDA0066126
		A. nidulans	47382	2014	Air	Damyang-gun	RDA0066075	RDA0066131
		A. nidulans	48025	2015	Nuruk	Seocheon-gun	RDA0066078	RDA0066134
		A. nidulans	48026	2015	Nuruk	Seocheon-gun	RDA0066079	RDA0066135
	A. protuberus	*A. versicolor*	42602	2007	Air	Seoul	RDA0066035	RDA0066092
		A. versicolor	43835	2008	Paddy soil	Jeju-do	RDA0066042	RDA0066098
		A. versicolor	43836	2008	Paddy soil	Jeju-do	RDA0066043	RDA0066099
		A. protuberus	49742	2020	Seawater	Gunsan-si	RDA0066083	RDA0066139
	A. puulaauensis	*A. versicolor*	46504	2011	Meju	Gimcheon-si	RDA0066057	RDA0066113
	A. sublatus	*A. nidulans*	46824	2012	Wheat straw	Sunchang-gun	RDA0066062	RDA0066118
	A. sydowii	*A. sydowii*	43841	2008	Fermented soybeans	Suwon-si	RDA0066048	RDA0066104
		A. sydowii	43838	2008	Powdered red pepper	Chuncheon-si	RDA0066045	RDA0066101
		A. sydowii	43839	2008	Bread	Suwon-si	RDA0066046	RDA0066102
		A. sydowii	44339	2009	Sputum	Seoul	RDA0066049	RDA0066105
		A. sydowii	44340	2009	Sputum	Seoul	RDA0066050	RDA0066106
		A. sydowii	44341	2009	Sputum	Seoul	RDA0066051	RDA0066107
		A. sydowii	44960	2010	Horse	Cheongju-si	RDA0066053	RDA0066109
		A. sydowii	46500	2011	Meju	Cheongju-si	RDA0066054	RDA0066110
		A. sydowii	46501	2011	Meju	Suwon-si	RDA0066055	RDA0066111
		A. sydowii	47136	2013	Soybean	Korea	RDA0066066	RDA0066122
		A. versicolor	47138	2013	Soybean	Korea	RDA0066067	RDA0066123
		A. sydowii	47275	2013	Rice straw	Yangyang-gun	RDA0066071	RDA0066127
		A. sydowii	47389	2014	Air	Sunchang-gun	RDA0066076	RDA0066132
	A. tabacinus	*A. versicolor*	47279	2013	Rice straw	Yangyang-gun	RDA0066072	RDA0066128
		A. versicolor	43830	2008	Paddy soil	Jeju-do	RDA0066036	RDA0066093
	A. unguis	*A. unguis*	43831	2008	Forest soil	Muju-gun	RDA0066037	RDA0066094
		A. unguis	43832	2008	Paddy soil	Jeju-do	RDA0066038	RDA0066095
		A. unguis	43833	2008	Forest soil	Muju-gun	RDA0066039	RDA0066096
Usti	*A. baeticus*	*A. baeticus*	48862	2019	Rhizosphere soil	Gwangju-si	RDA0062661	RDA0062662
	A. calidoustus	*A. calidoustus*	49737	2020	House dust	Gwangju-si	RDA0062659	RDA0062660
	A. insuetus	*A. insuetus*	49965	2021	Water	Nowon-gu	RDA0062657	RDA0062658

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

3. Results

3.1. Phylogenetic analyses

A combined *BenA* and *CaM* sequence dataset was used to understand the phylogenetic relationships of KACC strains with other publicly available (ex-) type species belonging to the subgenus *Nidulantes* (Figure 1). The concatenated alignment of 74 sequences including our strains as well as (ex-) type species spanning 20 taxa contained 1024 characters (including alignment gaps). Of these, 579 characters were from *CaM*, and 445 characters from *BenA* (Supplementary Table 2). The concatenated phylogenetic tree indicated that the 53 strains taken in this study were spread across 14 different *Aspergillus* species, of which eleven species were earlier reported in Korea, whereas three species (red colored bold text) were hitherto not described from Korea

