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RESEARCH ARTICLE

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Seven Undescribed *Aspergillus* Species from Different Niches in Korea

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ABSTRACT

An investigation of species of the genus *Aspergillus* present in arthropod, freshwater, and soil led to the discovery of seven undescribed species in Korea. Based on their morphological characteristics and molecular phylogeny analyses using a combined data set of β-tubulin (*BenA*) and calmodulin (*CaM*) sequences, the isolated strains CNUFC IGS2-5, CNUFC YJ1-19, CNUFC WD27, CNUFC U8-70, CNUFC AS2-24, CNUFC S32-1, and CNUFC U7-48, were identified as *Aspergillus brunneoviolaceus*, *A. capensis*, *A. floccosus*, *A. inflatus*, *A. parvulus*, *A. polyporicola*, and *A. spelaeus*, respectively. In the present study, the detailed morphological descriptions and phylogenetic relationships of these species are provided.

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Aspergillus; arthropod; freshwater; soil; morphology; phylogeny

1. Introduction

The genus *Aspergillus* (class: Eurotiomycetes; order: Eurotiales; family: Aspergillaceae) was first identified as asexual fungi for conidiophores resembling an aspergillum by Micheli in 1729 [1]. This genus classified into 6 subgenera and 27 sections [2,3]. Members of this genus are mainly environmental saprobes, acting as decomposers of organic materials and can also be found in vegetation, fruits, foods, indoor environments, water, soil, and air. Some *Aspergillus* species are of economic importance, producing itaconic acid used in polymer manufacturing and the cholesterol-lowering drug lovastatin [4], whereas others produce mycotoxins, cause food spoilage, promote development of allergies and other health problems, and also causes infections in humans and animals [3].

Aspergillus species identification presently relies on standardized methods based on morphological characteristics, multiloci DNA sequence analyses, and extrolite characterization. Molecular DNA markers are involved in sequencing of the internal transcribed spacer, *β*-tubulin (*BenA*), calmodulin (*CaM*), and the RNA polymerase II second largest subunit (*RPB2*) sequences. Currently, this genus consists of 446 species [2], only two of which is registered in Korea [5,6]. Furthermore, about 76 species of *Aspergillus* have been reported from Korea, in comparison to the recent publications of new species that have been discovered from other countries [7–10].

Therefore, the present study aimed to identify and provide a brief description of seven undescribed species belonging to five different sections of *Aspergillus* in Korea, that is, *A. brunneoviolaceus*, *A. capensis*, *A. floccosus*, *A. inflatus*, *A. parvulus*, *A. polyporicola*, and *A. spelaeus*, based on their morphological and molecular analyses. This study contributes to the knowledge on biodiversity of *Aspergillus* species in Korea.

2. Materials and methods

2.1. Sample collection and isolation

The samples collected from various locations, as listed in Table 1, were placed in sterile plastic bags and 50-mL falcon tubes and transferred to the laboratory. Serial dilutions were prepared for the isolations from freshwater and soil samples following the method described by Pangging *et al.* [11]. The body surface of arthropod was cut and placed onto potato dextrose agar (PDA; Difco™ Becton, Dickinson and Co., Sparks, MD, USA) supplemented with penicillin (50 mg/L) and streptomycin (50 mg/L) to inhibit the growth of bacteria.

Pure isolates were maintained in 20% glycerol at −80 °C and PDA slant tubes at the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, Korea as CNUFC IGS2-5, CNUFC YJ1-19, CNUFC WD27, CNUFC U8-70, CNUFC AS2-24, CNUFC S32-1, and CNUFC U7-48, as long-term preservations. Moreover, CNUFC IGS2-5, CNUFC U8-70, CNUFC AS2-24, CNUFC S32-1, and CNUFC U7-48 were deposited at the Collection of National Institute of Biological Resources (NIBR), Incheon, Korea.

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Table 1. Information of isolates used in this study.

Species	Strain no.	Source	Location
A. brunneoviolaceus	CNUFC IGS2-5 = QWJQFGC000000441	Arthropod	Imgok-dong, Gwangsan-gu, Gwangju, Korea (35°13′05.2″ N 126°44′43.7″ E)
A. capensis	CNUFC YJ1-19 = NNIBRFG9303	Freshwater	Jukrim-ri, Sora-myeon, Yeosu-si, Jeonnam Province, Korea (34°45′40.0″ N 127°37′21.8″ E)
A. floccosus	CNUFC WD27 = NNIBRFG9304	Freshwater	Jeongdo-ri, Gugyedeung, Wando, Korea (34°18′46.0″ N 126°45′20.0″ E)
A. inflatus	CNUFC U8-70 = QWJQFGC000000299	Rhizosphere soil	Hyeonpo-ri, Buk-myeon, Ulleung Island, Korea (37°31′13.9″ N 130°48′57.5″ E)
A. parvulus	CNUFC AS2-24 = IMYKFGC000000017	Dry soil	Anmyeon-eup, Taean-gun, Anmyeondo, Korea (36°44′43.5″ N 126°17′54.0″ E)
A. polyporicola	CNUFC S32-1 = IMYKFGC000000060	Rhizosphere soil	Miryang, Gyeongnam Province, Korea (35°29′48.4″ N 128°45′39.9″ E)
A. spelaeus	CNUFC U7-48 = QWJQFGC000000300	Wildgrapes rhizosphere soil	Gitdaebong, Ulleung Island, Korea (37°30′22.7″ N 130°51′25.0″ E)

CNUFC YJ1-19 and CNUFC WD27 were deposited at the Culture Collection of the Nakdonggang National Institute of Biological Resources (NNIBR), Sangju, Korea.

2.2. Morphological characteristics

The seven undescribed species were cultured onto Czapek yeast autolysate agar (CYA), Malt extract autolysate agar (MEA) and Yeast extract sucrose agar (YES) [12] and further incubated at 25 °C in the dark for 7 days. An Olympus BX51 microscope with differential interference contrast optics (Olympus, Tokyo, Japan) was used to capture digital image fragments of mycelia that were removed from the cultures and placed on microscope slides with lactic acid (60%).

2.3. DNA extraction, PCR, and sequencing

Fungal isolates were cultured on PDA at 25 °C for 5–7 days. Genomic DNA was extracted using the Solg TM Genomic DNA Preparation Kit (Solgent Co. Ltd., Daejeon, Korea). The primer pairs Bt2a/Bt2b, T10/Bt2b [13], Ben2f/T22 [14] for *BenA*; Cmd5/Cmd6 [6], CF1/CF4 [15] for *CaM* were used for amplification. PCR amplification was performed according to the conditions described by Visagie *et al.* [12]. Thereafter, the PCR products were purified with an Accuprep PCR Purification Kit (Bioneer Corp., Daejeon, South Korea). Sequencing was done using the same primer pairs and then analyzed using ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

2.4. Phylogenetic analysis

Sequences for the selected strains were aligned with reference sequences obtained from GenBank using Clustal_X version 2.1 [16] and were edited manually with Bioedit version 7.2.6.0 [17]. Maximum likelihood (ML) phylogenies were constructed using MEGA version X [18]. The sequence of *Talaromyces flavus* CBS 310.38 ^{T} was used as an out group. The sequences of the isolates in this study were deposited in the NCBI database under the accession numbers listed in Table 2.

3. Results

3.1. Phylogenetic analysis

A BLASTn search of the *BenA* regions of CNUFC IGS2-5, CNUFC YJ1-19, CNUFC WD27, CNUFC-U8-70, CNUFC AS2-24, CNUFC S32-1, and CNUFC U7-48, revealed similarities of 100% (574/574 bp), 97.5% (503/516 bp), 100% (528/528 bp), 99.8% (465/466 bp), 99.4% (511/514 bp), 99.6% (517/519 bp), and 100% (519/519 bp), with *A. brunneoviolaceus* (MH614578), *A. capensis* (KJ775072), *A. floccosus* (FJ491714), *A. inflatus* (FJ531007), *A. parvulus* (KX423625), *A. polyporicola* (EU014088), and *A. spelaeus* (LT798972), respectively. Similarly, BLASTn using *CaM* regions of CNUFC IGS2-5, CNUFC YJ1-19, CNUFC WD27, CNUFC U8-70, CNUFC AS2-24, CNUFC S32-1, and CNUFC U7-48, revealed similarities of 99.8% (473/474 bp), 98.4% (499/507 bp), 99.6% (538/540 bp), 99.4% (476/479 bp), 100% (448/448 bp), 99.5% (729/733 bp), and 100% (517/517 bp), with *A. brunneoviolaceus* (EF661147), *A. capensis* (KJ775279), *A. floccosus* (MH292833), *A. inflatus* (FJ531094), *A. kanagawaensis* (FJ491592), *A. polyporicola* (LM644252), and *A. spelaeus* (HG916745), respectively. Moreover, the ML tree for combined *BenA* and *CaM* sequences revealed that the strains, CNUFC IGS2-5, CNUFC YJ1-19, CNUF WD27, CNUFC U8-70, CNUFC AS2-24, CNUFC S32-1, and CNUFC U7-48, were placed in clade with *A. brunneoviolaceus, A. capensis*, *A. floccosus*, *A. inflatus*, *A. parvulus*, *A. polyporicola*, and *A. spelaeus*, in their respective five sections in *Aspergillus* (Figure 1).

3.2. Taxonomy

3.2.1. Taxonomy of CNUFC IGS2-5

A. brunneoviolaceus Bat. & H. Maia, Anais Soc. Biol. Pernambuco 13: 91 (1955) [MB#292838] (Figure 2)

Table 2. GenBank accession numbers for fungal strains used in this study.

