


## Four Unrecorded *Aspergillus* Species from the Rhizosphere Soil in South Korea

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### ABSTRACT

The genus *Aspergillus* is commonly isolated from various marine and terrestrial environments; however, only a few species have been studied in rhizosphere soil. As part of the Korean indigenous fungal excavation project, we investigated fungal diversity from rhizosphere soil, focusing on *Aspergillus* species. A total of 13 strains were isolated from the rhizosphere soil of three different plants. Based on phylogenetic analysis of  $\beta$ -tubulin and calmodulin and morphological characteristics, we identified five *Aspergillus* species. *A. calidouustus* and *A. pseudodeflectus* were commonly isolated from the rhizosphere soil. Four species were confirmed as unrecorded species in Korea: *A. calidouustus*, *A. dimorphicus*, *A. germanicus*, and *A. pseudodeflectus*. The detailed morphological descriptions of these unrecorded species are provided.

### ARTICLE HISTORY

Received 10 March 2021  
Revised 14 June 2021  
Accepted 14 June 2021

### KEYWORDS

*BenA*; *CaM*; morphology; new records; phylogeny

## 1. Introduction

*Aspergillus* is one of the most common fungi in various environments worldwide. *Aspergillus* is known as plant and human pathogen, mycotoxin producer, and food spoiler. However, it plays important roles in the ecological and industrial systems by producing antibiotics and organic acids, and degrading starches, celluloses, and other polysaccharides [1–5]. Morphological characters such as growth rate, color of the colony, thermotolerance, and size of conidial heads and conidia are known to be important features for initial identification of *Aspergillus* [6]. However, morphological feature is not enough to recognize species because their morphological characteristics vary by their ecological habitats [6,7]. For accurate identification of *Aspergillus*, standardized methods including morphology, molecular analysis, and extrolite profiling have been proposed. DNA markers such as the internal transcribed spacer region, calmodulin (*CaM*),  $\beta$ -tubulin (*BenA*), and the RNA polymerase II second largest subunit (*RPB2*) have been used in *Aspergillus* identification and phylogeny [8]. According to the current research, the genus consists of six subgenera, 27 sections, and 446 species worldwide [9]. In Korea, 69 *Aspergillus* species have been reported [10–14]. Although some species have been reported from soil in terrestrial, marine, and clinical environments [15–18], many *Aspergillus* were isolated from food fermentation such as meju and nuruk

[12,19–21]. Nonetheless, study on *Aspergillus* from rhizosphere soil in Korea is limited [22].

Fungi in rhizosphere environments play an important role in plant growth and adaptation [23,24]. *Aspergillus* is one of the common fungi in rhizosphere soil [25–31]. Some *Aspergillus* are known to produce plant promoting chemicals such as gibberellic acid and indole acetic acid [27,28]. Many *Aspergillus* strains were only identified at the genus level, as previous studies mainly focused on bioactive compounds [27–30]. Therefore, the diversity of *Aspergillus* in rhizosphere soil is unclear.

This project is organized by the National Institute of Biological Resources to excavate Korean indigenous fungi from the rhizosphere soil. We explored fungal diversity from rhizosphere soil of various plants; *Aspergillus*, *Penicillium*, *Trichoderma*, and *Fusarium* were common genera. Recently, we reported on diversity of *Penicillium*, revealing eight unrecorded species in Korea [32]. The main purpose of this study was to focus on *Aspergillus* in the rhizosphere of various plants and to identify them based on *BenA* and *CaM* loci. We discovered four unrecorded species: *A. calidouustus*, *A. dimorphicus*, *A. germanicus*, and *A. pseudodeflectus*.

## 2. Materials and methods

### 2.1. Sample collections and isolation

Rhizosphere soil of three plants (*Calystegia soldanella*, *Orobancha coerulescens*, and *Sorbus commixta*)

