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RESEARCH ARTICLE

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Additions to the Knowledge of the Fungal Order *Eurotiales* in Korea: Eight Undescribed Species

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ABSTRACT

Eurotiales is a relatively large order of *Ascomycetes*, well-known for their ability to produce secondary metabolites with potential beneficial applications. To understand their diversity and distribution, different environmental sources including soil, freshwater, insect, and indoor air were investigated. Eight strains of *Eurotiales* were isolated and identified based on their morphological characters and a multi-gene phylogenetic analysis of the ITS, *BenA*, *CaM*, and *RPB2* regions. We identified eight taxa that were previously not reported from Korea: *Aspergillus baeticus*, *A. griseoaurantiacus*, *A. spinulosporus*, *Penicillium anthracinoglaciei*, *P. labradorum*, *P. nalgiovense*, *Talaromyces atroroseus*, and *T. georgiensis*. Detailed descriptions, illustrations, and phylogenetic tree for the eight new records species are presented, and information regarding the records is also discussed.

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KEYWORDS
Eurotiales; freshwater; insect; soil; morphology; phylogeny

1. Introduction

Eurotiales is a relatively large order of *Ascomycetes*, which has both positive and negative impacts on human activities. The positive aspects include their utilization in food fermentation and biotechnology for the production of enzymes, organic acids, and medications [1–4]. The negative aspects include opportunistic infections, indoor growth, food spoilage, and mycotoxin production [1,5,6].

Eurotiales comprises 28 genera; 15 of them are classified under the *Aspergillaceae* family (*Aspergillago*, *Aspergillus*, *Evansstolkia*, *Hamigera*, *Leiothecium*, *Monascus*, *Penicilliopsis*, *Penicillium*, *Phialomyces*, *Pseudohamigera*, *Pseudopenicillium*, *Sclerocleista*, *Warcupiella*, *Xerochrysium*, and *Xeromyces*), two under *Elaphomycetaceae* (*Elaphomyces* and *Pseudotulostoma*), eight under *Trichocomaceae* (*Acidotalaromyces*, *Ascospirella*, *Dendrosphaera*, *Rasamsonia*, *Sagenomella*, *Talaromyces*, *Thermomyces*, *Trichocoma*), two under *Thermoascaceae* (*Paecilomyces*, *Thermoascus*), and one under *Penicillaginaceae* (*Penicillago*) [1].

The genera *Aspergillus*, *Penicillium*, and *Talaromyces* (*Eurotiomycetes*, *Eurotiales*) are considered to be among the most chemically inventive fungi, producing a wide array of secondary metabolites [1,2,7–9]. They are known for their ability to produce enzymes, organic acids, and antibacterial, anticancer, antifungal, antioxidative, and antiproliferative compounds [1,7,8,10–15]. In addition, they are effective biocontrol agents [16].

The genus *Aspergillus* was first described by Micheli [17]. Members of this genus are usually found in soil, water, decaying vegetation, seeds, grains, and indoor air environment [18–20]. Currently, the genus *Aspergillus* is divided into six subgenera, 27 sections, 75 series, and 446 species [1].

The genus *Penicillium* was erected by Link [21]. Members of the genus are isolated from diverse substrates, including soil, water, air, indoor environments, and food products [22–24]. Currently, this genus is divided into two subgenera, 32 sections, 89 series, and 483 accepted species [1].

The genus *Talaromyces* was initially described by Benjamin as a sexual state of the genus *Penicillium* [25]. *Talaromyces* species are distributed worldwide and are commonly isolated from soil, water, indoor environment, and human [8,26–29]. Currently, the genus *Talaromyces* is divided into eight sections and 171 accepted species [1,8,27].

Although *Aspergillus*, *Penicillium*, and *Talaromyces* species are of economic importance, the current number of the three genera found in Korea is still limited [23,28–31]. These genera are relevant and

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significant in human life, thus it is important to isolate and identify them.