Species	Collection no.	GenBank Accession no. BenA	CaM
A. acidohumus	DTO 340-H1 (T)	KX423623	KX423634
A. aculeatinus	CBS 121060 (T)	EU159220	EU159241
A. aculeatus	NRRL 5094 (T)	HE577806	AJ964877
A. alabamensis	CBS 125693 (T)	KP987049	EU147583
A. alboluteus	CBS 145855 (T)	MW478497	MW478511
A. allahabadii	NRRL 4539 (T)	EF669531	EF669559
A. ambiguus	NRRL 4737 (T)	EF669534	EF669564
A. ardalensis	CBS 134372 (T)	HG916683	HG916725
A. aureoterreus	NRRL 1923 (T)	EF669524	EF669538
A. barbosae	URM 5930 (T)	LR031377	LR031392
A. brunneo-uniseriatus	NRRL 4273 (T)	EF652123	EF652138
A. brunneoviolaceus	CBS 621.78 (T)	EF661105	EF661147
A. brunneoviolaceus	**CNUFC IGS2-5**	**OP168874**	**OP168867**
A. capensis	DTO 179-E6 (T)	KJ775072	KJ775279
A. capensis	**CNUFC YJ1-19**	**OP168879**	**OP168872**
A. carbonarius	NRRL 369 (T)	EF661099	EF661167
A. carneus	NRRL 527 (T)	EF669529	EF669569
A. cervinus	NRRL 5025 (T)	EF661251	EF661261
A. chaetosartoryae	NRRL 5501 (T)	EF652117	EF652129
A. christenseniae	CBS 122.56 (T)	FJ491639	FJ491608
A. chrysellus	NRRL 5084 (T)	EF652109	EF652136
A. citrinoterreus	CBS 138921 (T)	LN680657	LN680685
A. costaricaensis	CBS 115574 (T)	FJ629277	FN594545
A. cremeus	NRRL 5081 (T)	EF652120	EF652125
A. croceus	CCF 4405 (T)	LN873944	LN873957
A. dimorphicus	NRRL 3650 (T)	EF652111	EF652135
A. ellipticus	CBS 70779 (T)	AY585530	EF661170
A. elsenburgensis	CMV 011G4 (T)	MK451215	MK451513
A. europaeus	CCF 4409 (T)	LN909006	LN909007
A. flaschentraegeri	NRRL 5042 (T)	EF652113	EF652130
A. flavipes	NRRL 302 (T)	EU014085	EF669549
A. floccosus	CBS 116.37 (T)	FJ491714	KP987066
A. floccosus	**CNUFC WD27**	**OP168878**	**OP168871**
A. floridensis	NRRL 62478 (T)	HE984412	HE984429
A. fumigatiaffinis	CMV 001G1 (T)	MK450913	MK451390
A. giganteus	NRRL 10 (T)	EF669789	EF669857
A. gorakhpurensis	NRRL 3649 (T)	EF652114	EF652126
A. hortai	NRRL 274 (T)	FJ491706	KP987054
A. hydei	KUMCC 18-0196 (T)	MT161679	MT178247
A. iizukae	NRRL 3750 (T)	EU014086	EF669555
A. indologenus	CBS 11480 (T)	AY585539	AM419750
A. inflatus	CBS 682.70 (T)	FJ531008	FJ531090
A. inflatus	**CNUFC U8-70**	**OP168877**	**OP168870**
A. inusitatus	CBS 147044 (T)	MW478502	MW478517
A. iranicus	DTO 203-D7 (T)	KP987045	KP987060
A. itaconicus	NRRL 161 (T)	EF652118	EF652140
A. japonicus	CBS 114.51 (T)	HE577804	FN594551
A. lanuginosus	NRRL 4610 (T)	EU014080	EF669562
A. kanagawaensis	CBS 538.65 (T)	FJ491640	FJ491597
A. koreanus	EML-GSNP1-1 (T)	KX216530	KX216528
A. luppii	NRRL 6326 (T)	EU014079	EF669575
A. melleus	NRRL 5103 (T)	EF661326	EF661391
A. microcysticus	NRRL 4749 (T)	EF669515	EF669565
A. micronesiensis	DTO 267D5 (T)	KJ775085	KP987067
A. movilensis	CCF 4410 (T)	HG916697	HG916740
A. neoafricanus	NRRL 2399 (T)	EF669516	EF669543
A. neoflavipes	CBS 260.73 (T)	EU014084	EF669572
A. neoindicus	CBS 444.75 (T)	EF669532	EF669574
A. neoniger	CBS 115656 (T)	FJ491691	FJ491700
A. neoniveus	CBS 261.73 (T)	EU014098	EF669570
A. niger	NRRL 326 (T)	EF661089	EF661154
A. niveus	CBS 115.27 (T)	EF669528	EF669573
A. novoguineensis	CBS 906.96 (T)	FJ491641	FJ491605
A. nutans	NRRL 4364 (T)	EF661249	EF661262
A. olivimuriae	NRRL 66783 (T)	MH492010	MH492011
A. okavangoensis	CBS 147420 (T)	MW480789	MW480707
A. ostianus	NRRL420 (T)	EF661324	EF661385
A. oxumiae	CCDCA 11546 (T)	MN521388	MN531842
A. parvulus	NRRL 4753 (T)	EF661247	EF661259
A. parvulus	**CNUFC AS2-24**	**OP168873**	**OP168866**
A. polyporicola	NRRL 32683 (T)	EU014088	EF669553
A. polyporicola	NRRL 58570	LM644274	LM644252
A. polyporicola	**CNUFC S32-1**	**OP168875**	**OP168868**
A. pseudodeflectus	CMV 005H9	MK451064	MK451498
A. pseudoterreus	NRRL 4017 (T)	EF669523	EF669556
A. purpureocrustaceus	CMV 008B3 (T)	MK451138	MK451515
A. recifensis	URM 6605 (T)	LR031370	LR031385
A. saccharolyticus	CBS 127449 (T)	HM853553	HM853554
A. serratalhadensis	URM 7866 (T)	LT993222	LT993223
A. sigurros	CMV 005I4 (T)	MK451066	MK451512
A. spelaeus	CCF 4425 (T)	HG916698	HG916741
A. spelaeus	EMSL 4874	MW478506	MW478525
A. spelaeus	**CNUFC U7-48**	**OP168876**	**OP168869**
A. stromatoides	CBS 500.65 (T)	FJ531038	EF652127
A. subnutans	CBS 129386 (T)	KX528454	KX528455
A. suttoniae	UTHSCSA DI14-215 (T)	LT899536	LT899589
A. tardus	CBS 433.93 (T)	FJ531001	FJ531084
A. templicola	DTO 270 C-6 (T)	KJ775092	KJ775394
A. terreus	CBS 601.65 (T)	EF669519	EF669544
A. transcarpathiucs	CBS 423.68 (T)	FJ491632	FJ491610
A. trinidadensis	NRRL 62479 (T)	HE984420	HE984434
A. tubingensis	NRRL 4875 (T)	EF661086	EF661151
A. urmiensis	CBS 139558 (T)	KP987041	KP987056
A. uvarum	ITEM 4834 (T)	AM745751	AM745755
A. violaceofuscus	CBS 123.27 (T)	FJ491685	FJ491698
A. wentii	NRRL 375 (T)	EF652106	EF652131
A. wisconsinensis	CBS 413.64 (T)	FJ491638	FJ491609

Bold letters indicate isolates and accession numbers determined in our study.

CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CCDCA: culture collection at Federal University of Lavras, Minas Gerais, Brazil; CCF: Culture Collection of Fungi at the Department of Botany of Charles University in Prague; CMV: working collection housed at the PPRI; CNUFC: Chonnam National University Fungal Collection (Gwangju, South Korea); DTO: Internal collection of Dept. Applied and Industrial Mycology housed at CBS; ITEM: Microbial Culture Collection, Institute of Sciences of Food Production, Bari, Italy; KUMCC: Culture collection of Kunming Institute of Botany, Yunnan, China; NRRL: ARS culture collection, Peoria, IL, USA; URM: Padre Camille Torrend Herbarium, South America; UTHSCSA: Collection of Fungus Testing Laboratory, University of Texas, Health Science Center, San Antonio, USA; T: ex-type strain.

Colony characteristics: On CYA, the colonies initially appeared as white with flat mycelia and then turned brown, followed by reverse pale orange, and eventually reached 70–80 mm in diameter after 7 days at 25 °C. On MEA, colonies were dark brown, sporulation, widespread, and turned reverse colorless to light yellow, and further reached 82–85 mm in diameter after 7 days at 25 °C. On YES, colonies were initially cream with aerial mycelia, and further turned dark brown to black, followed by reverse ivory at margins to pale yellow toward center, and eventually reached 75–80 mm in diameter after 7 days at 25 °C.

Micromorphology: Conidiophores uniceriate, simple, smooth-walled, straight, occasionally sinuous, 232.4–1136.5 µm long. Vesicles spherical, subspherical, 33.5–60.8 × 43.9–63.5 µm. Phialides ampulliform, 6.8–10.4 × 2.8–4.6 µm. Conidia globose, often subglobose, rough, and echinulate on the surface, 3.6–5.8 × 3.8–5.7 µm in diameter.