**Table 1.** The information of *Aspergillus* strains isolated from rhizosphere soil.

Species	Section	Strain	Location	Substrate
<i>A. calidoustus</i> <sup>a</sup>	<i>Usti</i>	SFC20191113-NB113	Heunghae-eup, Buk-gu, Pohang-si, Gyeongsangbuk-do	<i>Calystegia soldanella</i>
		SFC20191113-NB116	Heunghae-eup, Buk-gu, Pohang-si, Gyeongsangbuk-do	<i>Calystegia soldanella</i>
		SFC20191113-NB135	Heunghae-eup, Buk-gu, Pohang-si, Gyeongsangbuk-do	<i>Calystegia soldanella</i>
		SFC20191113-NB146, NIBRFG0000509071	Heunghae-eup, Buk-gu, Pohang-si, Gyeongsangbuk-do	<i>Calystegia soldanella</i>
		SFC20191113-NB197, NIBRFG0000509286	Sannae-myeon, Miryang-si, Gyeongsangnam-do	<i>Sorbus commixta</i>
<i>A. dimorphicus</i>	<i>Cremeri</i>	SFC20191113-NB100, NIBRFG0000509072	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	<i>Orobanche coerulescens</i>
<i>A. germanicus</i>	<i>Usti</i>	SFC20191113-NB098, NIBRFG0000509073	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	<i>Orobanche coerulescens</i>
<i>A. insuetus</i>	<i>Usti</i>	SFC20191113-NB013	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	<i>Calystegia soldanella</i>
<i>A. pseudodeflectus</i>	<i>Usti</i>	SFC20191113-NB114, NIBRFG0000509074	Heunghae-eup, Buk-gu, Pohang-si, Gyeongsangbuk-do	<i>Calystegia soldanella</i>
		SFC20191113-NB115	Heunghae-eup, Buk-gu, Pohang-si, Gyeongsangbuk-do	<i>Calystegia soldanella</i>
		SFC20191113-NB136	Heunghae-eup, Buk-gu, Pohang-si, Gyeongsangbuk-do	<i>Calystegia soldanella</i>
		SFC20191113-NB156	Heunghae-eup, Buk-gu, Pohang-si, Gyeongsangbuk-do	<i>Calystegia soldanella</i>
		SFC20191113-NB199, NIBRFG0000509287	Sannae-myeon, Miryang-si, Gyeongsangnam-do	<i>Sorbus commixta</i>

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

were collected from five sites in Korea in 2019 (Table 1). Five grams of soil for each sample was diluted tenfold in sterile water. A 100 µL of each dilute was plated on dichloran rose bengal chloramphenicol agar (DRBC; Difco, Becton Dickinson). All plates were incubated at 25 °C for 7 days. Based on morphology, *Aspergillus*-like strains were transferred to potato dextrose agar (PDA; Difco, Becton Dickinson) plate. Strains were stored in 20% glycerol at -80 °C at the Seoul National University Fungus Collection (SFC).

## 2.2. DNA extraction, amplification, and sequencing

Genomic DNA was extracted from isolated *Aspergillus* using a modified cetyltrimethylammonium bromide extraction protocol [33]. For the primer sets, Bt2a/Bt2b for *BenA* and CF1/CF4 or cmd5/cmd6 for *CaM*, were used [34–36]. PCR was performed in a C1000 thermal cycler (Bio-Rad, Richmond, CA, USA) with previously described methods [37]. The PCR products were purified using the Expin<sup>TM</sup> PCR Purification Kit (GeneAll Biotechnology, Seoul, Korea), according to the guideline. DNA sequencing was performed using the PCR primers at Macrogen (Seoul, Korea), using an ABI Prism 3730 genetic analyzer (Life Technologies, Gaithersburg, MD, USA).

## 2.3. Phylogenetic analysis

The sequences were assembled, proofread, and aligned using MEGA7 [38] and were deposited in GenBank (accession numbers in Table 2). *Hamigera avellanea* CBS 295.48 was used as the outgroup [39]. Multiple alignments were performed using the default settings of the Multiple Alignment Fast Fourier Transform (MAFFT ver. 7) [40]. Then, each sequence was manually checked and adjusted. Maximum likelihood (ML) phylogenetic tree was performed with RAXML [41] implemented on

CIPRES web portal [42], using the GTR + GAMMA model of evolution with 1000 bootstrap replicates.

## 2.4. Morphological analysis

Morphological analysis of the four unrecorded species was performed on three different culture media using previously described methods: Czapek yeast autolysate agar (CYA; yeast extract, Difco), malt extract agar (MEA; Oxoid), and yeast extract sucrose agar (YES; yeast extract, Difco). The Methuen Handbook of Color was used for the color names and alphanumeric codes for macromorphological characteristics [43]. The microscopic observation was processed under a light microscope (Eclipse 80i, Nikon, Tokyo, Japan) using the samples grown on MEA and CYA.

## 3. Results

### 3.1. Species identification

A total of 13 *Aspergillus* strains were isolated from rhizosphere of three plants. Based on the combined dataset of *BenA* and *CaM* sequences, they were identified as five species in two sections with four unrecorded species in Korea (Table 1 and Figures 1 and 2). Twelve strains were included in section *Usti* and were identified as four species: *A. calidoustus* (5 strains), *A. germanicus* (1), *A. insuetus* (1), and *A. pseudodeflectus* (5). *A. calidoustus*, *A. germanicus*, and *A. pseudodeflectus* were unrecorded species in Korea. For section *Cremeri*, one strain was discovered and identified as *A. dimorphicus*, which was unrecorded species in Korea.