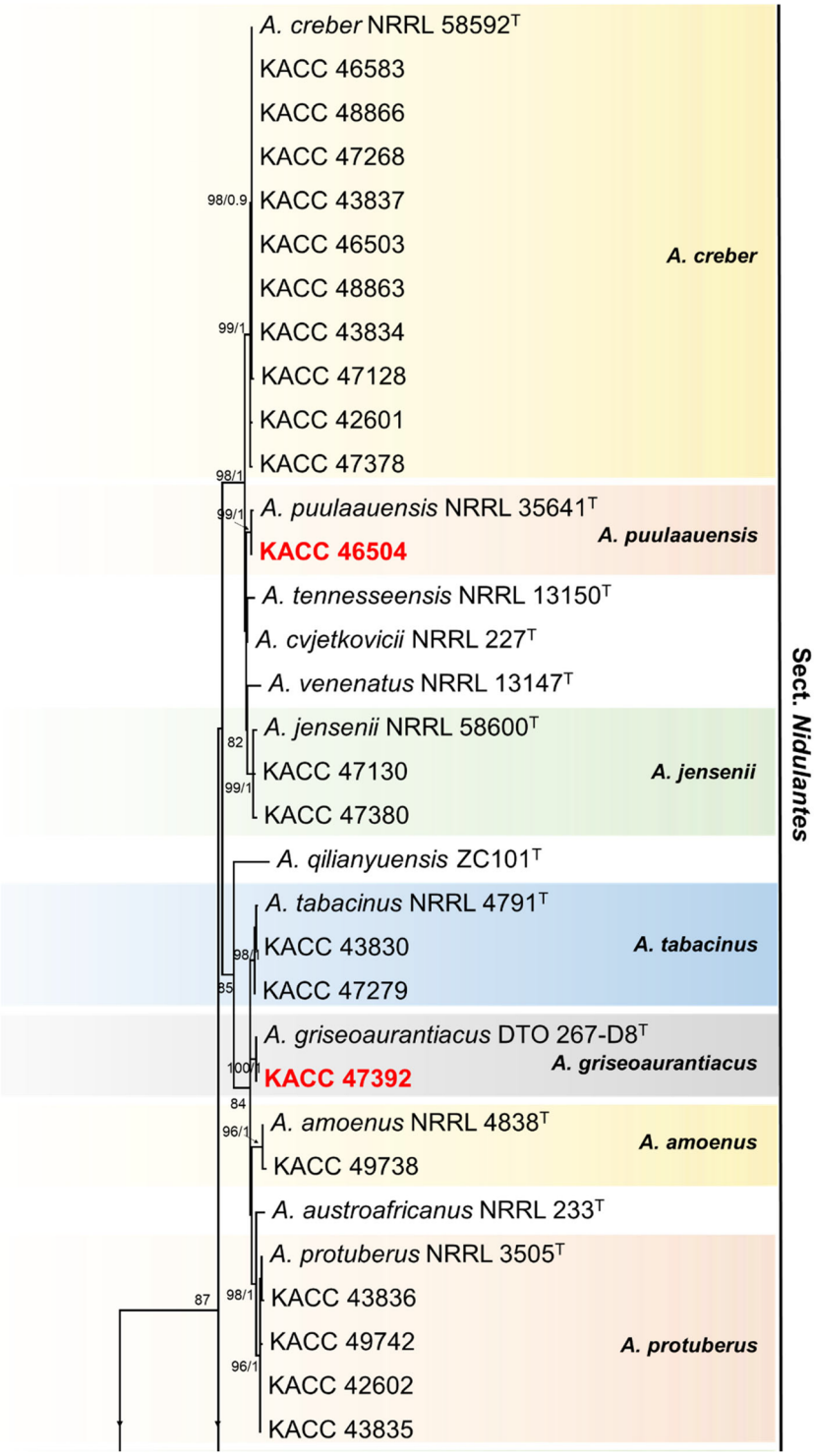

Figure 1. Phylogenetic position of *Aspergillus* subgenus *Nidulantes* strains from the KACC based on a combined data set containing partial *BenA* and *CaM* sequences. Bootstrap values ≥70 (left) and aBayes values ≥0.9 (right) are presented at the nodes. The scale bar indicates the number of substitutions per nucleotide. The unrecorded species are represented in bold & red in color. Ex-type strains are denoted by^{T}. The species *A. cibarius* was used as the outgroup.