3.2.2. Taxonomy of CNUFC YJ1-19

A. capensis Visagie, Hirooka & Samson, Studies in Mycology 78: 105 (2014) [MB#809193] (Figure 3)

Colony characteristics: On CYA, the colonies were floccose, with white mycelium, yellowish

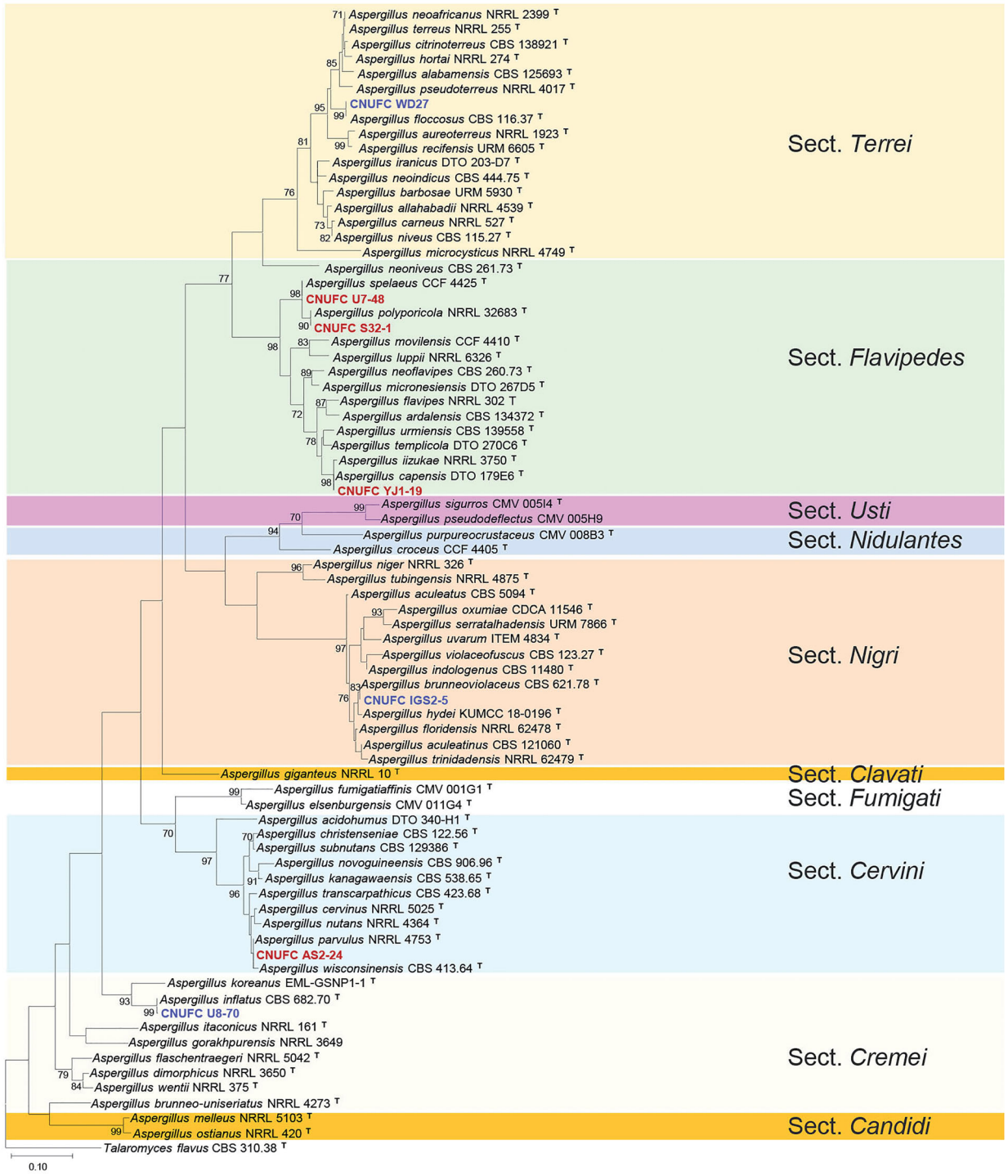

Figure 1. Phylogenetic tree of *Aspergillus brunneoviolaceus* CNUFC IGS2-5, *A capensis* CNUFC YJ1-19, *A. floccosus* CNUFC WD27, *A. inflatus* CNUFC U8-70, *A. parvulus* CNUFC AS2-24, *A. polyporicola* CNUFC S32-1, and *A. spelaeus* CNUFC U7-48, and related species based on ML analysis of the combined *BenA* and *CaM* sequences. Numbers at the nodes indicate the bootstrap values (≥70%) from 1000 replicates. The bar indicates the number of substitutions per nucleotide. The study isolates are presented in bold and are represented by different colors.

sporulation at the periphery, brown soluble pigment, and reverse pale brown, and eventually reached 20–24 mm in diameter after 7 days at 25 °C. On MEA, the colonies were floccose, mycelial areas were yellowish white to pale yellow, moderate sporulation, soluble pigment was absent, followed by reverse brown to dark brown coloration, and eventually reached 19–21 mm in diameter after 7 days at 25 °C. On YES, the colonies were floccose, with moderate sporulation, pale yellow mycelia, followed by reverse pale brown, and eventually reached 21–22 mm in diameter after 7 days at 25 °C.

Micromorphology: Conidiophores biseriate, 189–990 × 4.2–9.5 µm. Vesicles globose to elongated, 11–29 µm in diameter. Metulae, 5.3–9.4 × 3.8–4.1 µm. Phialides ampulliform, 3.7–5.8 × 2.5–3.6 µm. Conidia globose to subglobose, smooth, 2.3–3.1 × 2.3–3.1 µm in diameter. Sclerotia absent.

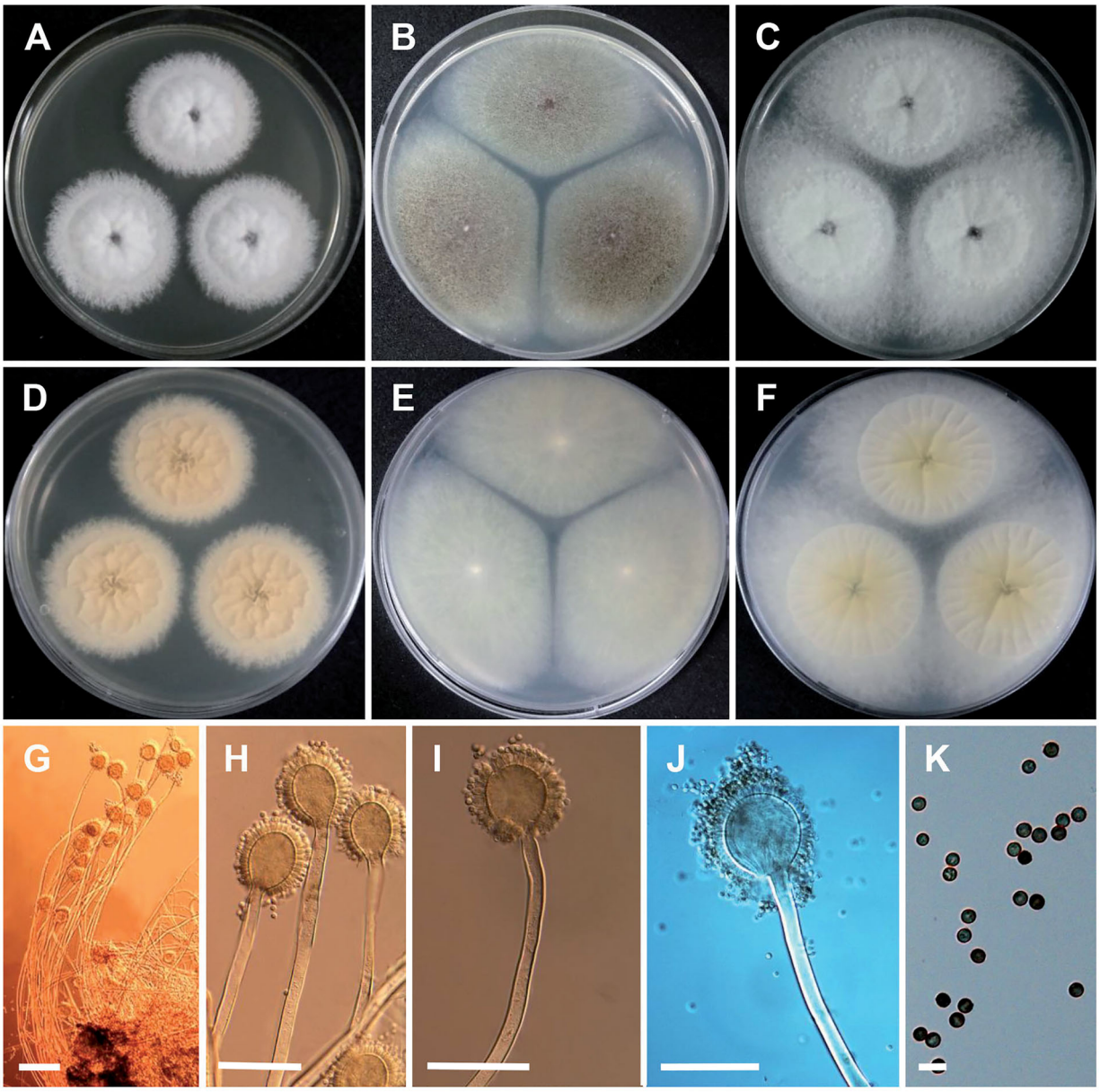

Figure 2. Morphology of *Aspergillus brunneoviolaceus*. (A,D) Colonies on CYA. (B,E) Colonies on MEA. (C,F) Colonies on YES. (A–C, Obverse view; D–F, reverse view). (G–J) Conidiophores; (K) Conidia (scale bars: G = 100 µm, H, I = 50 µm, J = 80 µm, and K = 10 µm).

3.2.3. Taxonomy of CNUFC WD27

A. floccosus (Y.K. Shih) Samson, S.W. Peterson, Frisvad & Varga, Studies in Mycology 69: 45 (2011) [MB#560393] (Figure 4).