*A. calidoustus* and *A. pseudodeflectus* were commonly isolated from the rhizosphere soil (Table 1). *Aspergillus* diversity was higher in rhizosphere soil of *Calystegia soldanella* compared to others. Although *A. pseudodeflectus* was commonly isolated from *C. soldanella* and *Sorbus commixta*, generally, the *Aspergillus* diversity was found unique for each plant.

**Table 2.** Strains used for phylogenetic analyses in this study.

Section of <i>Aspergillus</i>	Species	Strain	GenBank accession no.			
			<i>BenA</i>	<i>CaM</i>		
<i>Cremeri</i>	<i>A. arxii</i>	CBS 525.83 <sup>T</sup>	MN969365	MN969223		
	<i>A. brunneouniseriatus</i>	NRRL 4273 <sup>T</sup>	EF652123	EF652138		
	<i>A. chaetosartoryae</i>	NRRL 5501 <sup>T</sup>	EF652117	EF652129		
	<i>A. chrysellus</i>	NRRL 5084 <sup>T</sup>	EF652109	EF652136		
	<i>A. citocrescens</i>	CBS 140566 <sup>T</sup>	FR775317	LN878969		
	<i>A. cremeus</i>	NRRL 5081 <sup>T</sup>	EF652120	EF652125		
	<i>A. dimorphicus</i>	NRRL 3650 <sup>T</sup>	EF652111	EF652135		
		<b>SFC20191113-NB100</b>	<b>MW711172</b>	<b>MW711185</b>		
		CMV012C9	MK451246	MK451357		
		NRRL 35052	EU021672	EU021685		
		<i>A. europaeus</i>	CBS 134393 <sup>T</sup>	LN909006	LN909007	
		<i>A. flaschentraegeri</i>	NRRL 5042 <sup>T</sup>	EF652113	EF652130	
		<i>A. gorakhpurensis</i>	NRRL 3649 <sup>T</sup>	EF652114	EF652126	
		<i>A. inflatus</i>	CBS 682.70 <sup>T</sup>	FJ531008	FJ531090	
		<i>A. itaconicus</i>	NRRL 161 <sup>T</sup>	EF652118	EF652140	
		<i>A. koreanus</i>	EML-GSNP1-1 <sup>T</sup>	KX216530	KX216528	
		<i>A. pulvinus</i>	NRRL 5078 <sup>T</sup>	EF652121	EF652139	
		<i>A. stromatoides</i>	CBS 500.65 <sup>T</sup>	FJ531038	EF652127	
		<i>A. tardus</i>	CBS 433.93 <sup>T</sup>	FJ531001	FJ531084	
		<i>A. wentii</i>	NRRL 375 <sup>T</sup>	EF652106	EF652131	
	<i>Usti</i>	<i>A. asper</i>	CBS 140842 <sup>T</sup>	KT698838	KT698839	
		<i>A. baeticus</i>	NRRL 62501 <sup>T</sup>	HE615092	HE615117	
		<i>A. calidoustus</i>	CBS 121601 <sup>T</sup>	FJ624456	HE616559	
			<b>SFC20191113-NB113</b>	<b>MW711167</b>	<b>MW711176</b>	
			<b>SFC20191113-NB116</b>	<b>MW711161</b>	<b>MW711173</b>	
			<b>SFC20191113-NB135</b>	<b>MW711160</b>	<b>MW711175</b>	
			<b>SFC20191113-NB146</b>	<b>MW711162</b>	<b>MW711174</b>	
			<b>SFC20191113-NB197</b>	<b>MW711163</b>	<b>MW711177</b>	
			E449/MIO9	HG931688	HG931695	
			E460	HG964949	HG964950	
			<i>A. carlsbadensis</i>	IBT 14493 <sup>T</sup>	FJ531179	FJ531126
			<i>A. collinsii</i>	CBS 140843 <sup>T</sup>	KT698843	KT698844
			<i>A. contaminans</i>	CBS 142451 <sup>T</sup>	LT594443	LT594425
		<i>A. deflectus</i>	NRRL 2206 <sup>T</sup>	EF652261	EF652349	
		<i>A. elongatus</i>	NRRL 5176 <sup>T</sup>	EF652326	EF652414	
		<i>A. germanicus</i>	DTO 27-D9 <sup>T</sup>	FJ531172	FJ531141	
		<b>SFC20191113-NB098</b>	<b>MW711171</b>	<b>MW711184</b>		
		<i>A. granulosis</i>	NRRL 1932 <sup>T</sup>	EF652254	EF652342	
		<i>A. heterothallicus</i>	NRRL 5096 <sup>T</sup>	EF652323	EF652411	
		<i>A. insuetus</i>	NRRL 279 <sup>T</sup>	EF652281	EF652369	
		<i>A. insuetus</i>	<b>SFC20191113-NB013</b>	<b>MW711170</b>	<b>MW711183</b>	
		<i>A. keveii</i>	CBS 209.92 <sup>T</sup>	EU076376	EU076365	
		<i>A. keveioides</i>	CBS 132737 <sup>T</sup>	JN982694	JN982684	
		<i>A. lucknowensis</i>	NRRL 3491 <sup>T</sup>	EF652283	EF652371	
		<i>A. monodii</i>	CBS 435.93 <sup>T</sup>	FJ531171	FJ531142	
		<i>A. porphyreostipitatus</i>	DTO 266-D9 <sup>T</sup>	KJ775080	KJ775338	
		<i>A. pseudodeflectus</i>	ET1611	KY853416	KY853415	
			NRRL 6135 <sup>T</sup>	EF652331	EF652419	
			<b>SFC20191113-NB114</b>	<b>MW711169</b>	<b>MW711178</b>	
			<b>SFC20191113-NB115</b>	<b>MW711166</b>	<b>MW711179</b>	
			<b>SFC20191113-NB136</b>	<b>MW711168</b>	<b>MW711182</b>	
			<b>SFC20191113-NB156</b>	<b>MW711164</b>	<b>MW711181</b>	
			<b>SFC20191113-NB199</b>	<b>MW711165</b>	<b>MW711180</b>	
		AS3 15308	JN982689	JN982679		
		NRRL 278	EF652280	EF652368		
	<i>A. pseudoustus</i>	IBT 28161 <sup>T</sup>	FJ531168	FJ531129		
	<i>A. puniceus</i>	NRRL 5077 <sup>T</sup>	EF652322	EF652410		
	<i>A. sigurros</i>	CMV00514 <sup>T</sup>	MK451066	MK451512		
	<i>A. thesauricus</i>	NRRL 62487 <sup>T</sup>	HE615095	HE615120		
	<i>A. turkensis</i>	CBS 504.65 <sup>T</sup>	FJ531191	FJ531145		
	<i>A. ustus</i>	NRRL 275 <sup>T</sup>	EF652279	EF652367		