This work presents eight new records of *Aspergillus baeticus*, *A. griseoaurantiacus*, *A. spinulosporus*, *Penicillium anthracinoglaciei*, *P. labradorum*, *P. nalgiovense*, *Talaromyces atroroseus*, and *T. georgiensis* in Korea based on both morphological characteristics and phylogenetic analyses.

2. Materials and methods

2.1. Sample collection, and isolation

Soil samples were collected from Kunryang-ri, Cheongyang, Chungnam Province, Samgak-dong, Buk-gu, Gwangju, and Sangju, Gyeongsang Province in South Korea. Freshwater and insects samples were collected from Hanbat Arboretum located in Daejeon, and Kunryang-ri, Cheongyang, Chungnam Province. The samples were kept at 4 °C until further use. Fungal isolations from soil, freshwater, and insect were carried out as previously detailed [28, 32]. Strains were isolated using potato dextrose agar (PDA; Becton, Dickinson and Co., Sparks, MD) and malt extract agar (MEA; Becton, Dickinson and Co., Sparks, MD) amended with 50 ppm of the antibiotic neomycin. For indoor air, colonies were picked from the contaminated plates kept at 25 °C. Individual colonies with various morphologies were picked and transferred to new PDA plates. All the pure isolates were maintained in PDA slant tubes and 20% glycerol at −80 °C at the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, South Korea. The strains were also deposited at the Culture Collection of the Nakdonggang National Institute of Biological Resources (NNIBR), Sangju, South Korea under the numbers NNIBRFG46707 and NNIBRFG46718, and the Collection of the National Institute of Biological Resources (NIBR), Incheon, Korea under the numbers NIBRFGC000508631, NIBRFG0000509598, NIBRFG0000509600, EKFTFGC000000138, and EKFTFGC000000142.

2.2. DNA extraction, PCR, and sequencing

Genomic DNA extractions were performed on seven-day-old colonies grown on PDA using the Solg™ Genomic DNA Prep Kit (Solgent Co. Ltd., Daejeon, South Korea). The internal transcribed spacer (ITS) rDNA, β-tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB2*) gene regions were amplified by PCR using the primer pairs ITS5/ITS4 [33] and V9G/ITS4 [33,34], Tub2Fd/Tub4Rd [35] and T1/T22 [36], CF1L/CF4 [36], Cmd5/Cmd6 [37], and RPB2-5F/RPB2-7cR [38], respectively. PCR was done in 20 µL volumes with AccuPower PCR PreMix (BioneerCorp., Daejeon, South Korea), 2 µL genomic DNA, 1.5 µL of each primer (5 pmol/µL), and 14 µL deionized water. The PCR thermal cycle programs for ITS, *BenA*, *CaM*, and *RPB2* amplification were as follows: initial denaturing step of 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C (ITS) or 55 °C (*BenA*, *CaM*, *RPB2*) for 30 s, elongation at 72 °C for 45 s, and final extension at 72 °C for 7 min. The PCR products were then purified using an Accuprep PCR Purification Kit (BioneerCorp., Daejeon, South Korea). The purified PCR products were sequenced in both directions with the same primers by Macrogen (Daejeon, South Korea).

2.3. Molecular analyses

The taxa used in the phylogenetic analysis were obtained from previous studies [1, 28, 39,40], and downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank/). SeqMan v. 7.0.0 (DNAstar, Madison, WI) was used to assemble the consensus sequences. The sequences were aligned using the MAFFT v.7 online program (http://mafft.cbrc.jp/alignment/server/) [41] and manually optimized using MEGA v.7 [42]. Maximum-likelihood (ML) analysis was performed using the RAxML-HPC2 on XSEDE (v.8.2.12) in the CIPRES Science Gateway (https://www.phylo.org/portal2) using a GTRGAMMA model with rapid bootstrap analysis followed by 1000 bootstrap replicates. The consensus trees were viewed in FigTree v. 1.3.1 [43]. The newly generated sequences in this study were deposited in GenBank (Table 1).