Colony characteristics: On CYA, the colonies were floccose, wrinkled, pale white, with no soluble pigment, moderate sporulation, followed by reverse pale yellow coloration, and eventually reached 25–28 mm in diameter after 7 days at 25 °C. On MEA, the colonies were floccose, regular, lemonade pink, with no soluble pigment, strong sporulation, reverse yellowish orange, and eventually reached 22–27 mm in diameter after 7 days at 25 °C. On YES, the colonies were plane, wrinkled, pale white, with moderate sporulation, no soluble pigment, reverse pale brown, and eventually reached 27–32 mm in diameter after 7 days at 25 °C.

Micromorphology: Conidial heads long, densely columnar, 45–95 µm in diameter. Conidiophores biseriate, 150–375 × 4.5–5.2 µm. Vesicles globose, 12–16 µm in diameter. Metulae closely packed, 5.5–8.8 × 1.8–2.1 µm. Phialides, 4.6–6.5 × 1.8–2.1 µm. Conidia globose, elliptical, 2.0–2.6 µm in diameter.

3.2.4. Taxonomy of CNUFC U8-70

A. inflatus (Stolk & Malla) Samson, Frisvad, Varga, Visagie & Houbraken, Studies in Mycology 78: 155 (2014) [MB#809590] (Figure 5)

Colony characteristics: On CYA, the colonies were furrowed, wrinkled, grayish green, with no soluble pigment, moderate sporulation, reverse yellowish brown coloration, and eventually reached 15–18 mm in diameter after 7 days at 25 °C. On MEA, the colonies were plane, regular, pale yellow,

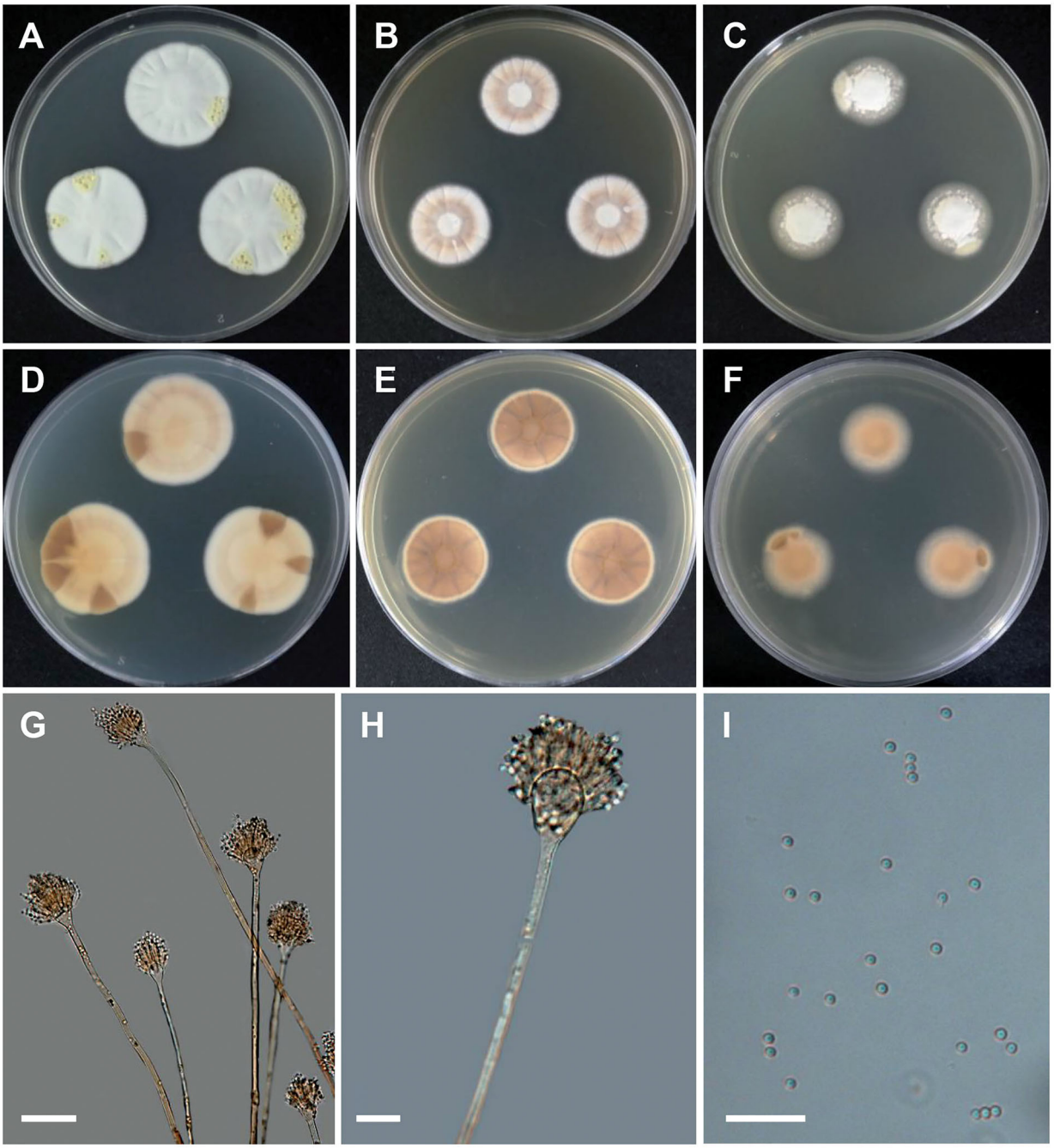

Figure 3. Morphology of *Aspergillus capensis*. (A,D) Colonies on CYA. (B,E) Colonies on MEA. (C,F) Colonies on YES. (A–C: obverse view, D–F: reverse view). (G,H) Conidiophores; (I) Conidia (scale bars: G–I = 20 µm).

with no soluble pigment, reverse pale brown, and eventually reached 15–17 mm in diameter after 7 days at 25 °C. On YES, the colonies were plane, wrinkled toward center, grayish blue, with moderate sporulation, no soluble pigment, followed by reverse pale yellow coloration, and eventually reached 16–18 mm in diameter after 7 days at 25 °C.

Micromorphology: Conidiophores biseriate, smooth walled, 120–480 × 1.6–3.0 µm. Vesicles pyriform, 3.0–6.1 µm in diameter. Metulae, 4.2–9.2 × 1.6–2.0 µm. Phialides ampulliform, 5.2–7.5 × 2–3 µm. Conidia mostly globose, subglobose, 1.5–2.4 µm in diameter.

3.2.5. Taxonomy of CNUFC AS2-24

A. parvulus G. Sm., Transactions of the British Mycological Society 44(1): 45 (1961) [MB#121074] (Figure 6)

Colony characteristics: On CYA, the colonies were plane, regular, pale yellow, with no soluble pigment, moderate sporulation, reverse pale yellow coloration, and eventually reached 15–17 mm in diameter after 7 days at 25 °C. On MEA, the colonies were plane, regular, light purple, with no soluble pigment, good sporulation, reverse pale yellow, and eventually reached 22–26 mm in diameter after 7 days at 25 °C. On YES, the colonies were plane, brownish yellow, slightly wrinkled toward center,

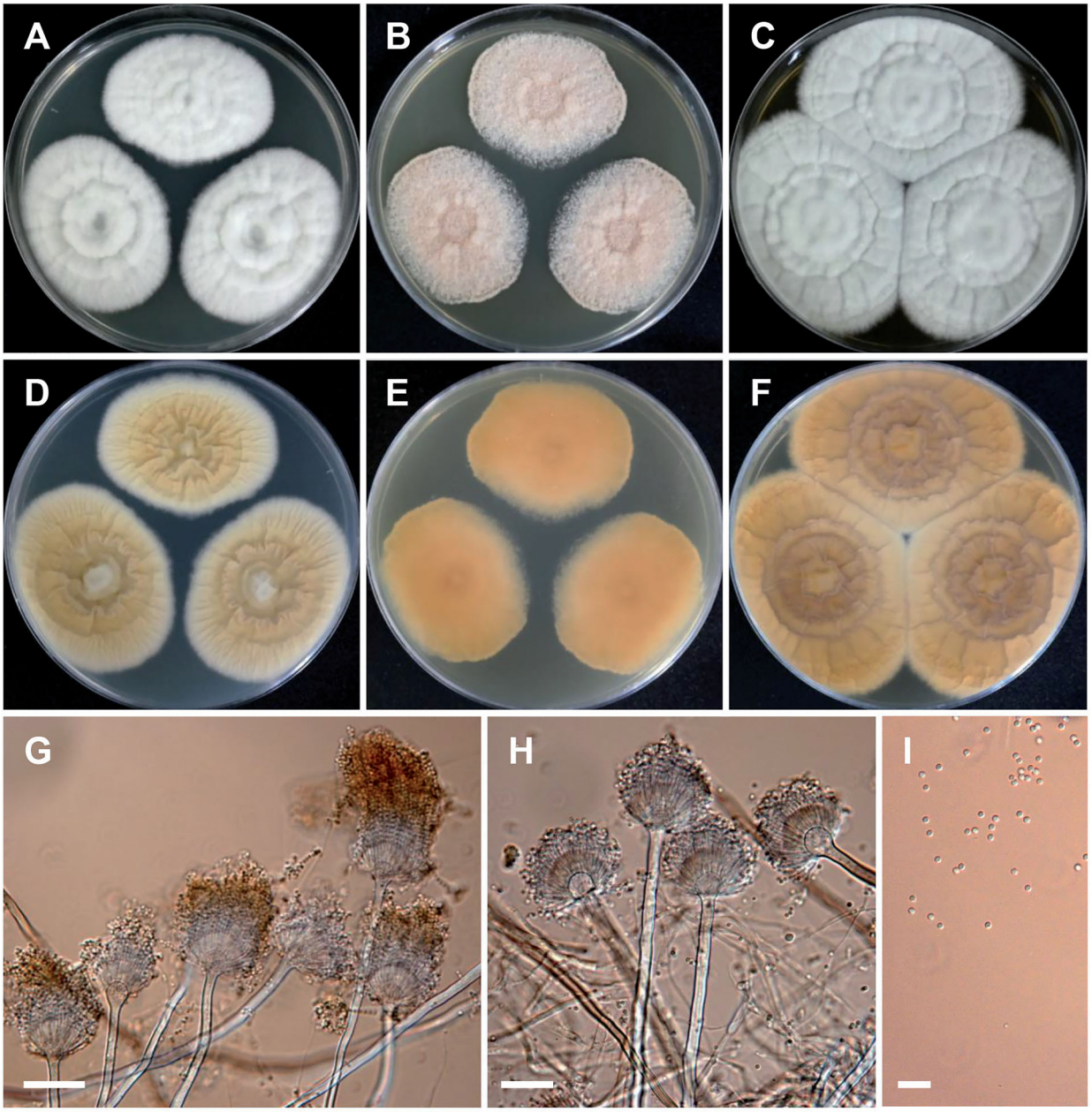

Figure 4. Morphology of *Aspergillus floccosus*. (A,D) Colonies on CYA. (B,E) Colonies on MEA. (C,F) Colonies on YES. (A–C: obverse view, D–F: reverse view). (G,H) Conidial heads, Conidiophores; (I) Conidia (scale bars: G–I = 20 µm).

