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RESEARCH ARTICLE

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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 β-tubulin and calmodulin and morphological characteristics, we identified five *Aspergillus* species. *A. calidoustus* and *A. pseudodeflectus* were commonly isolated from the rhizosphere soil. Four species were confirmed as unrecorded species in Korea: *A. calidoustus*, *A. dimorphicus*, *A. germanicus*, and *A. pseudodeflecuts*. The detailed morphological descriptions of these unrecorded species are provided.

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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*), *β*–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 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. calidoustus*, *A. dimorphicus*, *A. germanicus*, and *A. pseudodeflectus*.

2. Materials and methods

2.1. Sample collections and isolation

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

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

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

[a]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 ExpinTM 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 *Cremei*, 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 *Calytegia 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.

			GenBank accession no.	
Section of *Aspergillus*	Species	Strain	*BenA*	*CaM*
Cremei	*A. arxii*	CBS 525.83^T	MN969365	MN969223
	A. brunneouniseriatus	NRRL 4273^T	EF652123	EF652138
	A. chaetosartoryae	NRRL 5501^T	EF652117	EF652129
	A. chrysellus	NRRL 5084^T	EF652109	EF652136
	A. citocrescens	CBS 140566^T	FR775317	LN878969
	A. cremeus	NRRL 5081^T	EF652120	EF652125
	A. dimorphicus	NRRL 3650^T	EF652111	EF652135
		SFC20191113-NB100	**MW711172**	**MW711185**
		CMV012C9	MK451246	MK451357
		NRRL 35052	EU021672	EU021685
	A. europaeus	CBS 134393^T	LN909006	LN909007
	A. flaschentraegeri	NRRL 5042^T	EF652113	EF652130
	A. gorakhpurensis	NRRL 3649^T	EF652114	EF652126
	A. inflatus	CBS 682.70^T	FJ531008	FJ531090
	A. itaconicus	NRRL 161^T	EF652118	EF652140
	A. koreanus	EML-GSNP1-1^T	KX216530	KX216528
	A. pulvinus	NRRL 5078^T	EF652121	EF652139
	A. stromatoides	CBS 500.65^T	FJ531038	EF652127
	A. tardus	CBS 433.93^T	FJ531001	FJ531084
	A. wentii	NRRL 375^T	EF652106	EF652131
Usti	*A. asper*	CBS 140842^T	KT698838	KT698839
	A. baeticus	NRRL 62501^T	HE615092	HE615117
	A. calidoustus	CBS 121601^T	FJ624456	HE616559
		SFC20191113-NB113	**MW711167**	**MW711176**
		SFC20191113-NB116	**MW711161**	**MW711173**
		SFC20191113-NB135	**MW711160**	**MW711175**
		SFC20191113-NB146	**MW711162**	**MW711174**
		SFC20191113-NB197	**MW711163**	**MW711177**
		E449/MI09	HG931688	HG931695
		E460	HG964949	HG964950
	A. carlsbadensis	IBT 14493^T	FJ531179	FJ531126
	A. collinsii	CBS 140843^T	KT698843	KT698844
	A. contaminans	CBS 142451^T	LT594443	LT594425
	A. deflectus	NRRL 2206^T	EF652261	EF652349
	A. elongatus	NRRL 5176^T	EF652326	EF652414
	A. germanicus	DTO 27-D9^T	FJ531172	FJ531141
		SFC20191113-NB098	**MW711171**	**MW711184**
	A. granulosus	NRRL 1932^T	EF652254	EF652342
	A. heterothallicus	NRRL 5096^T	EF652323	EF652411
	A. insuetus	NRRL 279^T	EF652281	EF652369
	A. insuetus	**SFC20191113-NB013**	**MW711170**	**MW711183**
	A. keveii	CBS 209.92^T	EU076376	EU076365
	A. keveioides	CBS 132737^T	JN982694	JN982684
	A. lucknowensis	NRRL 3491^T	EF652283	EF652371
	A. monodii	CBS 435.93^T	FJ531171	FJ531142
	A. porphyreostipitatus	DTO 266-D9^T	KJ775080	KJ775338
	A. pseudodeflectus	ET1611	KY853416	KY853415
		NRRL 6135^T	EF652331	EF652419
		SFC20191113-NB114	**MW711169**	**MW711178**
		SFC20191113-NB115	**MW711166**	**MW711179**
		SFC20191113-NB136	**MW711168**	**MW711182**
		SFC20191113-NB156	**MW711164**	**MW711181**
		SFC20191113-NB199	**MW711165**	**MW711180**
		AS3 15308	JN982689	JN982679
		NRRL 278	EF652280	EF652368
	A. pseudoustus	IBT 28161^T	FJ531168	FJ531129
	A. puniceus	NRRL 5077^T	EF652322	EF652410
	A. sigurros	CMV005I4^T	MK451066	MK451512
	A. thesauricus	NRRL 62487^T	HE615095	HE615120
	A. turkensis	CBS 504.65^T	FJ531191	FJ531145
	A. ustus	NRRL 275^T	EF652279	EF652367