2.4. Morphological studies

Methods used for morphological observations followed previous study [8, 44–46]. Colony characteristics were recorded after seven days of incubation. Micro-morphological characters were observed under a light microscope using a differential interference contrast microscope (Olympus BX53, Tokyo, Japan) and Olympus DP74 digital camera.

3. Results

3.1. Phylogenetic analyses

The phylogenetic relationship of isolates CNUFC SF23, CNUFC CY2264, and CNUFC GRS15 with accepted *Aspergillus* species was determined by analysis of concatenated sequence datasets of four loci (ITS, *BenA*, *CaM*, and *RPB2*). The concatenated

Table 1. Fungal species and sequences used in phylogenetic analyses.

Species	Strain	GenBank accession no.			
		ITS	*BenA*	*CaM*	*RPB2*
Aspergillus aeneus	NRRL 4769 T	EF652474	EF652298	EF652386	EF652210
A. amoenus	NRRL 4838 T	EF652480	JN853946	JN854035	JN853824
A. baeticus	CCF 4226 T	HE615086	HE615092	HE615117	HE615124
A. baeticus	CCF 4150	HE615087	HE615093	HE615118	HE615125
A. baeticus	**CNUFC GRS15**	**OR875254**	**OR885883**	**OR885884**	**OR940304**
A. aurantiopurpureus	CBS 140608 T	KU866588	KU866824	KU866711	KU866966
A. austroafricanus	NRRL 233 T	JQ301891	JN853963	JN854025	JN853814
A. creber	NRRL 58592 T	JQ301889	JN853980	JN854043	JN853832
A. crustosus	NRRL 4988 T	EF652489	EF652313	EF652401	EF652225
A. desertorum	CBS 653.73 T	EF652505	EF652329	EF652417	EF652241
A. discophorus	CBS 469.88 T	EU448272	AY339999	EU443970	MN969069
A. foeniculicola	CBS 156.80 T	EU448274	EU443990	EU443968	MN969071
A. foveolatus	CBS 279.81 T	KX423658	KX423622	MN969229	KU867034
A. fruticulosus	CBS 486.65 T	EF652483	EF652307	EF652395	EF652219
A. glaucus	NRRL 116 T	EF652052	EF651887	EF651989	EF651934
A. granulosus	NRRL 1932 T	EF652430	EF652254	EF652342	EF652166
A. griseoaurantiacus	DTO 267-D8 T	KJ775553	KJ775086	KJ775357	KU866988
A. griseoaurantiacus	**CNUFC SF23**	**OR826527**	**OR885885**	**OR885886**	**OR940305**
A. heterothallicus	NRRL 5096 T	EF652499	EF652323	EF652411	EF652235
A. hongkongensis	HKU49 T	AB987907	LC000552	MN969320	LC000578
A. jaipurensis	CBS 952.97 T	MN431371	AY339988	KU866761	KU867024
A. nidulans	CBS 589.65 T	EF652427	EF652251	EF652339	EF652163
A. ochraceoroseus	NRRL 28622 T	EF661224	EF661113	EF661137	EF661074
A. pepii	AV11051B IX	KU613368	KU613371	KU613365	
A. protuberus	NRRL 3505 T	EF652460	EF652284	EF652372	EF652196
A. pseudoustus	IBT 28161 T	FJ531147	FJ531168	FJ531129	KU866978
A. puniceus	NRRL 5077 T	EF652498	EF652322	EF652410	EF652234
A. puulaauensis	NRRL 35641 T	JQ301893	JN853979	JN854034	JN853823
A. quadrilineatus	CBS 591.65 T	EF652433	EF652257	EF652345	EF652169
A. recurvatus	CBS 496.65 T	EF652482	EF652306	EF652394	EF652218
A. rugulosus	CBS 133.60 T	EF652434	EF652258	EF652346	EF652170
A. spinulosporus	CBS 120.55 T	EF652445	EF652269	EF652357	EF652181
A. spinulosporus	**CNUFC CY2264**	**OR826528**	**OR892783**	**OR892786**	**OR885887**