<sup>T</sup> indicates the ex-type strains.

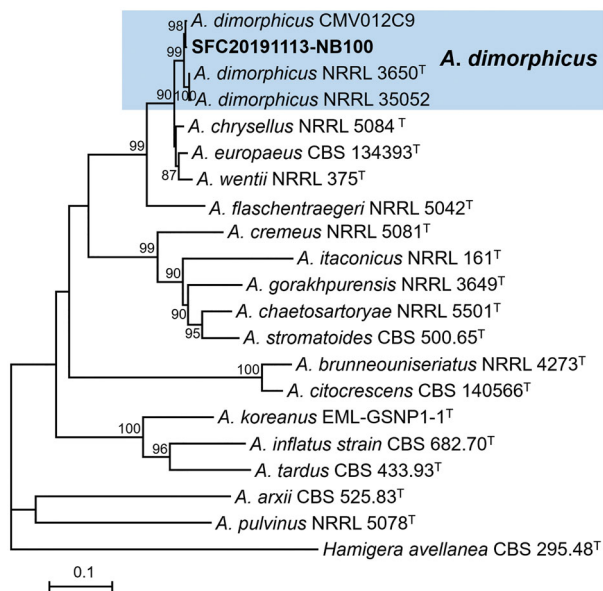
Sequences produced in this study are presented in bold letters.

### 3.2. Taxonomy

*Aspergillus calidoustus* Varga, Houbraken, & Samson (2008)

Description: Colony diameter, at 25 °C for 7 days, in mm: CYA 50–51; CYA 15 °C 12–13; CYA 30 °C 60–66; CYA 37 °C 8–12; MEA 51–54; YES 50–51 (Figure 3).

Colonies on CYA, lightly sulcate, moderate to good sporulation, floccose, greenish gray (27D2) elsewhere with 1 mm white margin, exudate brownish orange (6D8) to dark brown (6F8) droplets, soluble pigment absent, reverse color olive brown (4D3) at center and light yellow (1A4) elsewhere. Colonies on MEA, lightly sulcate, moderate sporulation, velvety with floccose at center, central part



**Figure 1.** Maximum likelihood (ML) phylogenetic tree of *Aspergillus* sect. *Cremei* based on the combined data set of *BenA* and *CaM* sequences. Bootstrap values >70 are presented at the nodes. The scale bar represents the number of nucleotide substitutions per site. "T" indicates the ex-type strains. *Aspergillus* reported in this study are represented in bold. The unrecorded *Aspergillus* species are accented in color box.

gray (27B1) at center and greenish gray (27E2) elsewhere with 1 mm white margin, no exudates, soluble pigment absent, reverse color olive brown (4E4) and orange yellow (4B8) elsewhere. Colonies on YES, lightly sulcate, moderated sporulation, central floccose, gray (3C1) to yellowish gray (3C2) elsewhere with 1 mm white margin, no exudates, soluble pigment light yellow (3A4), reverse color olive (3D3) to yellow (3A7).