"T" 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

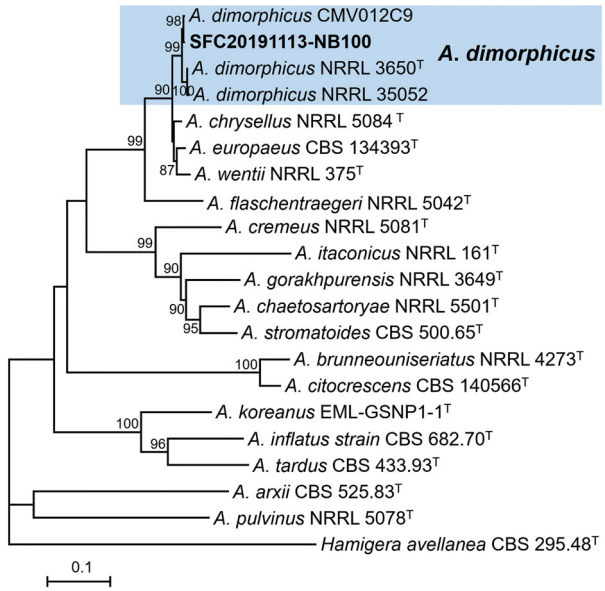

Figure 1. Maximum likelihood (ML) phylogenetic tree of *Aspergillus* sect. *Cremei* based on the combined data set of *BenA* and *CaM* sequences. Bootstrap values >70 are presented at the nodes. The scale bar represents the number of nucleotide substitutions per site. "T" indicates the ex-type strains. *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) µm wide; *vesicles* pyriform to broadly spathulate, (8.6–) 9.3 × 9.8 (–10.5) µm; *conidial heads* loosely columnar; *metulae* covering the upper half to three-fourths of upper surface, (3.1–) 4.3 × 5.6 (–7.1) µm; *phialides* (2.8–) 3.2 × 5.4 (–6.7) µm; *conidia* rough walls, inner and outer wall visible, globose, (3.2–) 3.6 to 3.7 (–4.1) µm.

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) µm wide; *vesicles* mostly globose to subglobose, (8.0–) 9.9 × 10.3 (–13.4) µm; *conidial heads* globose to loosely radiate, *phialides* (3.0–) 3.6 × 9.6 (–11.5) µm; *conidia* subglobose to globose with rough wall, 3.5 to 4.7 µm.

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) µm wide; *vesicles* septulate, (8.5–) 10.1 × 10.3 (–12.4) µm; *conidial heads* loosely columnar; *metulae* covering the upper half to three-fourths of upper surface, (2.3–) 3.2 × 5.2 (–5.8) µm; *phialides* (2.3–) 2.7 × 5.0 (–6.1) µm; *conidia* globose with brown smooth wall, (2.8–) 3.1 to 3.4 (–3.9) µm.