A. spectabilis	NRRL 6363 T	EF652510	EU482437	EF652422	EF652246
A. striatus	CBS 283.67 T	EF652470	EF652294	EF652382	EF652206
A. subversicolor	NRRL 58999 T	JQ301894	JN853970	JN854010	JN853799
A. sulphureoviridis	CBS 140626 T	KU866673	KU866911	KU866793	KU867058
A. sydowii	NRRL 250 T	EF652450	EF652274	EF652362	EF652186
A. tabacinus	NRRL 4791 T	EF652478	EF652302	EF652390	EF652214
A. tennesseensis	NRRL 13150 T	JQ301895	JN853976	JN854017	JN853806
A. ustus	NRRL 275 T	EF652455	EF652279	EF652367	EF652191
A. versicolor	NRRL 238 T	EF652442	EF652266	EF652354	EF652178
A. violaceus	CBS 138.55 T	EF652438	EF652262	EF652350	EF652174
P. anthracinoglaciei	EXF-11456 T		MT080506	MT080565	MT080526
P. anthracinoglaciei	EXF-11218	MK460414	MT080469	MT080528	MT080510
P. anthracinoglaciei	EXF-11216	MK460412	MT080468	MT080527	MT080509
P. anthracinoglaciei	**CNUFC CY2234**	**OR826529**	**OR892784**	**OR892785**	**OR885888**
P. astrolabium	CBS 122427 T	DQ645804	DQ645793	DQ645808	JN406634
P. bialowiezense	CBS 227.28 T	EU587315	AY674439	AY484828	JN406604
P. brevicompactum	CBS 257.29 T	AY484912	AY674437	AY484813	JN406594
P. brevistipitatum	AS 3.6887	DQ221696	DQ221695	KU896824	JN406528
P. buchwaldii	CBS 117181 T	JX313164	MN969374	JX313148	JN406637
P. canescens	NRRL 910 T	AF033493	JX140946	MN969241	JN121485
P. canis	NRRL 62798 T	KJ511291	KF900167	KF900177	KF900196
P. chrysogenum	CBS 306.48 T	AF033465	JF909955	JX996273	JN121487
P. compactum	AS 3.15411 T	KM973207	KM973203	KM973200	KT698909
P. concentricum	CBS 477.75 T	KC411763	AY674413	DQ911131	KT900575
P. confertum	CBS 171.87 T	JX997081	AY674373	JX996963	JX996708
P. corvianum	KAS 3618 T	KT887875	KT887836	KT887797	MN969170
P. desertorum	DTO 148-I6 T	JX997011	JX996818	JX996937	JX996682
P. dimorphosporum	NRRL 5207 T	AF081804	KJ834448	KP016783	JN121517
P. dipodomyis	CBS 110412 T	MN431359	AY495991	JX996950	JF909932
P. erubescens	NRRL 6223 T	AF033464	HQ646566	EU427281	JN121490
P. fennelliae	CBS 711.68 T	JX313169	MN969382	JX313151	JN406536
P. flavigenum	CBS 419.89 T	JX997105	AY495993	JX996281	JN406551
P. griseoazureum	CBS 162.42 T	KC411679	KP016919	KP016823	KP016852
P. griseofulvum	NRRL 2300 T	AF033468	JF909942	KT900574	JN121449
P. halotolerans	DTO 148-H9 T	JX997005	JX996816	JX996935	JX996680
P. hermansii	DTO 079-D5 T	MG333472	MG386214	MG386229	MG386242
P. janczewskii	CBS 221.28 T	AY157487	MN969386	MN969267	JN406612
P. jensenii	NRRL 909 T	AY443470	JX140954	AY443490	JN406614
P. labradorum	UTHSCSA DI19-20 T	MK881918	MK887898	MK887899	MK887900
P. labradorum	**CNUFC MOP1**	**OR826530**	**OR837013**	**OR837014**	**OR940306**
P. menonorum	NRRL 50410 T	HQ646591	HQ646573	HQ646584	KF900194
P. mononematosum	CBS 172.87 T	JX997082	AY495997	JX996964	JX996709
P. nalgiovense	CBS 328.59 T	GU981587	GU981631	KX961269	KX961301
P. nalgiovense	**CNUFC CY224**	**OR826531**	**OR837015**	**OR837016**	**OR837017**