(Figure 1). Phylogenetic tree was constructed based on single gene were provided in the supplementary figure 1 and 2. During BLAST analysis, we observed the *BenA* sequence of strain KACC 46504 to express 100% similarity with *A. puulaauensis* NRRL35641^{T} followed by 99.2% similarity with *A. cvjetkovicii* NRRL227^{T} (98.94%) and *A. jensenii* NRRL58600^{T} (98.14%). This was also consistent with the BLAST results of the strain using *CaM* sequence where the top nearest hits were *A. puulaauensis* NRRL35641^{T} (99.6%), *A. cvjetkovicii* NRRL227^{T} (99.4%) and *A. tennesseensis* NRRL13150^{T} (99.19%). Interestingly, in case of strain KACC 46824, the top BLAST hits for similarity with its *BenA* sequence were found to be *A. sublatus* CBS140630^{T} (100%) and *A. quadrilineatus* NRRL201^{T} (100%). However, BLAST results

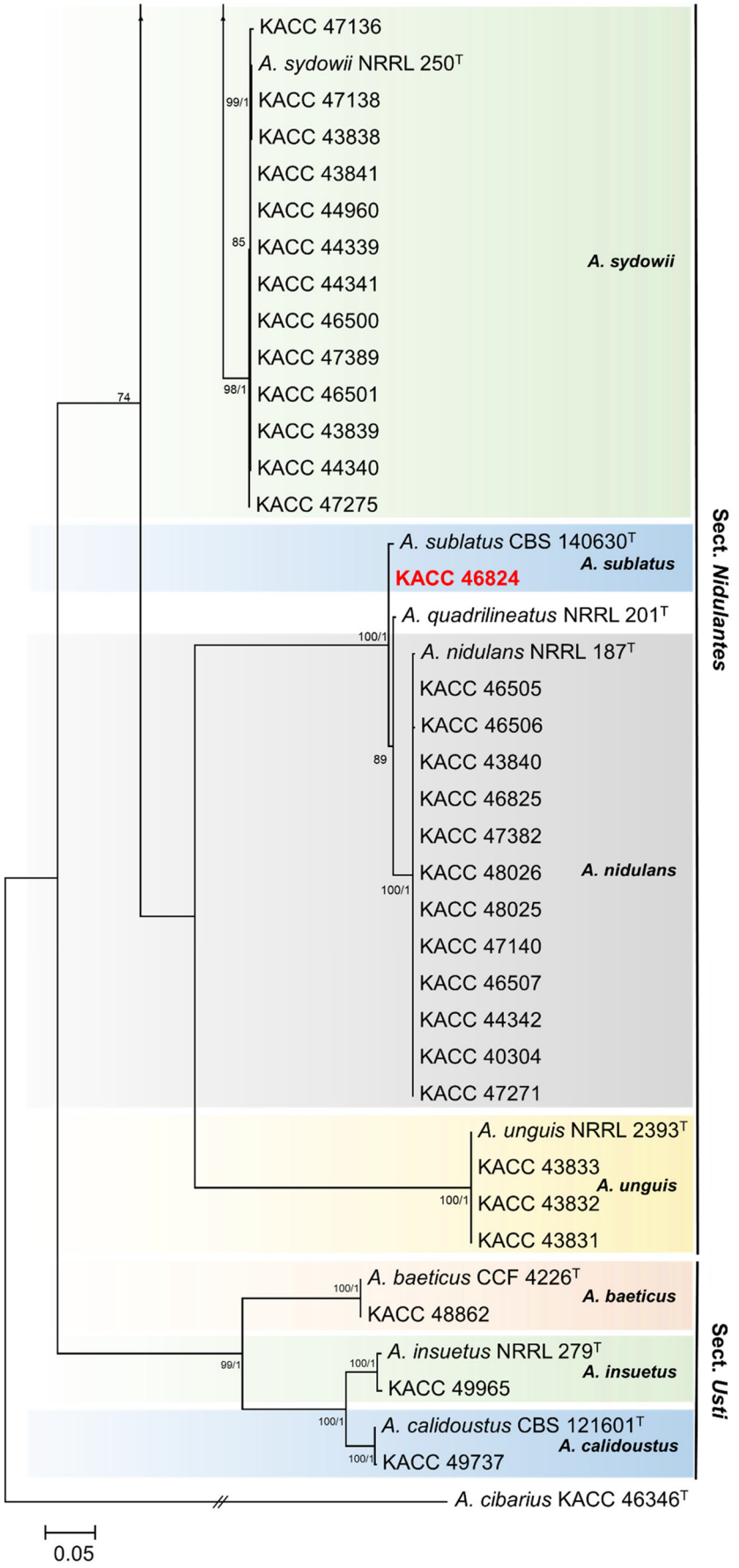

Figure 1. Continued.