*Conidiophores* biseriate with thick, smooth-walled, brown, (2.4–) 3.3 (–4.9)  $\mu\text{m}$  wide; *vesicles* pyriform to broadly spatulate, (8.6–) 9.3  $\times$  9.8 (–10.5)  $\mu\text{m}$ ; *conidial heads* loosely columnar; *metulae* covering the upper half to three-fourths of upper surface, (3.1–) 4.3  $\times$  5.6 (–7.1)  $\mu\text{m}$ ; *phialides* (2.8–) 3.2  $\times$  5.4 (–6.7)  $\mu\text{m}$ ; *conidia* rough walls, inner and outer wall visible, globose, (3.2–) 3.6 to 3.7 (–4.1)  $\mu\text{m}$ .

**Strains examined:** SFC20191113-NB113, SFC20191113-NB116, SFC20191113-NB135, SFC20191113-NB146, and SFC20191113-NB197

**Remarks:** *A. calidoustus* is morphologically similar to *A. pseudodeflectus* and *A. ustus*. *A. calidoustus* was able to grow at 37 °C, but *A. ustus* was not [44]. *A. calidoustus* can be distinguished from *A. pseudodeflectus* by narrow margin and moderate or good sporulation in CYA.

***Aspergillus dimorphicus*** B.S. Mehrotra & R. Prasad (1969)

**Description:** Colony diameter, at 25 °C for 7 days, in mm: CYA 40–43; CYA 15 °C 10–12; CYA 30 °C 37–39; No growth at CYA 37 °C; MEA 30–31; YES 74–75 (Figure 3).

Colonies on CYA, moderately sulcate, moderate sporulation, floccose, yellowish gray (3C2) at center, pastel yellow (3A5) elsewhere with 1 mm white margin exudates yellowish white (3A2), soluble pigment absent, reverse color yellowish white (2A2). Colonies on MEA, lightly sulcate, moderate sporulation, floccose, pastel yellow (3A4) elsewhere with 1 mm white margin, exudates yellowish white (3A2), soluble pigment absent, reverse color light orange (5A5). Colonies on YES, lightly sulcate, good sporulation, floccose, olive yellow (3D6) elsewhere with 1 mm white margin, no exudates, soluble pigment absent, reverse color pastel yellow (3A4).

*Conidiophores* biseriate with smooth-walled, sinuous, light yellow, (3.7–) 4.4 (–6.1)  $\mu\text{m}$  wide; *vesicles* mostly globose to subglobose, (8.0–) 9.9  $\times$  10.3 (–13.4)  $\mu\text{m}$ ; *conidial heads* globose to loosely radiate, *phialides* (3.0–) 3.6  $\times$  9.6 (–11.5)  $\mu\text{m}$ ; *conidia* subglobose to globose with rough wall, 3.5 to 4.7  $\mu\text{m}$ .

**Strain examined:** SFC20191113-NB100

**Remarks:** *A. dimorphicus* is morphologically similar to *A. wentii*, it can be distinguished from *A. wentii* by branched conidiophore with two vesicles [45].