(*Continued*)

Table 1. Continued.

Species	Strain	GenBank accession no.			
		ITS	*BenA*	*CaM*	*RPB2*
P. neocrassum	CBS 122428 T	DQ645805	DQ645794	DQ645809	JN406633
P. olsonii	CBS 232.60 T	EU587341	AY674445	DQ658165	JN121464
P. ovatum	DTO 270G7 T	KF667370	KF667366	KF667368	KF667372
P. parvum	NRRL 2095 T	AF033460	HQ646568	KF900173	JN406559
P. pimiteouiense	NRRL 25542 T	AF037431	HQ646569	HQ646580	JN406650
P. robsamsonii	CBS 140573 T	KU904339	KT698885	KT698894	KT698904
P. rubens	DTO 098-E8 T	JX997057	JF909949	JX996263	JX996658
P. tularense	CBS 430.69 T	AF033487	KC427175	JX313135	JN121516
P. vinaceum	NRRL 739 T	AF033461	HQ646575	HQ646586	JN406555
T. atroroseus	CBS 133442 T	KF114747	KF114789	KJ775418	KM023288
T. atroroseus	**CNUFC SJ322**	**OR826532**	**OR837018**	**OR837019**	
T. austrocalifornicus	CBS 644.95 T	JN899357	KJ865732	KJ885261	MN969147
T. bohemicus	CBS 545.86 T	JN899400	KJ865719	KJ885286	JN121532
T. boninensis	CBS 650.95 T	JN899356	KJ865721	KJ885263	KM023276
T. borbonicus	CBS 141340	MG827091	MG855687	MG855688	MG855689
T. cecidicola	CBS 101419 T	AY787844	FJ753295	KJ885287	KM023309
T. chlorolomus	DAOM 241016 T	FJ160273	GU385736	KJ885265	KM023304
T. cnidii	KACC 46617 T	KF183639	KF183641	KJ885266	KM023299
T. cinnabarinus	CBS 267.72	JN899376	AY753377	KJ885256	JN121477
T. dendriticus	CBS 660.80 T	JN899339	JX091391	KF741965	KM023286
T. derxii	CBS 412.89 T	JN899327	JX494306	KF741959	KM023282
T. diversiformis	CBS 141931 T	KX961215	KX961216	KX961259	KX961274
T. diversus	CBS 320.48 T	KJ865740	KJ865723	KJ885268	KM023285
T. erythromellis	CBS 644.80	JN899383	HQ156945	KJ885270	KM023290
T. euchlorocarpius	DTO 176-I3 T	AB176617	KJ865733	KJ885271	KM023303
T. flavus	CBS 310.38 T	JN899360	JX494302	KF741949	JF417426
T. fusiformis	CBS 140637	KU866656	KU866843	KU866740	KU867000
T. georgiensis	DI16-145 T	LT558967	LT559084		LT795606
T. georgiensis	**CNUFC DW211**	**OR826533**	**OR885889**		**OR885890**
T. gwangjuensis	CNUFC WT19-1 T	MK766233	MZ318448		MK912174
T. helicus	CBS 335.48 T	JN899359	KJ865725	KJ885289	KM023273
T. iowaense	NRRL 66822 T	MH281565	MH282578	MH282579	MH282577
T. koreanus	CNUFC YJW2-13 T	MZ315100	MZ318450	MZ332529	MZ332533
T. minnesotensis	UTHSC DI16-144	LT558966	LT559083	LT795604	LT795605
T. pigmentosus	CBS 142805 T	MF278330	LT855562	LT855565	LT855568
T. pseudostromaticus	CBS 470.70 T	JN899371	HQ156950	KJ885277	KM023298
T. purpureus	CBS 475.71 T	JN899328	GU385739	KJ885292	JN121522
T. purpureogenus	CBS 286.36 T	JN899372	JX315639	KF741947	JX315709
T. ptychoconidius	DAOM 241017 T	FJ160266	GU385733	JX140701	KM023278
T. rademirici	CBS 140.84 T	JN899386	KJ865734		KM023302
T. ramulosus	DAOM 241660 T	EU795706	FJ753290	JX140711	KM023281
T. reverso-olivaceus	CBS 140672 T	KU866646	KU866834	KU866730	KU866990
T. stipitatus	CBS 375.48 T	JN899348	KM111288	KF741957	KM023280
T. tabacinus	NRRL 66727 T	MG182613	MG182627	MG182606	MG182620
T. teleomorphus	CNUFC YJW2-5 T	MZ315102	MZ318452	MZ332531	MZ332535
T. tenuis	CBS 141840 T	MN864275	MN863344	MN863321	MN863333
T. trachyspermus	CBS 373.48 T	JN899354	KF114803	KJ885281	JF417432
T. ucrainicus	CBS 162.67 T	JN899394	KF114771	KJ885282	KM023289
T. varians	CBS 386.48 T	JN899368	KJ865731	KJ885284	KM023274
T. viridulus	CBS 252.87 T	JN899314	JX091385	KF741943	JF417422
T. verruculosus	NRRL 1050 T	KF741994	KF741928	KF741944	KM023306
Trichocoma paradoxa	CBS 788.83 T	JN899398	KF984556	KF984670	JN121550