from *CaM* sequence analysis of the strain revealed a higher similarity with *A. sublatus* CBS140630^T (99.34%) compared to *A. quadrilineatus* NRRL201^T (98.68%). It also indicated one-hundred percent sequence similarity with another strain of *A. sublatus* DTO421-D[4]. To further confirm its identity, we

studied the strain KACC 46824 for its macro and micro-morphology. The macromorphology of the strain was consistent with the type strain of *A. sublatus*/*A. latus* and in its micromorphology, comparing the vesicle shape indicated its likeness to *A. sublatus*/*A. latus* compared to *A. quadrilineatus* type strain. Finally, BLAST analysis in case of strain KACC 47392 indicated its top hits of *BenA* sequence to be *A. griseoaurantiacus* DTO:267-D8^T (100%) followed by *A. tabacinus* NRRL4791^T (99.47%) and *A. amoenus* NRRL4838^T (98.93%). This was substantiated by the BLAST of its *CaM* sequence which showed the highest similarity of the sequence to be with *A. griseoaurantiacus* DTO:267-D8^T (100%) followed by *A. tabacinus* NRRL4791^T (98.99%) and *A. amoenus* NRRL4838^T (98.91%).

In the section *Nidulantes*, 50 strains grouped into eleven clusters, with *A. amoenus*, *A. creber*, *A. griseoaurantiacus*, *A. jensenii*, *A. nidulans*, *A. protuberus, A. puulaauensis, A. sublatus*, *A. sydowii*, *A. tabacinus* and *A. unguis* as their nearest neighbors. Until now, 76 species of section *Nidulantes* have been reported worldwide [1, 5]. Among them, *A. amoenus*, *A. creber*, *A. jensenii*, *A. nidulans*, *A. protuberus, A. subversicolor, A. sydowii*, *A. tabacinus*, *A. tennesseensis, A. unguis* and *A. versicolor* are recorded in Korea [16, 23]. Species *A. puulaauensis*, *A. griseoaurantiacus* and *A. sublatus* were not previously recorded in Korea, and are incorporated now in the present report.

Among 26 known species from the section *Usti* [1, 12]; five of them, viz., *A. baeticus, A. calidoustus*, *A. germanicus*, *A. insuetus* and *A. pseudodeflectus* have been reported from Korea [16]. Three strains from the section *Usti* in Korea grouped with type strains of *A. calidoustus*, *A. insuetus* and *A. baeticus* as their closest neighbors and identified respectively.

3.2. Taxonomy

Aspergillus puulaauensis Jurjević, S.W. Peterson & B.W. Horn, IMA Fungus 3 (1): 71 (2012) [MB#800602] [24]

Colony characteristics: The fungal colonies attain 20–21 mm diameter in a span of 7 days at 25 °C on CYA, sulcate with artemisia green conidial heads, no soluble pigment, reverse brown. The colonies were sulcate, centrally raised light sporulation with funicular hyphal clumps, surrounded by yellowish sporulation with white mycelium at periphery, yellowish brown color in reverse and reaches 17-18 mm diameter in 7 days on MEA. The colonies were clear white fine sporulation, white in reverse and extended 15-16 mm in diameter at 25 °C after 7 days on DG18. Irregular colony appearance with artemisia green conidial heads seen on further incubation. Colonies attain 32–33 mm diameter on YES at 25 °C after 7 days; light grayish green sporulation at center followed by yellowish sporulation with white mycelium at margins, reverse pale yellow.

Micromorphology: Presence of biseriate conidial heads, smooth-walled, stipes hyaline, 150–320 × 4–5 µm. Pyriform to spatulate vesicles, 5–11 µm. Metulae covering half to entire surface of the vesicle, 4–6 × 3–4 µm. Flask-shaped phialides, 5–7 × 2–3.5 µm, fragmentary heads resembling penicillate fructifications were occasionally present. Conidia spherical to ellipsoidal, often covered by a thick layer (about 0.3 µm), rough, 2–3 µm (Figure 2).