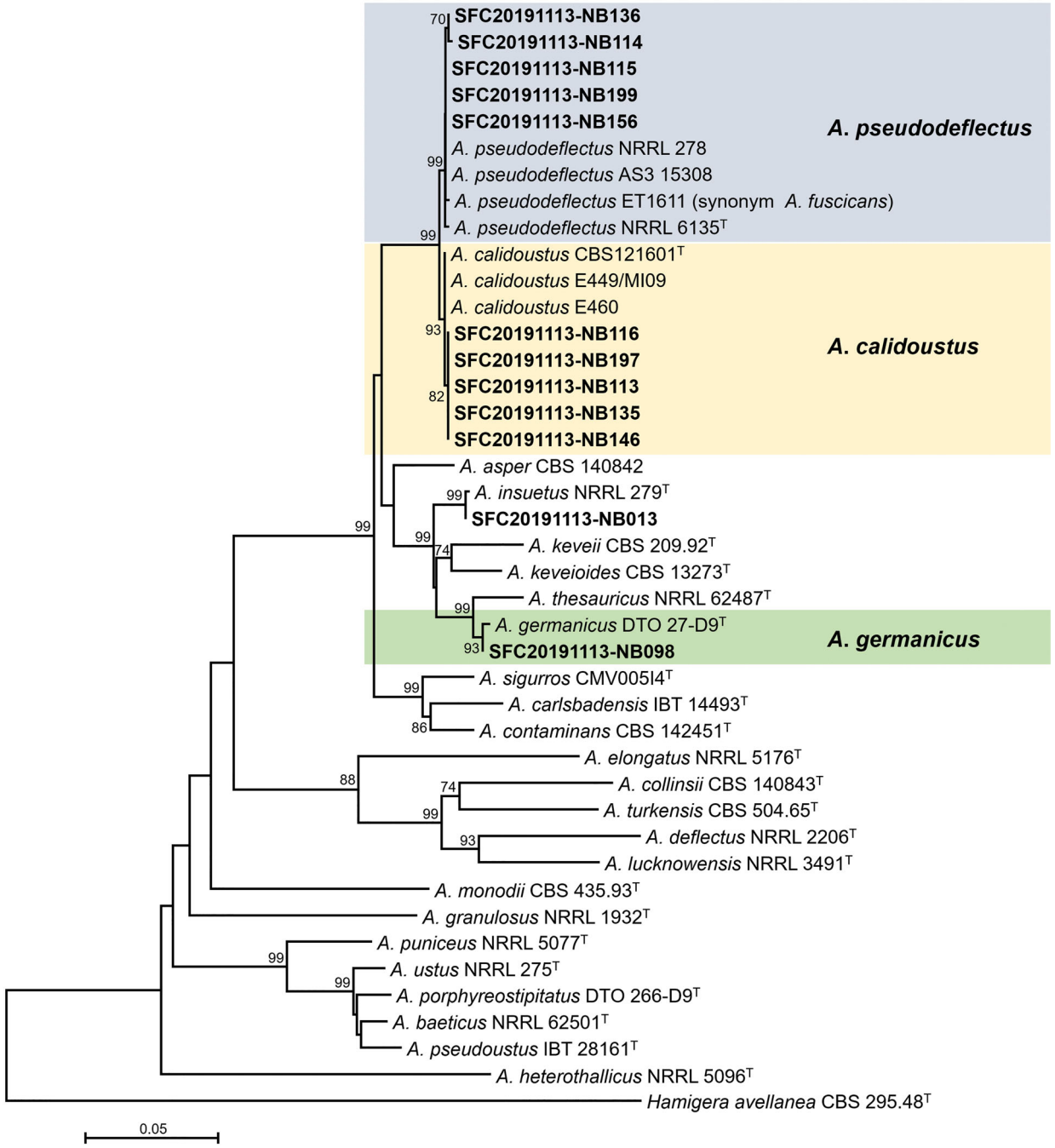

Figure 2. Maximum likelihood (ML) phylogenetic tree of *Aspergillus* sect. *Usti* based on the combined data set of *BenA* and *CaM* sequences. Bootstrap values >70 are presented at the nodes. The scale bar represents the number of nucleotide substitutions per site. "T" indicates the ex-type strains. *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. thesauricus*, it can be distinguished from *A. thesauricus* by growth at 37 °C, thicker conidiophore, 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)