with moderate sporulation, no soluble pigment, followed by reverse pale yellow coloration, and eventually reached 16–17 mm in diameter after 7 days at 25 °C.

Micromorphology: Conidiophores uniseriate, bent, smooth, 12–72 × 2.5–3.2 µm. Vesicles globose, occasionally subclavate, 6–11 µm in diameter. Phialides ampulliform, 4–6 × 2–3 µm. Conidia globose, 2.6–3.6 µm in diameter.

3.2.6. Taxonomy of CNUFC S32-1

A. polyporicola Hubka, A. Nováková, M. Kolařík, S.W. Peterson, Mycologia 107(1): 194 (2015) [MB#808145] (Figure 7)

Colony characteristics: On CYA, the colonies were floccose, grayish brown, granular, with moderate sporulation, followed by reverse pale brown coloration, and eventually reached 19–21 mm in diameter after 7 days at 25 °C. On MEA, the colonies were granulose, pale yellow to yellowish toward center, with soluble pigment, reverse pale yellow coloration, and eventually reached 19–20 mm in diameter after 7 days at 25 °C. On YES, the colonies were plane, white mycelia, wrinkled toward center, with moderate sporulation, reverse white to pale yellow toward center, and eventually reached 21–22 mm in diameter after 7 days at 25 °C.

Micromorphology: Conidiophores biseriate, smooth walled, 270–820 × 3.2–6.0 µm. Vesicles globose to subglobose, pyriform, 7–17 µm in diameter. Metulae, 4.2–8.5 µm. Phialides, 3–5 µm. Conidia globose to subglobose, 2.1–3.1 µm in diameter. No ascospores or ascomata observed.

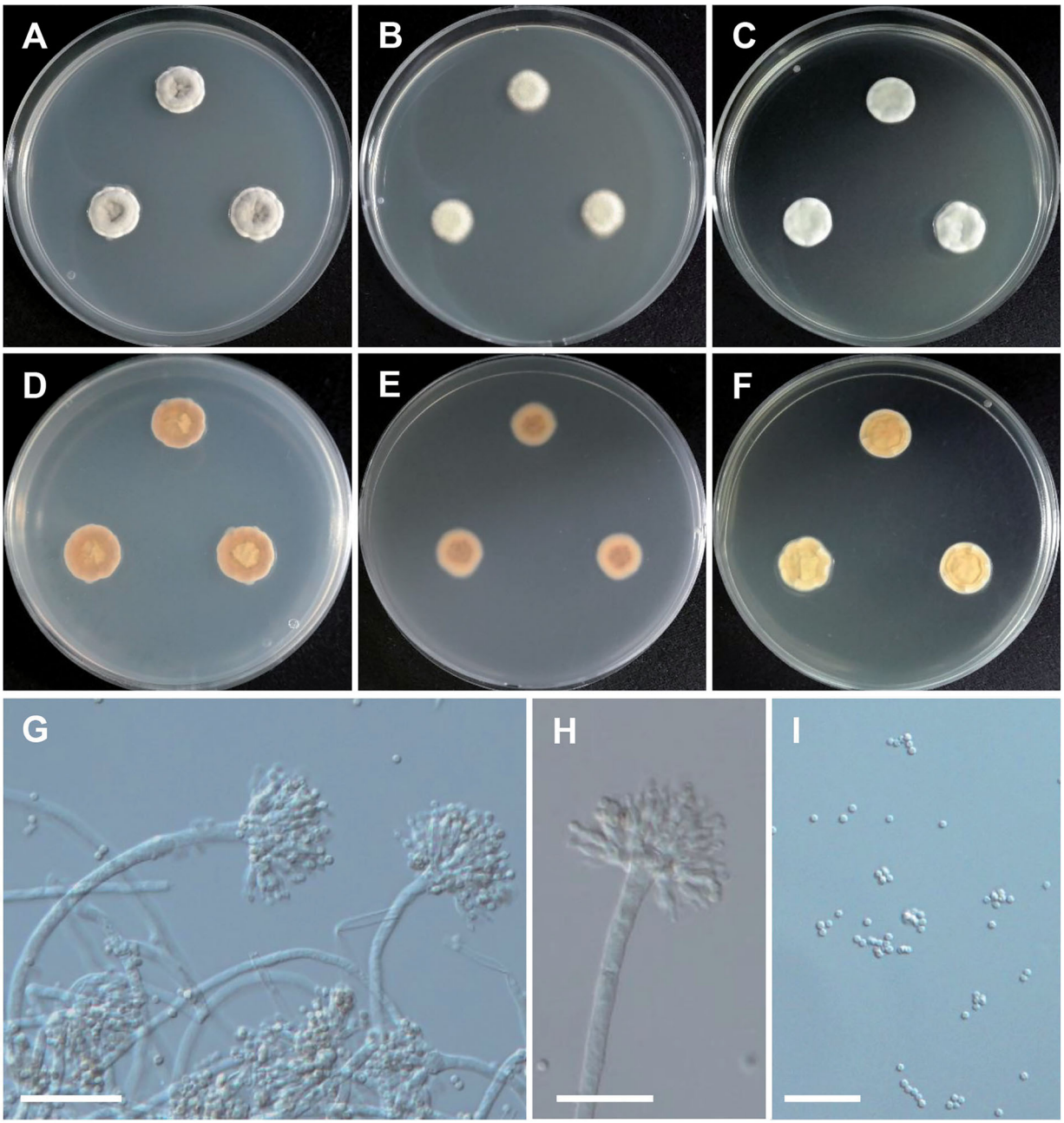

Figure 5. Morphology of *Aspergillus inflatus*. (A,D) Colonies on CYA. (B,E) Colonies on MEA. (C,F) Colonies on YES. (A–C: obverse view, D–F: reverse view). (G,H) Conidiophores; (I) Conidia (scale bars: G–I = 20 µm).

3.2.7. Taxonomy of CNUFC U7-48

A. spelaeus A. Nováková, Hubka, M. Kolařík, S.W. Peterson, Mycologia 107(1): 194 (2015) [MB#808146] (Figure 8)

Colony characteristics: On CYA, the colonies were floccose, light grayish yellowish brown, with no soluble pigment, followed by reverse pale yellow coloration, and eventually reached 20–22 mm in diameter after 7 days at 25 °C. On MEA, the colonies were plane, delicately granular to granular, with moderate sporulation, abundant small colorless or pale yellow droplets on the colony surface, no soluble pigment, reverse light orange, and eventually reached 19–22 mm in diameter after 7 days at 25 °C. On YES, the colonies were plane, wrinkled toward center, pale white, with no soluble pigment, reverse pale brown coloration, and eventually reached 21–23 mm in diameter after 7 days at 25 °C.

Micromorphology: Conidiophores biseriate, 231–890 × 4.1–7.6 µm. Vesicles pyriform, 9.9–26.1 µm in diameter. Metulae mostly covering the entire surface of the vesicle, 5.0–11.2 × 3.1–4.0 µm. Phialides, 3.5–7.2 × 2–3 µm. Conidia smooth, mostly globose, few subglobose, 2.5–3.1 µm in diameter. No ascospores or ascomata observed.