Examined strain: KACC 46504

Remarks: KACC 46504 was alike *A. puulaauensis* described by Jurjević et al. [24]. However, conidia were slightly smaller (2–3 µm) than that of *A. puulaauensis* NRRL 35641 (2.5–5.5 µm) [24]. Moreover, colonies on YES media showed soluble pink pigment after 14 days at 25 °C.

Aspergillus griseoaurantiacus Visagie, Hirooka & Samson, Studies in Mycology 78: 112 (2014) [MB#809197] [22]

Colony characteristics: In medium CYA, the colonies were floccose, mycelial areas white to light brown with bluish green sporulation, reverse brown and reached 16–17 mm diameter after 7 days at 25 °C. Colonies on MEA were found to be floccose, dark bluish green with dull brown sporulation at center, encircled by white mycelium at margins, exudate minute brown droplets on further incubation of the colony, reverse yellow and attains 14–15 mm diameter at 25 °C after 7 days. Colonies were white in appearance, slow growth, reverse yellow and extended 7-8 mm in diameter at the end of 7 days at 25 °C on DG18. On further incubation, colonies exhibited artemisia green conidial heads and irregular colony margin. Colonies on YES attain 26–27 mm diameter after 7 days at 25 °C; Colony surface floccose, moderately radially sulcate toward the center, mycelium white, light greenish ash sporulation, reverse yellow.

Micromorphology: The fungal conidial heads were radiating biseriate, smooth-walled, stipes hyaline, 130–350 × 4–6 µm. Vesicles spatulate, 10–16 µm. Metulae covering 85% of the head, 4–7 × 3–4 µm. Ampulliform phialides, 5–7 × 2.5–3.5 µm. Conidia were found to be globose to ellipsoidal and smooth, 2–3 µm (Figure 3).

Examined strain: KACC 47392

Remarks: KACC 47392 was morphologically close to *A. griseoaurantiacus* earlier described by Visagie et al. [22]. However, KACC 47392 showed globose to