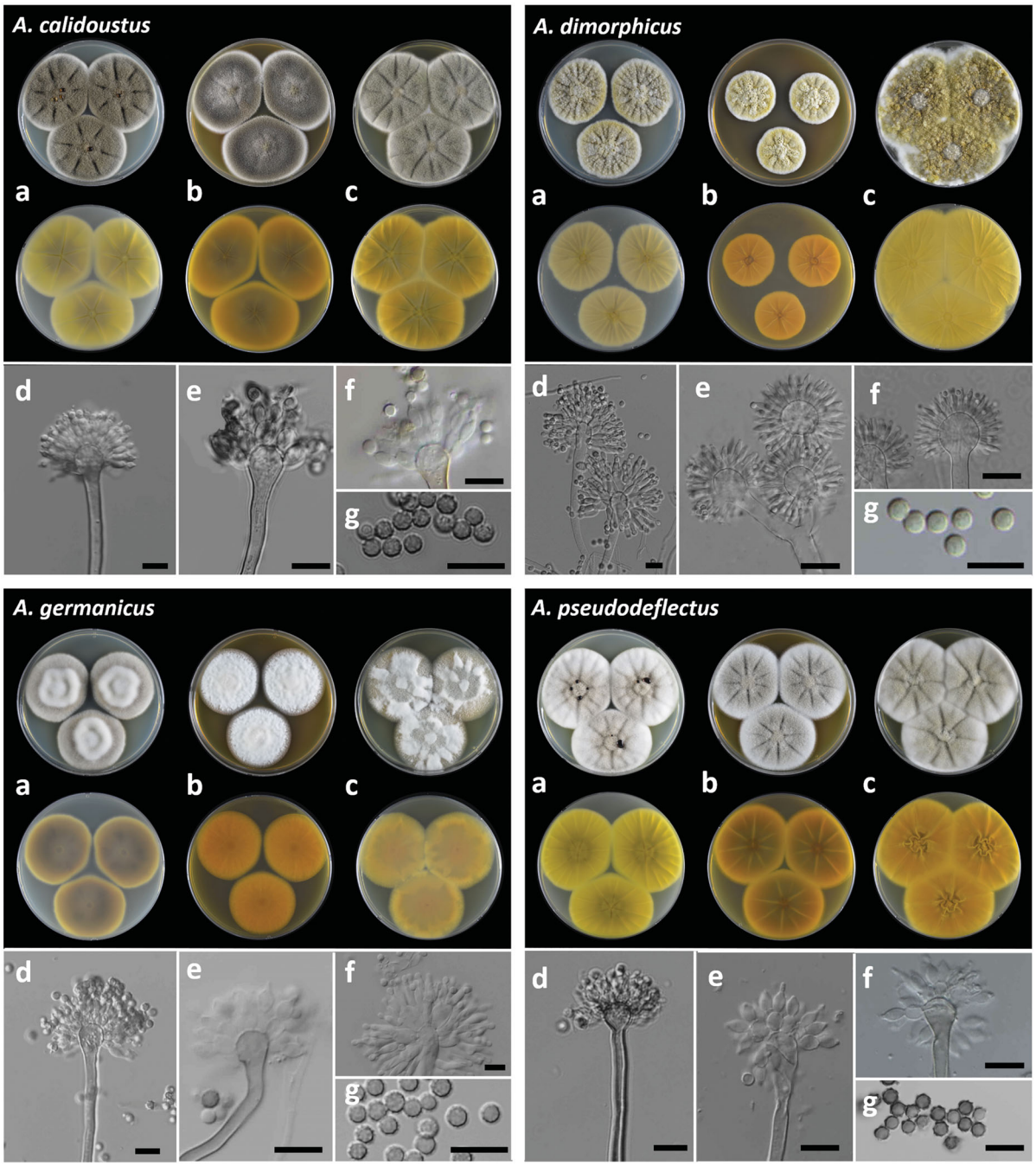

Figure 3. The unrecorded *Aspergillus* species in Korea: *A. calidoustus* (SFC20191113-NB146), *A. dimorphicus* (SFC20191113-NB100), *A. germanicus* (SFC20191113-NB098) and *A. pseudodeflectus* (SFC20191113-NB114). (a–c) Colonies grown on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and 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 biseriate 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. germaincus*, *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).