4. Discussion

To date, there have been few reports on undescribed *Aspergillus* species in Korea despite having a cosmopolitan distribution. Moreover, several new *Aspergillus* species have been introduced worldwide;

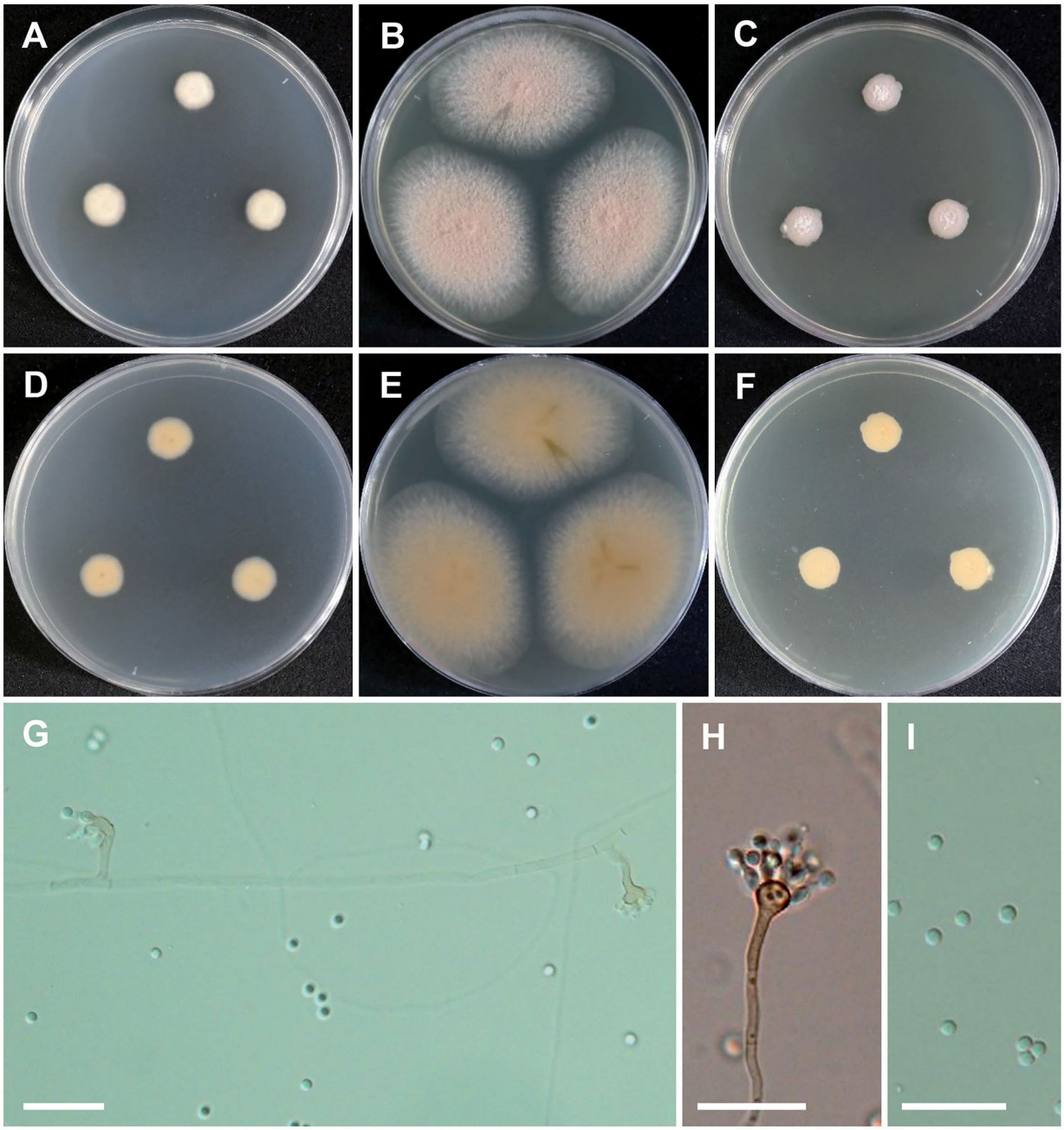

Figure 6. Morphology of *Aspergillus parvulus*. (A,D) Colonies on CYA. (B,E) Colonies on MEA. (C,F) Colonies on YES. (A–C: obverse view, D–F: reverse view). (G,H) Conidiophores; (I) Conidia (scale bars: G–I = 20 μm).

therefore, collecting and expanding samples from different habitats is needed for identification of *Aspergillus* species from the Korean peninsula owing to their economic benefits. The present study provides a comprehensive account of the occurrence and distribution of *Aspergillus* species in Korea, particularly *A. brunneoviolaceus, A. capensis*, *A. floccosus*, *A. inflatus*, *A. parvulus*, *A. polyporicola*, and *A. spelaeus*. In this study, seven *Aspergillus* species in five different sections were identified and compared to their most closely related species. Analysis of the combined *BenA* and *CaM* datasets revealed that the strains CNUFC IGS2-5, CNUFC YJ1-19, CNUFC WD27, CNUFC U8-70, CNUFC AS2-24, CNUFC S32-1, and CNUFC U7-48 were placed into their respective type species of *A. brunneoviolaceus, A. capensis*, *A. floccosus*, *A. inflatus*, *A. parvulus*, *A. polyporicola*, and *A. spelaeus*.

As shown in Figure 1, CNUFC IGS2-5 aligned with *A. brunneoviolaceus* NRRL4912 (ex-type strain) in section *Nigri*. Morphologically, the isolated strains present similar characters with type strain NRRL 4912 of *A. brunneoviolaceus* described by Batista and da Silva [19]. These include good sporulation with dark brown conidia; uniseriate conidiophores; globular, subglobular, and spherical vesicle, (30–)35–70(–90) μm; and conidia globose to ellipsoidal, smooth, and slightly roughened, 3.5–4.5(–6)×3.5–4.5(–5) μm. Moreover, section *Nigri*, known as black aspergilli includes species

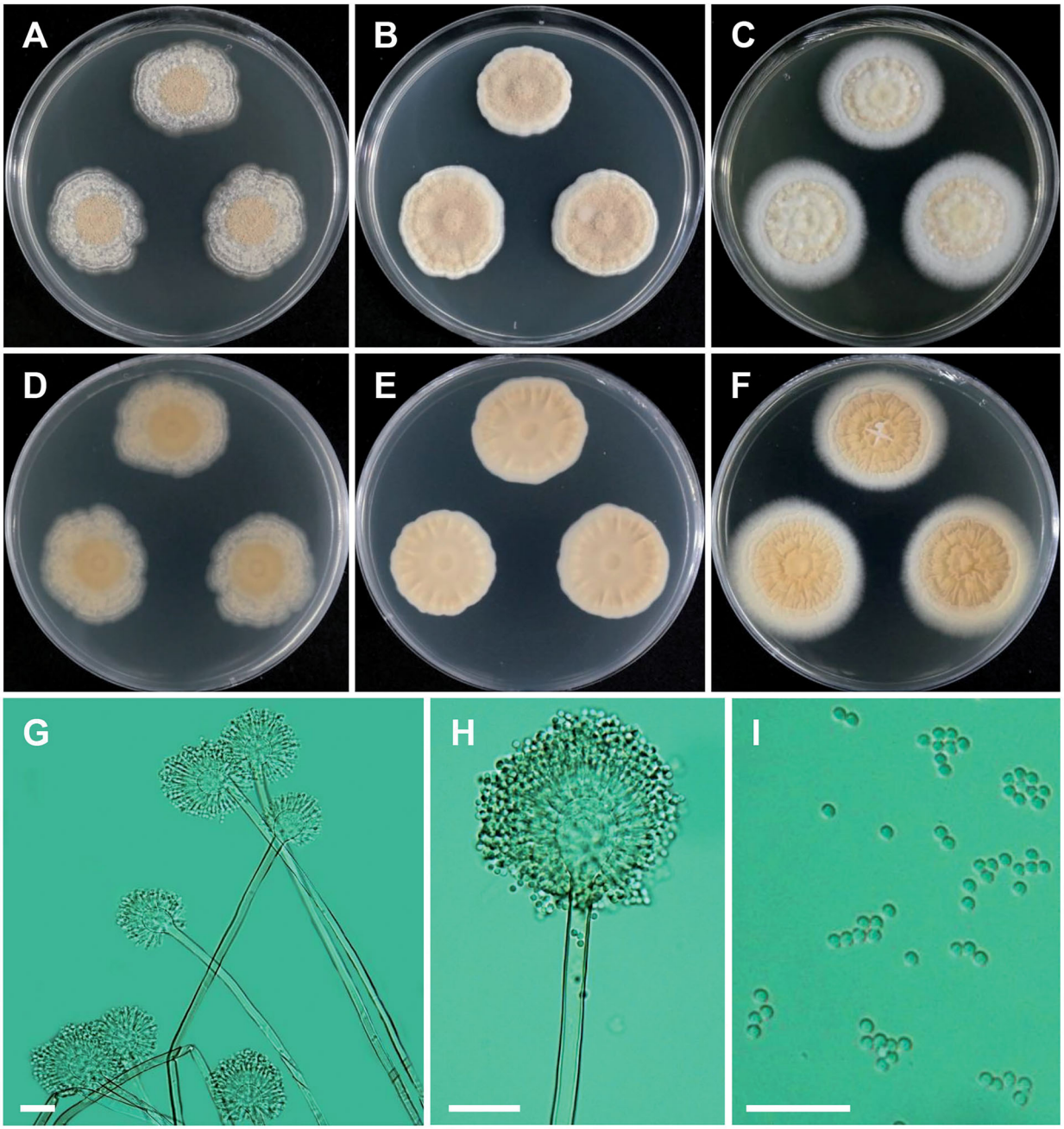

Figure 7. Morphology of *Aspergillus polyporicola*. (A,D) Colonies on CYA. (B,E) Colonies on MEA. (C,F) Colonies on YES. (A–C: obverse view, D–F: reverse view). (G,H) Conidiophores; (I) Conidia (scale bars: G–I = 20 µm).

with smooth conidiophores and hyaline or pigmentation below the vesicle; globose, subglobose, and pyriform vesicles; typically radiating conidial heads; or divergent columns in certain species [20]. These aspergilli have been isolated from contaminated materials, indoor air environments, soil samples, and plants [21]. In general, 27 species were accepted in this section [22]. Three additional new species, *A. hydei*, *A. oxumiae,* and *A. labruscus*, were discovered from air under *Quercus variabilis*, in soil cultivated with *Agave sisalana*, and on the surface of grape berries [23–25]. *A. brunneoviolaceus* is a rare member of the group of black aspergilli, which has utmost significance in the industry [26]. To date, *A. brunneoviolaceus* was isolated from soil (CBS 313.89), thumb nail (PW4048), bronchoalveolar lavage (PW4122), sputum (PW4213), wound (PW4049), *Lactuca sativa* (CBS 119.49), guano (IHEM 18675), corneal scraping keratitis (IHEM 18675), dropping of *Coenobita* sp. (IHEM 4062), industrial material (CCF 108), and indoor environment (ITEM 14794, ITEM 14799, and ITEM 14802) [14,27–29]. This is first study to isolate *A. brunneoviolaceus* from a spider in Korea, thereby revealing its significance as a member of the ecosystem of an arthropod.