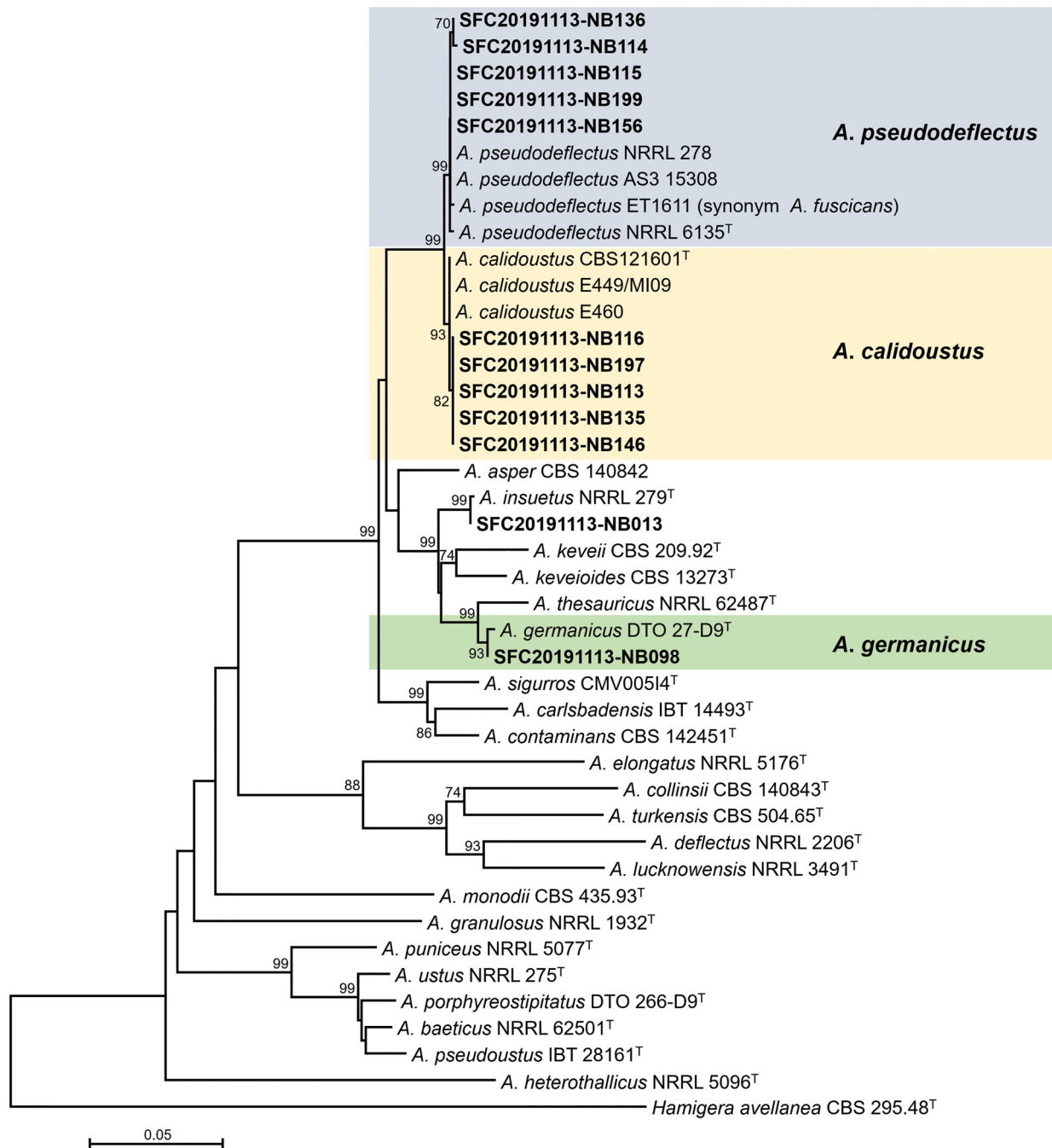
***Aspergillus germanicus*** Varga, Frisvad & Samson (2011)

**Description:** Colony diameter, at 25 °C for 7 days, in mm: CYA 42–43; CYA 15 °C 13–14; CYA 30 °C 47–48; CYA 37 °C 8–9; MEA 40–42; YES 45–48 (Figure 3).

Colonies on CYA, poor sporulation, floccose, orange gray (6B2) to white (26C1) at center, no exudates, soluble pigment yellowish gray (3B2), reverse color brownish gray (4E4) to pale yellow at margin (3A3). Colonies on MEA, poor sporulation, velvety, white, no exudates, soluble pigment absent, reverse color orange (5A7). Colonies on YES, poor sporulation, floccose to velvety, central part color white to grayish yellow (4B4) to white at center, no exudates, soluble pigment pale yellow (3A3), reverse color grayish orange (5B4) at center and grayish yellow (3C4) and light yellow (3A5).

*Conidiophores* biseriate with thick, smooth-walled, brown, (3.7–) 4.7 (–5.4)  $\mu\text{m}$  wide; *vesicles* septulate, (8.5–) 10.1  $\times$  10.3 (–12.4)  $\mu\text{m}$ ; *conidial heads* loosely columnar; *metulae* covering the upper half to three-fourths of upper surface, (2.3–) 3.2  $\times$  5.2 (–5.8)  $\mu\text{m}$ ; *phialides* (2.3–) 2.7  $\times$  5.0 (–6.1)  $\mu\text{m}$ ; *conidia* globose with brown smooth wall, (2.8–) 3.1 to 3.4 (–3.9)  $\mu\text{m}$ .





**Figure 2.** Maximum likelihood (ML) phylogenetic tree of *Aspergillus* sect. *Usti* based on the combined data set of *BenA* and *CaM* sequences. Bootstrap values >70 are presented at the nodes. The scale bar represents the number of nucleotide substitutions per site. "T" indicates the ex-type strains. *Aspergillus* reported in this study are represented in bold. The unrecorded *Aspergillus* species are accented in color box.