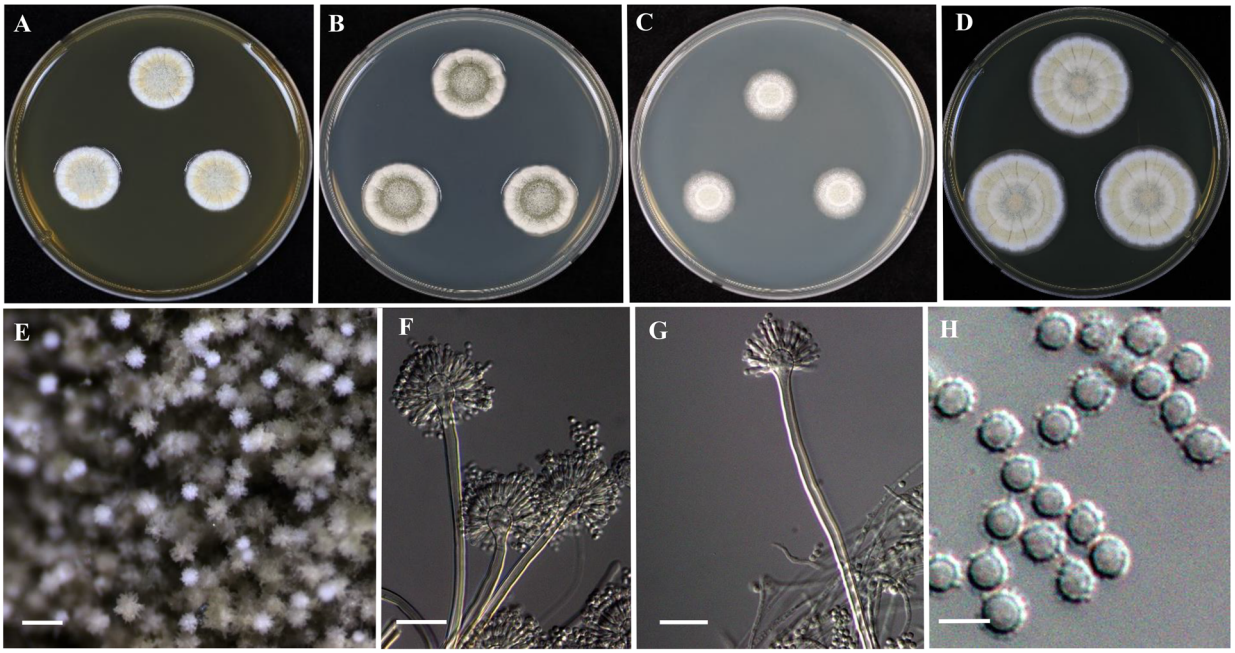

Figure 2. Morphology of *Aspergillus puulaauensis* (KACC 46504). (A–D) Colonies grown on MEA, CYA, DG18 and YES media after 7 days at 25 °C from left to right. (E) Conidial head on MEA, (F,G) Conidiophores with conidial head & (H) Conidia. Scale bars: E = 125 µm, F, G = 25 µm, H = 5 µm.

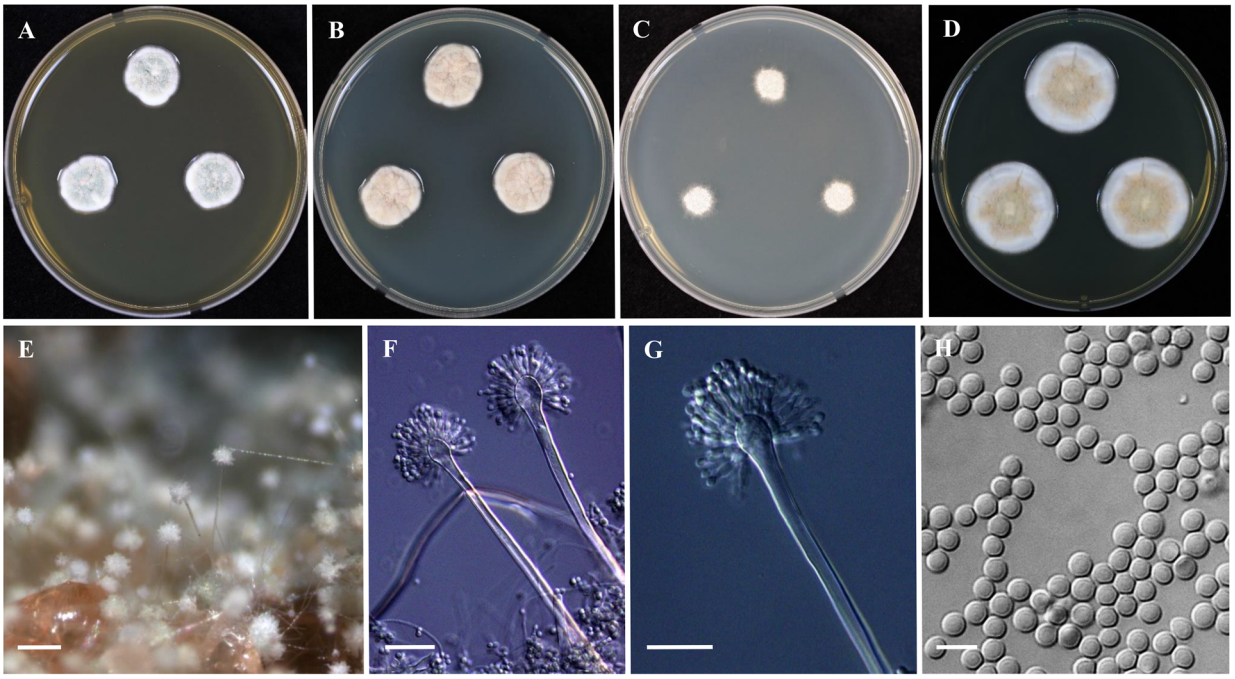

Figure 3. Morphology of *Aspergillus griseoaurantiacus* (KACC 47392). (A-D) Colonies grown on MEA, CYA, DG18 and YES media after 7 days at 25 °C from left to right. (E) Conidial head on MEA, (F,G) Conidiophores with conidial head & (H) Conidia. Scale bars: E = 125 µm, F, G = 25 µm, H = 5 µm.

ellipsoidal, smooth conidia but *A. griseoaurantiacus* CBS 138191 has ellipsoidal, finely roughened conidia [22]. In addition, colonies on YES media, indicated minute brown droplets like exudate after 14 days at 25 °C.