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References

[1] Planchot V, Colonna P, Gallant DJ, et al. Extensive degradation of native starch granules by alpha-amylase from *Aspergillus fumigatus*. J Cereal Sci. 1995;21(2):163–171.
[2] Hrmová M, Biely P, VršAnská M. Cellulose-and xylan-degrading enzymes of *Aspergillus terreus* and *Aspergillus niger*. Enzyme Microb Technol. 1989; 11(9):610–616.
[3] de Vries RP, Visser J. *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. Microbiol Mol Biol Rev. 2001;65(4):497–522.
[4] Scheckermann C, Wagner F, Fischer L. Galactosylation of antibiotics using the *β*-galactosidase from *Aspergillus oryzae*. Enzyme Microb Technol. 1997;20(8):629–634.
[5] Yang L, Lübeck M, Lübeck PS. *Aspergillus* as a versatile cell factory for organic acid production. Fungal Biol Rev. 2017;31(1):33–49.
[6] Geiser DM, Klich MA, Frisvad JC, et al. The current status of species recognition and identification in *Aspergillus*. Stud Mycol. 2007;59:1–10.
[7] Balajee SA, Houbraken J, Verweij PE, et al. *Aspergillus* species identification in the clinical setting. Stud Mycol. 2007;59:39–46.
[8] Samson RA, Visagie CM, Houbraken J, et al. Phylogeny, identification and nomenclature of the genus *Aspergillus*. Stud Mycol. 2014;78:141–173.
[9] Houbraken J, Kocsubé S, Visagie CM, et al. Classification of *Aspergillus*, *Penicillium*, *Talaromyces* and related genera (Eurotiales): an overview of families, genera, subgenera, sections, series and species. Stud Mycol. 2020;95:5–169.
[10] Kim HJ, Kim JS, Cheon KH, et al. Species list of *Aspergillus*, *Penicillium* and *Talaromyces* in Korea, Based on one fungus one name system. Kor J Mycol. 2016;44(4):207–219.
[11] Oh JY, Mannaa M, Han GD, et al. First report of *Aspergillus awamori* as a fungal pathogen of garlic (*Allium sativum* L.). Crop Prot. 2016;85:65–70.
[12] Kim KM, Lim J, Lee JJ, et al. Characterization of *Aspergillus sojae* isolated from meju, Korean traditional fermented soybean brick. J Microbiol Biotechnol. 2017;27(2):251–261.
[13] Nguyen TT, Pangging M, Bangash NK, et al. Five new records of the family Aspergillaceae in Korea, *Aspergillus europaeus*, *A. pragensis*, *A. tennesseensis*, *Penicillium fluviserpens*, and *P. scabrosum*. Mycobiology. 2020;48(2):81–94.
[14] National List of Species of Korea. 2019. National Institute of Biological Resources. [Internet] [cited 2021 Mar 5]. Available from: http://kbr.go.kr.