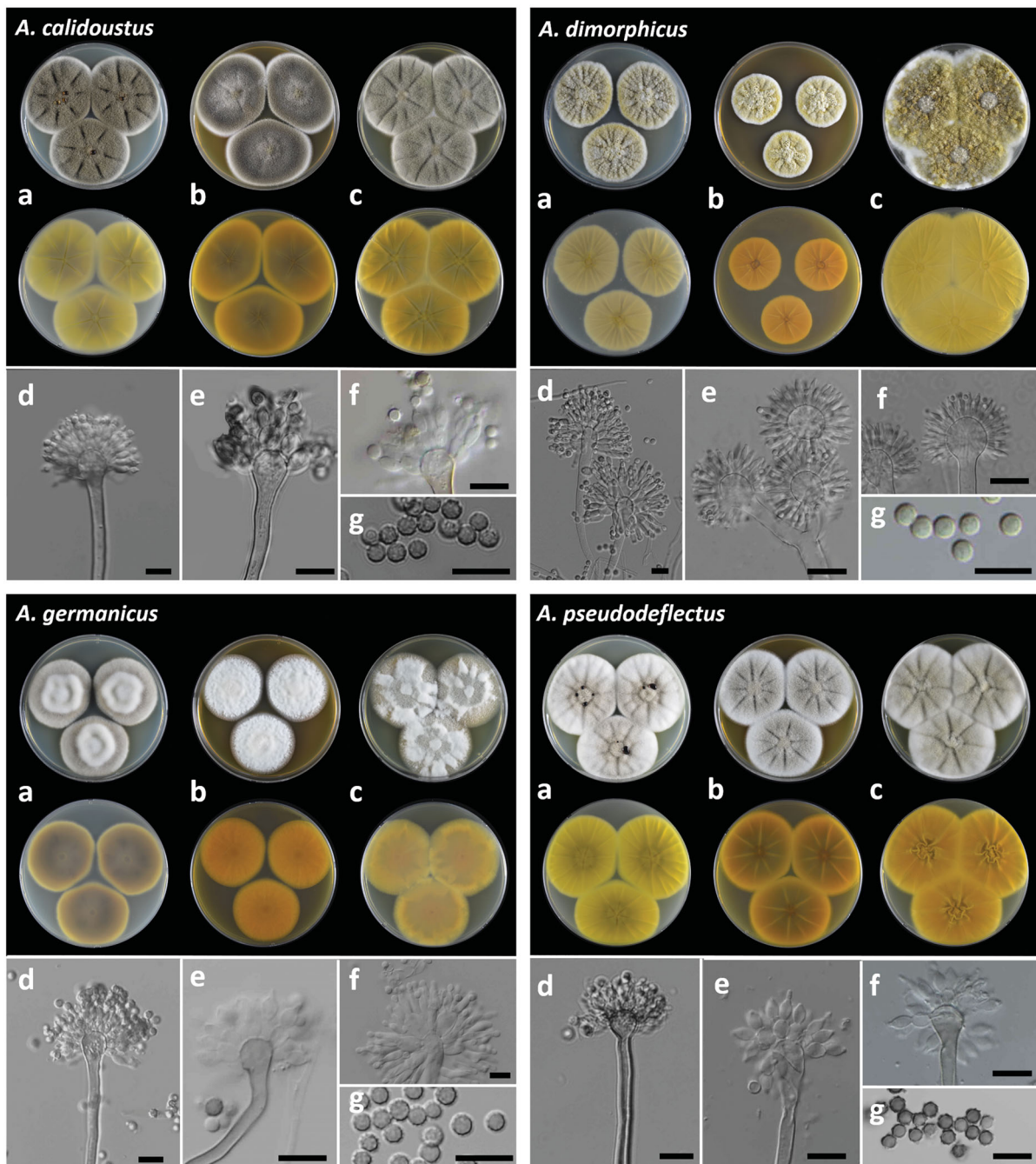
**Strain examined:** SFC20191113-NB098

**Remarks:** *A. germanicus* is morphologically similar to *A. thesausicus*, it can be distinguished from *A. thesausicus* by growth at 37 °C, thicker conidiphore, and smaller vesicle diameter [46].

*Aspergillus pseudodeflectus* Samson & Mouchacca (1975)

**Description:** Colony diameter, at 25 °C for 7 days, in mm: CYA 49–51; CYA 15 °C 10–13; CYA 30 °C 57–59; CYA 37 °C 14–18; MEA 47–48; YES 52–54 (Figure 3).