Aspergillus sublatus Y. Horie, Transactions of the Mycological Society of Japan 20: 481 (1979) [MB#118407] [25]

Colony characteristics: The colonies were moderately deep with white mycelium, no soluble pigment, light brown sporulation, reverse brown and eventually reaching a diameter of 36-37 mm at the end of 7 days at 25 °C on CYA medium. The surface of the colonies was found to be floccose with white mycelial areas and light brown to green sporulation, no soluble pigment, brownish-yellow in reverse, and

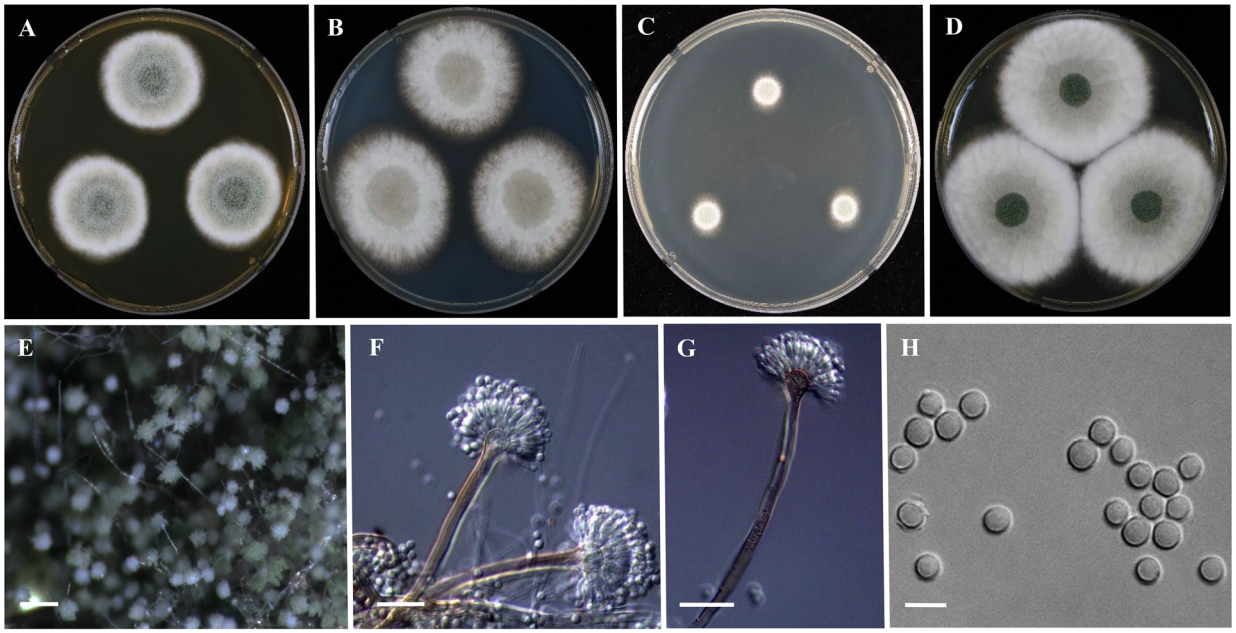

Figure 4. Morphology of *Aspergillus sublatus* (KACC 46824). (A–D) Colonies grown on MEA, CYA, DG18 and YES media after 7 days at 25 °C from left to right. (E) Conidial head on MEA, (F,G) Conidiophores with conidial head & (H) Conidia. Scale bars: E = 125 µm, F, G = 25 µm, H = 5 µm

further reached 30–32 mm in diameter on MEA at 25 °C after 7 days. On DG18, white colony mycelium and sporulation green in color and white in reverse with 10–12 mm in diameter at 25 °C after 7 days. On YES medium, the colonies were floccose, white mycelial areas, wrinkled with 58–60 mm in diameter after 7 days at 25 °C; conidia green, reverse wrinkled, yellowish orange that faded into light yellow toward the margins.

Micromorphology: Microscopic observation found the conidial heads to be biseriate and at instances reduced Penicillium-like structures were present, Brown hyaline stipes, smooth-walled, 90-250 × 4–6 µm. Vesicles were subglobose to subclavate, brown, measuring 8–11 µm and covered the upper half of the head; Metulae 3–7 × 2–4 µm. Phialides were flask-shaped, 5–9 × 2–3.5 µm. Conidia were observed to be globose to subglobose, smooth, 3–4 µm (Figure 4).