[15] Kim JD. Keratinolytic activity of five *Aspergillus* species isolated from poultry farming soil in Korea. Mycobiology. 2003;31(3):157–161.
[16] Kim DH, Kim SH, Kim YK, et al. Reidentification of *Aspergillus* spp. isolated from clinical specimens of patients suspected as pulmonary aspergillosis in Korea. Korean J Med Mycol. 2009;14(3):133–144.
[17] Hong SB, Kim DH, Park IC, et al. Isolation and identification of *Aspergillus* section *Fumigati* strains from arable soil in Korea. Mycobiology. 2010;38(1):1–6.
[18] Lee S, Park MS, Lim YW. Diversity of marine-derived *Aspergillus* from tidal mudflats and sea sand in Korea. Mycobiology. 2016;44(4):237–247.
[19] Hong SB, Lee M, Kim DH, et al. *Aspergillus cibarius* sp. nov., from traditional meju in Korea. J Microbiol. 2012;50(4):712–714.
[20] Yang S, Choi SJ, Kwak J, et al. *Aspergillus oryzae* strains isolated from traditional Korean nuruk: fermentation properties and influence on rice wine quality. Food Sci Biotechnol. 2013;22(2):425–432.
[21] Kim HR, Kim JH, Bai DH, et al. Identification and characterization of useful fungi with α-amylase activity from the Korean traditional nuruk. Mycobiology. 2011;39(4):278–282.
[22] Hong SB, Shin HD, Hong J, et al. New taxa of *Neosartorya* and *Aspergillus* in *Aspergillus* section *Fumigati*. Antonie Van Leeuwenhoek. 2008;93(1-2):87–98.
[23] Coats VC, Rumpho ME. The rhizosphere microbiota of plant invaders: an overview of recent advances in the microbiomics of invasive plants. Front Microbiol. 2014;5:368.
[24] Ehrmann J, Ritz K. Plant: soil interactions in temperate multi-cropping production systems. Plant Soil. 2014;376(1-2):1–29.
[25] Wijeratne EK, Turbyville TJ, Zhang Z, et al. Cytotoxic constituents of *Aspergillus terreus* from the rhizosphere of *Opuntia versicolor* of the Sonoran Desert. J Nat Prod. 2003;66(12):1567–1573.
[26] Jain R, Saxena J, Sharma V. Solubilization of inorganic phosphates by *Aspergillus awamori* S19 isolated from rhizosphere soil of a semi-arid region. Ann Microbiol. 2012;62(2):725–735.
[27] Islam S, Akanda AM, Sultana F, et al. Chilli rhizosphere fungus *Aspergillus* spp. PPA1 promotes vegetative growth of cucumber (*Cucumis sativus*) plants upon root colonisation. Arch Phytopathol. 2014;47(10):1231–1238.
[28] Pandya ND, Desai PV, Jadhav HP, et al. Plant growth promoting potential of *Aspergillus* sp. NPF7, isolated from wheat rhizosphere in South Gujarat, India. Environ Sustain. 2018;1(3):245–252.
[29] He W, Xu Y, Fu P, et al. Cytotoxic indolyl diketopiperazines from the *Aspergillus* sp. GZWMJZ-258, endophytic with the medicinal and edible plant *Garcinia multiflora*. J Agric Food Chem. 2019;67(38):10660–10666.
[30] Orfali R, Perveen S. Secondary metabolites from the *Aspergillus* sp. in the rhizosphere soil of *Phoenix dactylifera* (Palm tree). BMC Chem. 2019;13(1):1–6.
[31] Tavakol Noorabadi M, Babaeizad V, Zare R, et al. Isolation, Molecular identification, and mycotoxin production of *Aspergillus* species isolated from the rhizosphere of sugarcane in the South of Iran. Toxins. 2020;12(2):122.
[32] Park MS, Lee JW, Kim SH, et al. *Penicillium* from rhizosphere soil in terrestrial and coastal environments in South Korea. Mycobiology. 2020;48(6):431–442.
[33] Rogers SO, Bendich AJ. Extraction of total cellular DNA from plants, algae and fungi. In: Gelvin S, Schilperoort R, editors. Plant molecular biology manual. Dordrecht: Kluwer Academic; 1994.
[34] Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol. 1995;61(4):1323–1330.
[35] Hong SB, Go SJ, Shin HD, et al. Polyphasic taxonomy of *Aspergillus fumigatus* and related species. Mycologia. 2005;97(6):1316–1329.
[36] Peterson SW, Vega FE, Posada F, et al. *Penicillium coffeae*, a new endophytic species isolated from a coffee plant and its phylogenetic relationship to *P. fellutanum, P. thiersii* and *P. brocae* based on parsimony analysis of multilocus DNA sequences. Mycologia. 2005;97(3):659–666.
[37] Park MS, Lee S, Oh SY, et al. Diversity and enzyme activity of *Penicillium* species associated with macroalgae in Jeju Island. J Microbiol. 2016;54(10):646–654.
[38] Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870–1874.
[39] Visagie CM, Hirooka Y, Tanney JB, et al. *Aspergillus*, *Penicillium* and *Talaromyces* isolated from house dust samples collected around the world. Stud Mycol. 2014;78:63–139.
[40] Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30(4):772–780.
[41] Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 2006;22(21):2688–2690.
[42] Miller MA, Pfeiffer W, Schwartz T. Creating the CIPRES science gateway for inference of large phylogenetic trees. Paper presented at: SC10 workshop on gateway computing environments (GCE10), New Orleans, LA; 2010. p. 1–8.
[43] Kornerup A, Wanscher JH. Methuen handbook of colour. 3rd ed. London: Methuen; 1978.
[44] Varga J, Houbraken J, Van Der Lee HA, et al. *Aspergillus calidoustus* sp. nov., causative agent of human infections previously assigned to *Aspergillus ustus*. Eukaryot Cell. 2008;7(4):630–638.
[45] Peterson SW. Phylogenetic analysis of *Aspergillus* sections *Cremei* and *Wentii*, based on ribosomal DNA sequences. Mycol Res. 1995;99(11):1349–1355.
[46] Novakova A, Hubka V, Saiz-Jimenez C, et al. *Aspergillus baeticus* sp. nov. and *Aspergillus thesauricus* sp. nov., two species in section *Usti* from Spanish caves. Int J Syst Evol Microbiol. 2012;62(Pt 11):2778–2785.
[47] Yoo SJ, Shin DJ, Won HY, et al. *Aspergillus terreus* JF27 promotes the growth of tomato plants and induces resistance against *Pseudomonas syringae* pv. *tomato*. Mycobiology. 2018;46(2):147–153.