Colonies on CYA, lightly sulcate, poor to moderate sporulation, floccose, grayish orange (5B3) at center and white elsewhere, exudates dark brown (8F4), soluble pigment pale yellow (3A3), reverse color olive (3D3) at center and light yellow (2A5) elsewhere. Colonies on MEA, moderately sulcate, poor to moderate sporulation, floccose, brownish gray (5C2) and white at margin, no exudates, soluble pigment absent, reverse color brown (6E5) and golden yellow at margin (5B7). Colonies on YES, radially sulcate and wrinkled at center, poor to moderate sporulation, floccose, yellowish gray (4B2)



**Figure 3.** The unrecorded *Aspergillus* species in Korea: *A. calidoustus* (SFC20191113-NB146), *A. dimorphicus* (SFC20191113-NB100), *A. germanicus* (SFC20191113-NB098) and *A. pseudodeflectus* (SFC20191113-NB114). (a–c) Colonies grown on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and yeast extract sucrose agar (YES) from left to right (top = obverse, bottom = reverse). (d–f) Conidiophores; (g) Conidia (scale bar = 10 µm).

and white at margin, no exudates, soluble pigment pale yellow (3A3), reverse color deep yellow (4A8).

*Conidiophores* biserial with short, curved, rough-walled, brown, (3.4–) 3.9 (–4.2) µm wide; *vesicles* globose to clavate, (6.7–) 7.9 × 9.5 (–10.5) µm; *conidial heads* brown, radiate; *metulae* more or less cylindrical, (2.4–) 3.6 × 4.6 (–5.4) µm; *phialides* (2.8–) 3.4 × 6.4 (–8.1) µm; *conidia* globose to ellipsoidal with thick-walled, brown, rough wall, (3.3–) 3.7 to 4.5 (–5.3) µm.

**Strains examined:** SFC20191113-NB114, SFC20191113-NB115, SFC20191113-NB136, SFC20191113-NB156, and SFC20191113-NB199

**Remarks:** *A. pseudodeflectus* is morphologically similar to *A. calidoustus*. *A. pseudodeflectus* can be distinguished from *A. calidoustus* by wide margin and poor sporulation in CYA.

#### 4. Discussion

The rhizosphere soil is a complex and dynamic environment that provides a close relationship between plants and microbes. A total of 13 *Aspergillus* strains were isolated from rhizosphere soil of three plants and were identified as five

species in two sections including four unrecorded species based on *BenA* and *CaM* sequences. Although 12 species in *Aspergillus* section *Fumigati* have been reported from arable soil [17], many previous studies only focused on limited environments, such as meju and nuruk [12,19–21]. Only *Aspergillus terreus* has previously been reported from rhizosphere soil of paprika plants in Korea [47]. Five additional species (*A. calidoustus*, *A. dimorphicus*, *A. germanicus*, *A. insuetus*, and *A. pseudodeflectus*) are reported for the first time in this study, from the rhizosphere soil in Korea.

Four species were unrecorded in Korea: *A. calidoustus*, *A. dimorphicus*, *A. germanicus*, and *A. pseudodeflectus*. *A. calidoustus* is commonly found in clinical environments, indoor air, and forest soil [44,48,49]. It has been isolated from *Acanthospermum austral* and is known for its antifungal and cytotoxic activity [50]. In this study, *A. calidoustus* was isolated from the rhizosphere soil of *Calystegia soldanella* and *Sorbus commixta*. *A. pseudodeflectus* was previously isolated from desert soil and seaweed [51,52]. It produced pseudodeflectusin, which exhibited cytotoxic activity [51]. In this study, *A. pseudodeflectus* strains were isolated from rhizosphere soil of *Calystegia soldanella* and *Sorbus commixta*. The *A. pseudodeflectus* strains isolated from Korea exhibited faster growth on YES compared to the other reported strains [53,54]. Some fungi isolated from different environments exhibit different metabolism and growth rates due to environment adaptation [55,56]. *A. germanicus* was first isolated from indoor air, but there are few reports of the species so far [57]. In this study, *A. germanicus* was isolated from the rhizosphere soil of *Orobanche coerulescens*. Our study is the first report of the species from rhizosphere soil. *A. dimorphicus* was isolated from garden soil, loess soil, and deep-sea sediment [58–61]. *A. dimorphicus* showed antitumor activities [62] and proteolytic activities [63]. *A. dimorphicus* strain was isolated from the rhizosphere soil of *Orobanche coerulescens* in this study.

*Aspergillus* species are well known for their potential for usage in industrial and medical compounds, but many strains remain at the genus level. Therefore, we believe that our study will provide the basis for the discovery of new compound based on accurate identification of *Aspergillus*. Although many *Aspergillus* species have been found in rhizosphere soils using the NGS method [64–66], the role of *Aspergillus* in rhizosphere soil is unclear. To understand the interaction between *Aspergillus* and plants, further studies are needed to investigate the function of *Aspergillus* in rhizosphere soil.

### Disclosure statement

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

### Funding

This research was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea [grant number NIBR202002104 and NIBR202102107].

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