Examined strain: KACC 46824

Remarks: *A. sublatus* was synonymized with *A. latus* [6]. KACC 46824 showed excellent growth at 37 °C on CYA with dense sporulation. Green sporulation was enhanced on colonies after incubation of 14 days at 25 °C.

4. Discussion

In the current study, we aimed at re-identifying the strains previously submitted to KACC as *Aspergillus* or its earlier related sexual state genera (e.g. *Emericella*), based on a combination of molecular and morphological data. In earlier days, morphological characteristics alone were used for identification of these fungal strains and then ITS was recognized as a universal DNA marker for a more accurate identification of fungi. However, earlier studies have shown that the ITS sequence was insufficient for the accurate identification of *Aspergillus* species and recommended *BenA* and *CaM* gene as the suitable markers [1, 22]. In the current study, *BenA* and *CaM* gene based phylogeny analysis led us to recognize three previously undescribed species of Korea. Until now, only few reports have been made on unrecorded *Aspergillus* species from Korea despite the ubiquitous distribution. However, there has also been an increase in the reports of several new *Aspergillus* species throughout the world [13, 17].

The previously unrecorded species of Korea, KACC 46504, KACC 47392 and KACC 46824 strains belonged to the section *Nidulantes* (Figure 1). *Aspergillus* section *Nidulantes* species are ubiquitous in the environment and are believed to perform significant roles in everyday life of humans [6]. Recently, *Aspergillus* section *Nidulantes* underwent a taxonomic revision by means of a polyphasic approach, and a series classification was introduced. The revised section harbors 67 species and seven series [1]. In the present study, the three unrecorded species were found to be associated with the *Versicolores* (two species) and *Nidulantes* (one species) series. The fungi belonging to the series *Versicolores* are often mentioned to be as ubiquitous as they are frequently

isolated from a wide range of environmental niches including soil, indoors, foods, animal feed, plant sources, caves, and even from clinical material [24, 26]. In our case, *A. sublatus*, *A. puulaauensis* and *A. griseoaurantiacus* mainly originated from food and air. The initial report describing *A. griseoaurantiacus* indicated the species to be present in house dust from various indoor environments of Mexico and Micronesia [22]. Moreover, *A. griseoaurantiacus* has also been reported to produce the enzyme chitinase and the mycotoxin, sterigmatocystin, making it relevant to biotechnology as well as human health [11, 27]. *A. puulaauensis* however, has been isolated from a comparatively wider spectrum of ecological niches including dead hardwood branch, clinical areas, mold damaged homes, grapes and once from indoor air [24, 28]. Previous reports of *A. puulaauensis* have recorded production of norsolorinic acid, Versicolorin A, 5-methoxyterigmatocystin & sterigmatocystin and also reported cellular cytotoxicity against A549 and HaCaT cell lines [6, 28, 29]. Few studies showed that *A. puulaauensis* extrolites to have a potential to be used as a ingredient in cosmetics or used to lower oxidative stress [30]. *A. sublatus* was treated as a synonym of *A.latus* [6] but failed to have any priority over *A. sublatus*. Later, fungal taxonomists considered that the correct name for this species is *A. sublatus* as prosposed by Y. Horie [25]. Several extrolites such as asperthecin, asperugins, emericellin, shamixanthones, sterigmatocystin, Nidulalin A & B, an emindol, a violaceol and versicolorins production have been reported in *A. sublatus* [6]. Moreover, *A. sublatus* is a well-known etiological agent of aspergillosis [13].

The second largest section in subgenus *Nidulantes* is *Usti* which encompasses 26 species and 4 series [1, 12]. Most fungi from the section *Usti,* are found to produce close to brown-pigmented conidiophores with coarsely roughened conidia [12, 31]. These group of aspergilli have an history of being isolated from cave sediments, indoor environments, soil as well as clinical samples [12, 32]. Many species of the section *Usti* especially *A. calidoustus* and *A. insuetus* have been recognized as causal agent of Invasive Aspergillosis [32].

To date, fourteen species have been reported from Korea in the subgenus *Nidulantes*. The KACC maintains 13 species from several provinces, and most of them originated from soil, air, water, food and clinical samples. In this study, we have described three more unrecorded species in the subgenus *Nidulantes* which now extends the count to 17 species in Korea. The results of this study will improve knowledge on the distribution of *Aspergillus* species in Korea and promote development of applications of the various species in the subgenus *Nidulantes*.

Disclosure statement

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

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