[48] Slack GJ. Identification of secondary metabolites from some Eurotium species, *Aspergillus insuetus* and *A. calidoustus* from Canadian homes. Ottawa, Canada: Carleton University; 2008.
[49] dos Reis Celestino J, de Carvalho LE, da Paz Lima M, et al. Bioprospecting of Amazon soil fungi with the potential for pigment production. Process Biochem. 2014;49(4):569–575.
[50] Rodrigues de Carvalho C, Vieira MDLA, Cantrell CL, et al. Biological activities of ophiobolin K and 6-epi-ophiobolin K produced by the endophytic fungus *Aspergillus calidoustus*. Nat Prod Res. 2016; 30(4):478–481.
[51] Samson RA, Mouchacca J. Additional notes on species of *Aspergillus*, *Eurotium* and *Emericella* from Egyptian desert soil. Antonie Van Leeuwenhoek. 1975;41(3):343–351.
[52] Ogawa A, Murakami C, Kamisuki S, et al. Pseudodeflectusin, a novel isochroman derivative from *Aspergillus pseudodeflectus* a parasite of the sea weed, *Sargassum fusiform*, as a selective human cancer cytotoxin. Bioorg Med Chem Lett. 2004; 14(13):3539–3543.
[53] Houbraken J, Due M, Varga J, et al. Polyphasic taxonomy of *Aspergillus* section *Usti*. Stud Mycol. 2007;59:107–128.
[54] Romero SM, Comerio RM, Barrera VA, et al. *Aspergillus fuscicans* (Aspergillaceae, Eurotiales), a new species in section *Usti* from Argentinean semi-arid soil. Phytotaxa. 2018;343(1):67–74.
[55] Bidochka MJ, Menzies FV, Kamp AM. Genetic groups of the insect-pathogenic fungus *Beauveria bassiana* are associated with habitat and thermal growth preferences. Arch Microbiol. 2002;178(6): 531–537.
[56] Baakza A, Vala AK, Dave BP, et al. A comparative study of siderophore production by fungi from marine and terrestrial habitats. J Exp Mar Biol Ecol. 2004;311(1):1–9.
[57] Samson RA, Varga J, Meijer M, et al. New taxa in *Aspergillus* section *Usti*. Stud Mycol. 2011;69(1): 81–97.
[58] Mehrotra B, Prasad R. *Aspergillus dimorphicus* and *Emericella cleisto-minuta* spp. nov. from Indian soils. Trans Brit Mycol Soc. 1969;52(2):331–336.
[59] Tuthill DE, Christensen M. *Aspergillus sepultus*, a new species in the *Aspergillus ochraceus* group. Mycologia. 1986;78(3):475–477.
[60] Deshmukh SK, Prakash V, Ranjan N. Marine fungi: a source of potential anticancer compounds. Front Microbiol. 2017;8:2536.
[61] Visagie CM, Houbraken J. Updating the taxonomy of *Aspergillus* in South Africa. Stud Mycol. 2020; 95:253–292.
[62] Xu R, Xu GM, Li XM, et al. Characterization of a newly isolated marine fungus *Aspergillus dimorphicus* for optimized production of the anti-tumor agent wentilactones. Mar Drugs. 2015;13(11): 7040–7054.
[63] Rodarte MP, Dias DR, Vilela DM, et al. Proteolytic activities of bacteria, yeasts and filamentous fungi isolated from coffee fruit (*Coffea arabica* L.). Acta Sci Agron. 2011;33(3):457–464.
[64] Noor SO, Al-Zahrani DA, Hussein RM, et al. Assessment of fungal diversity in soil rhizosphere associated with *Rhazya stricta* and some desert plants using metagenomics. Arch Microbiol. 2020; 202(9):1–9.
[65] Pattnaik SS, Busi S. Rhizospheric fungi: diversity and potential biotechnological applications, in recent advancement in white biotechnology through fungi. In: Yadav A, Mishra S, Singh S, Gupta A, editors. Recent advancement in white biotechnology through fungi. Fungal biology. Cham, Switzerland: Springer; 2019. p. 63–84.
[66] Wu N, Li Z, Wu F, et al. Microenvironment and microbial community in the rhizosphere of dioecious *Populus cathayana* at Chaka Salt Lake. J Soils Sediments. 2019;19(6):2740–2751.