Based on the phylogeny, CNUFC YJ1-19 clustered with *A. capensis* DTO 179-E6 (ex-type strain); CNUFC S32-1 with *A. polyporicola* NRRL32683 (ex-type strain); and CNUFC U7-48 with *A. spelaeus*

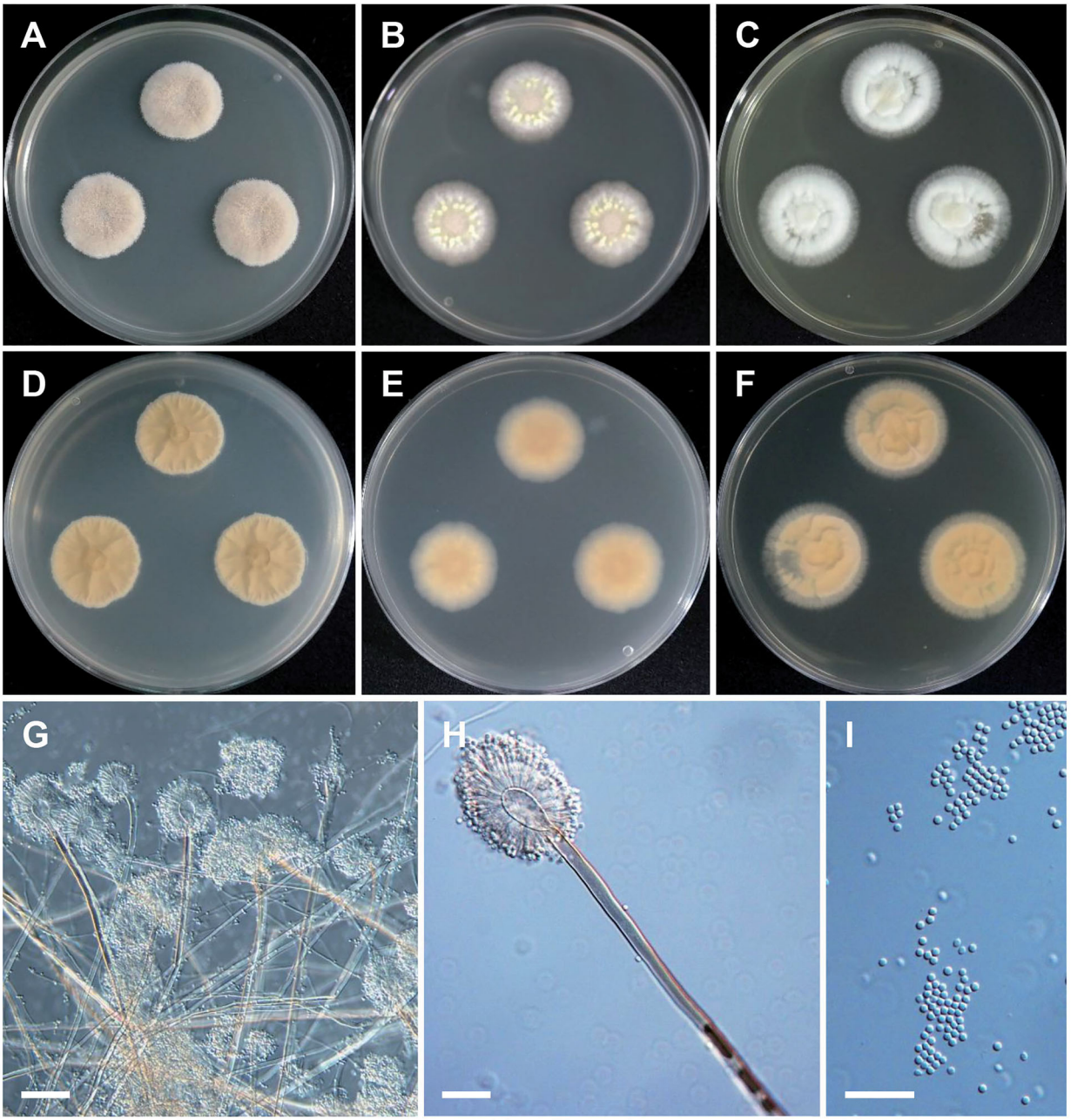

Figure 8. Morphology of *Aspergillus spelaeus*. (A,D) Colonies on CYA. (B,E) Colonies on MEA. (C,F) Colonies on YES. (A–C: obverse view, D–F: reverse view). (G,H) Conidiophores; (I) Conidia (scale bars: G–I = 20 µm).

CCF4425 (ex-type strain), in section *Flavipedes* (Figure 1). The isolate CNUFC YJ1-19 were morphologically similar to *A. capensis*, as described by Visagie *et al.* [12], although the length of conidiophores differed. The conidiophores described by Visagie *et al.* [12] were 235–1400 × 6.5–11 µm in length, whereas the isolate in the present study was 189–990 × 4.2–9.5 µm in length. The morphological characteristics of the isolates *A. polyporicola* and *A. spelaeus* in this study were consistent with those previously described by [30]. Section *Flavipedes* was expanded to include informal *A. flavus* group species [20,31,32]. Species belonging to section *Flavipedes* and *Terrei* are related phenotypically, and moreover, some of the species in the section *Terrei* were earlier placed in section *Flavipedes* due to overlapping cultural and morphological characteristics [30,32,33]. The genomic sequences have been useful in providing a robust tool for appropriate identification and delineation of species boundaries [30,34–36]. About 21 species were accepted in the section *Flavipedes* [37–40]. Members in this section are reported from foods, as endophytes from soils and rhizospheres, from indoor and cave environments, and occasionally as clinical specimens. *A. capensis* was reported from house dust samples [12], and a healthy plant of oilseed rape (*Brassica napus* L.) and produced three antifungal metabolites namely, methyl dichloroasterrate, penicillither, and rosellichalasin [41]. These metabolites exhibit antifungal activity toward major plant pathogens such as *Botrytis cinerea*, *Monilinia fructicola*, *Sclerotinia*

sclerotiorum, and *S. trifoliorum*. Rosellichalasin produced by *Aspergillus* sp. has revealed anticancer activities against human tumor cell lines, including A549, Hela, BEL-7402, and RKO [42]. The taxonomy of *A. capensis* and *A. iizukae* needs careful attention. More isolates with DNA sequences to be generated that would be helpful for a better resolution in identification of these two species.

Furthermore, CNUFC U8-70 clustered with *A. inflatus* in section *Cremei* (Figure 1). The isolate revealed similar morphological characters as that of *A. inflatus* CBS682.70 (ex-type strain) [22]. Section *Cremei* (known as the *A. cremeus* group) was first described by Raper and Fennell [32] with five species. Recently, about 13 species were included in this section [5,43]. Species belonging to this section are characterized by their yellowish–brown to brown or gray–green colony color, biseriate conidial heads, long conidiophores, and pale gray–green to yellow-brown conidia [22]. Species in this section are frequently found in soil and foods associated with spoilage of cereals and nuts. *A. inflatus* is reported to produce sterigmatocystin—a precursor to highly potent compounds, namely aflatoxins [44]. *A. inflatus* isolates were found in root surface of *Picea abies*, forest soil under *Quercus rubra*, as well as in scalp and sputum of humans [45].

The isolate CNUFC WD27 was phylogenetically related to the type of *A. floccosus* clade belonging to section *Terrei* (Figure 1). Moreover, morphological characters of the isolate were consistent with those of *A. floccosus* described by Samson *et al.* [46]. Section *Terrei* was introduced by Gams *et al.* [20], for Raper and Fennell (*A. terreus* group) [32] having buff to brown columnar conidial heads. They have a cosmopolitan distribution and are particularly important in fermentation industries [46]. Two new species were introduced in this section recently, and thus, the accepted number of species increased to 19 in total [4]. *A. floccosus* was earlier named as *Aspergillus terreus* var. *floccosus*, isolated from waste cloth from Wuchang, China, and was used as a clinical specimen in immunocompromised patients [46,47]. In the present study, *A. floccosus* was isolated from freshwater samples. *A. floccosus* was found to produce extrolites, aszonalenin, austalides, butyrolactones, hepatotoxic citrinin, decaturin, dihydrocitrinone, isocoumarin, and serantrypinone [46].

In the phylogeny, CNUFC AS2-24 aligned with *A. parvulus* clade in section *Cervini* (Figure 1). The morphological characters of the isolate were consistent with those of *A. parvulus,* as described by Chen *et al.* [48]. Section *Cervini* was established by Gams in 1985 for species with radiate or short columnar, fawn colored, uniseriate conidial heads. This section is economically less important, less studied in comparison to other sections, and comprises 10 species [48]. *A. parvulus* was originally isolated from different soil environments in USA, UK, The Netherlands, and feed ingredients from Argentina [48–50]. Furthermore, in this study, the isolate was obtained from rhizosphere soil. Previous studies reported that *A. parvulus* exhibits a wide spectrum of antibiotic activities against various bacteria [51], phytotoxic activities [52], produces parvulenone [53], naphthalenone [54], and asparvenone derivatives [55]. Species of section *Cervini* have not been found to be important human pathogens; however, Hubka *et al.* [56] reported an isolate (closely related to *A. parvulus*) as the possible cause of human onychomycosis.

Our study presents undescribed species of *Aspergillus* from different environmental habitats as well as new sources of isolation from arthropods populations. Further studies should focus on investigating more unique habitats and on sampling across Korea. Our work needs to be coupled with antifungal and antibacterial activity of the discovered species to produce novel metabolites for industrial applications.

Disclosure statement

No potential conflict of interest is reported by the authors.

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References

[1] Micheli PA. Nova plantarum genera juxta tournefortii methodum disposita. Florence (Italy): Typis Bernardi Paperinii; 1729.

[2] 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.

[3] Sklenář F, Jurjević Ž, Peterson SW, et al. Increasing the species diversity in the *Aspergillus* section *Nidulantes*: Six novel species mainly from

the indoor environment. Mycologia. 2020;112(2): 342–370.
[4] Correia ACR, Barbosa RN, Frisvad JC, et al. The polyphasic re-identification of a Brazilian *Aspergillus* section *Terrei* collection led to the discovery of two new species. Mycol Prog. 2020; 19(9):885–903.
[5] Hyde KD, Hongsanan S, Jeewon R, et al. Fungal diversity notes 367-491: taxonomic and phylogenetic contributions to fungal taxa. Fungal Divers. 2016;80(1):1–270.
[6] Hong SB, Cho HS, Shin HD, et al. Novel *Neosartorya* species isolated from soil in Korea. Int J Syst Evol Microbiol. 2006;56(Pt 2):477–486.
[7] 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;44:207–219.
[8] Nguyen TTT, 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.
[9] Nguyen TTT, Noh JK, Lee HB. New species and eight undescribed species belonging to the families Aspergillaceae and Trichocomaceae in Korea. Mycobiology 2021;49(6):534–550.
[10] Lee JW, Kim SH, You YH, et al. Four unrecorded *Aspergillus* species from the rhizosphere soil in South Korea. Mycobiology 2021;49(4):346–354.
[11] Pangging M, Nguyen TTT, Lee HB. New records of four species belonging to Eurotiales from soil and freshwater in Korea. Mycobiology 2019;47(2): 154–164.
[12] 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.
[13] 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.
[14] Hubka V, Kolarik M. *β*-tubulin paralogue tubC is frequently misidentified as the benA gene in *Aspergillus* section *Nigri* taxonomy: primer specificity testing and taxonomic consequences. Persoonia 2012;29:1–10.
[15] Peterson SW, Vega F, 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.
[16] Thompson JD, Gibson TJ, Plewniak F, et al. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997;25(24): 4876–4882.
[17] Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95–98.
[18] Kumar S, Stecher G, Li M, et al. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35(6): 1547–1549.
[19] Batista AC, da Silva MH. Alguns aspergillales de contaminação. Anais da Sociedade de Biologia de Pernambuco. 1955;13:91–100.
[20] Gams W, Christensen M, Onions AH, et al. Infrageneric taxa of *Aspergillus*. In: Samson RA, Pitt JI, editors. Advances in *Penicillium* and *Aspergillus* systematics. New York (NY): Plenum Press; 1985. p. 55–62.
[21] Serra R, Cabañes FJ, Perrone G, et al. *Aspergillus ibericus*: a new species of section *Nigri* isolated from grapes. Mycologia 2006;98(2):295–306.
[22] Samson RA, Visagie CM, Houbraken J, et al. Phylogeny, identification and nomenclature of the genus *Aspergillus*. Stud Mycol. 2014;78:141–173.
[23] Doilom M, Guo JW, Phookamsak R, et al. Screening of phosphate-solubilizing fungi from air and soil in Yunnan, China: four novel species in *Aspergillus*, *Gongronella*, *Penicillium*, and *Talaromyces*. Front Microbiol. 2020;11:585215.
[24] Crous PW, Wingfield MJ, Chooi YH, et al. Fungal planet description sheets: 1042–1111. Persoonia 2020;44:301–459.
[25] Fungaro MHP, Ferranti LS, Massi FP, et al. *Aspergillus labruscus* sp. nov., a new species of *Aspergillus* section *Nigri* discovered in Brazil. Sci Rep. 2017;7(1):6203.
[26] Ismail MA. Incidence and significance of black aspergilli in agricultural commodities: a review, with a key to all species accepted to-date. Eur J Biol Res. 2017;7:207–222.
[27] Varga J, Frisvad JC, Kocsubé S, et al. New and revisited species in *Aspergillus* section *Nigri*. Stud Mycol. 2011;69(1):1–17.
[28] Hendrickx M, Beguin H, Detandt M. Genetic re-identification and antifungal susceptibility testing of *Aspergillus* section *Nigri* strains of the BCCM/IHEM collection. Mycoses. 2012;55(2): 148–155.
[29] Jurjević Ž, Peterson SW, Stea G, et al. Two novel species of *Aspergillus* section *Nigri* from indoor air. IMA Fungus. 2012;3(2):159–173.
[30] Hubka V, Nováková A, Kolařík M, et al. Revision of *Aspergillus* section *Flavipedes*: seven new species and proposal of section *Jani* sect. nov. Mycologia. 2015;107(1):169–208.
[31] Thom C, Church MB. 1926. The aspergilli. Baltimore: Williams & Wilkins. p. 272.
[32] Raper KB, Fennell DI. The genus *Aspergillus*. Baltimore (MD): Williams & Wilkins; 1965.
[33] Samson RA. A compilation of the aspergilli described since 1965. Stud Mycol. 1979;18:1–40.
[34] Varga J, Tóth B, Kocsubé S, et al. Evolutionary relationships among *Aspergillus terreus* isolates and their relatives. Antonie Leeuwenhoek. 2005;88(2): 141–150.
[35] Peterson SW. Phylogenetic relationships in *Aspergillus* based on rDNA sequence analysis. In Samson RA, Pitt JI, editors. Integration of modern taxonomic methods for *Penicillium* and *Aspergillus* classification. Amsterdam: Harwood Academic Publishers; 2000. p. 163–178.
[36] Peterson SW. Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. Mycologia 2008;100(2):205–226.
[37] Crognale S, Pesciaroli L, Felli M, et al. *Aspergillus olivimuriae* sp. nov., a halotolerant species isolated

from olive brine. Int J Syst Evol Microbiol. 2019; 69(9):2899–2906.
[38] Sklenář F, Jurjević Z, Houbraken J, et al. Re-examination of species limits in *Aspergillus* section *Flavipedes* using advanced species delimitation methods and description of four new species. Stud Mycol. 2021;99:100120.
[39] Visagie CM, Goodwell M, Nkwe DO. *Aspergillus* diversity from the gcwihaba cave in Botswana and description of one new species. Fungal Syst Evol. 2021;8:81–89.
[40] Wang XC, Zhuang WY. New species of *Aspergillus* (Aspergillaceae) from tropical islands of China. J Fungi 2022;8:225.
[41] Qin J, Lyu A, Zhang QH, et al. Strain identification and metabolites isolation of *Aspergillus capensis* CanS-34A from *Brassica napus*. Mol Biol Rep. 2019;46(3):3451–3460.
[42] Xiao L, Liu H, Wu N, et al. Characterization of the high cytochalasin E and rosellichalasin producing-*Aspergillus* sp. nov. F1 isolated from marine solar saltern in China. World J Microbiol Biotechnol. 2013;29(1):11–17.
[43] Hubka V, NovaKova A, Samson RA, et al. *Aspergillus europaeus* sp. nov., a widely distributed soil-borne species related to *A. wentii* (section *Cremei*). Plant Syst Evol. 2016;302(6):641–650.
[44] Rank C, Nielsen KF, Larsen TO, et al. Distribution of sterigmatocystin in filamentous fungi. Fungal Biol. 2011;115(4-5):406–420.
[45] Stolk AC, Malla DS. *Penicillium inflatum* sp. nov. Persoonia 1971;6:197–200.
[46] Samson RA, Peterson SW, Frisvad JC, et al. New species in *Aspergillus* section *Terrei*. Stud Mycol. 2011;69(1):39–55.
[47] Zoran T, Sartori B, Sappl L, et al. Azole-resistance in *Aspergillus terreus* and related species: an emerging problem or a rare phenomenon. Front Microbiol. 2018;9:516.
[48] Chen AJ, Varga J, Frisvad JC, et al. Polyphasic taxonomy of *Aspergillus* section *Cervini*. Stud Mycol. 2016;85:65–89.
[49] Magnoli C, Dalcero AM, Chiacchiera SM, et al. Enumeration and identification of *Aspergillus* group and *Penicillium* species in poultry feeds from Argentina. Mycopathologia 1998;142(1): 27–32.
[50] Smith G. Some new and interesting species of micro-fungi. British Mycol Soc Trans. 1961;44: 12–50.
[51] Tsyganenko KS, Zaĭchenko OM. Antibiotic and phytotoxic properties of some *Aspergillus parvulus* Smith strains. Mikrobiol Z. 2004;66(5):62–67.
[52] Tsyganenko KS, Zaichenko OM. Antibiotic properties of some species of genus *Aspergillus* MICH. Mikrobiolohichnyĭ Zhurnal. 2004;66:56–61.
[53] Chao PD, Schifff P Jr, Slatkin DJ. Metabolites of aspergilli. 4. New naphthalenones and 6-ethyl-7-methoxyjuglone from *Aspergillus parvulus*. J Chem Res. 1979;236.
[54] Bartman CD, Campbell IM. Naphthalenone production in *Aspergillus parvulus*. Can J Microbiol. 1979;25(2):130–137.
[55] Bös M, Canesso R, Inoue-Ohga N, et al. O-methylasparvenone, a nitrogen-free serotonin antagonist. Bioorg Med Chem. 1997;5(12):2165–2171.
[56] Hubka V, Kubatova A, Mallatova N, et al. Rare and new etiological agents revealed among 178 clinical *Aspergillus* strains obtained from Czech patients and characterized by molecular sequencing. Med Mycol. 2012;50(6):601–610.