Bold letters indicate strains and accession numbers determined in this study. CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, The Netherlands; CCF: Culture Collection of Fungi, Charles University, Prague, Czech Republic; CNUFC: Chonnam National University Fungal Collection, Gwangju, Korea; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; DTO: Internal Culture Collection of the CBS-Fungal Biodiversity Center; KACC: Korean Agricultural Culture Collection, Republic of Korea; NRRL: Agricultural Research Service Culture Collection, Peoria, IL, USA; T: ex-type strain.

alignment consisted of 2748 nucleotides, including inserted gaps (ITS: 675 bp, *BenA*: 459 bp, *CaM*: 603 bp, and *RPB2*: 1011 bp).

The phylogenetic relationship of isolates CNUFC CY2234, CNUFC MOP1, and CNUFC CY224 with accepted *Penicillium* species was determined by analysis of concatenated sequence datasets of four loci (ITS, *BenA*, *CaM*, and *RPB2*). The concatenated alignment consisted of 2619 nucleotides, including inserted gaps (ITS: 584 bp, *BenA*: 499 bp, *CaM*: 581 bp, and *RPB2*: 955 bp).

The phylogenetic relationship of isolates CNUFC SJ322 and CNUFC DW211 with accepted *Talaromyces* species was determined by analysis of concatenated sequence datasets of four loci (ITS, *BenA*, *CaM*, and *RPB2*). The concatenated alignment consisted of 2806 nucleotides, including inserted gaps (ITS: 653 bp, *BenA*: 573 bp, *CaM*: 694 bp, and *RPB2*: 886 bp).

Isolates CNUFC GRS15, CNUFC SF23, and CNUFC CY2264 were clustered with the ex-type strain of *A. baeticus*, *A. griseoaurantiacus*, and *A. spinulosporus*, respectively, with strong statistical support (Figure 1). In Figure 2, isolates CNUFC CY2234, CNUFC MOP1, and CNUFC CY224 were clustered with the ex-type strain of *P. anthracinoglaciei*, *P. labradorum*, and *P. nalgiovense* with 100%

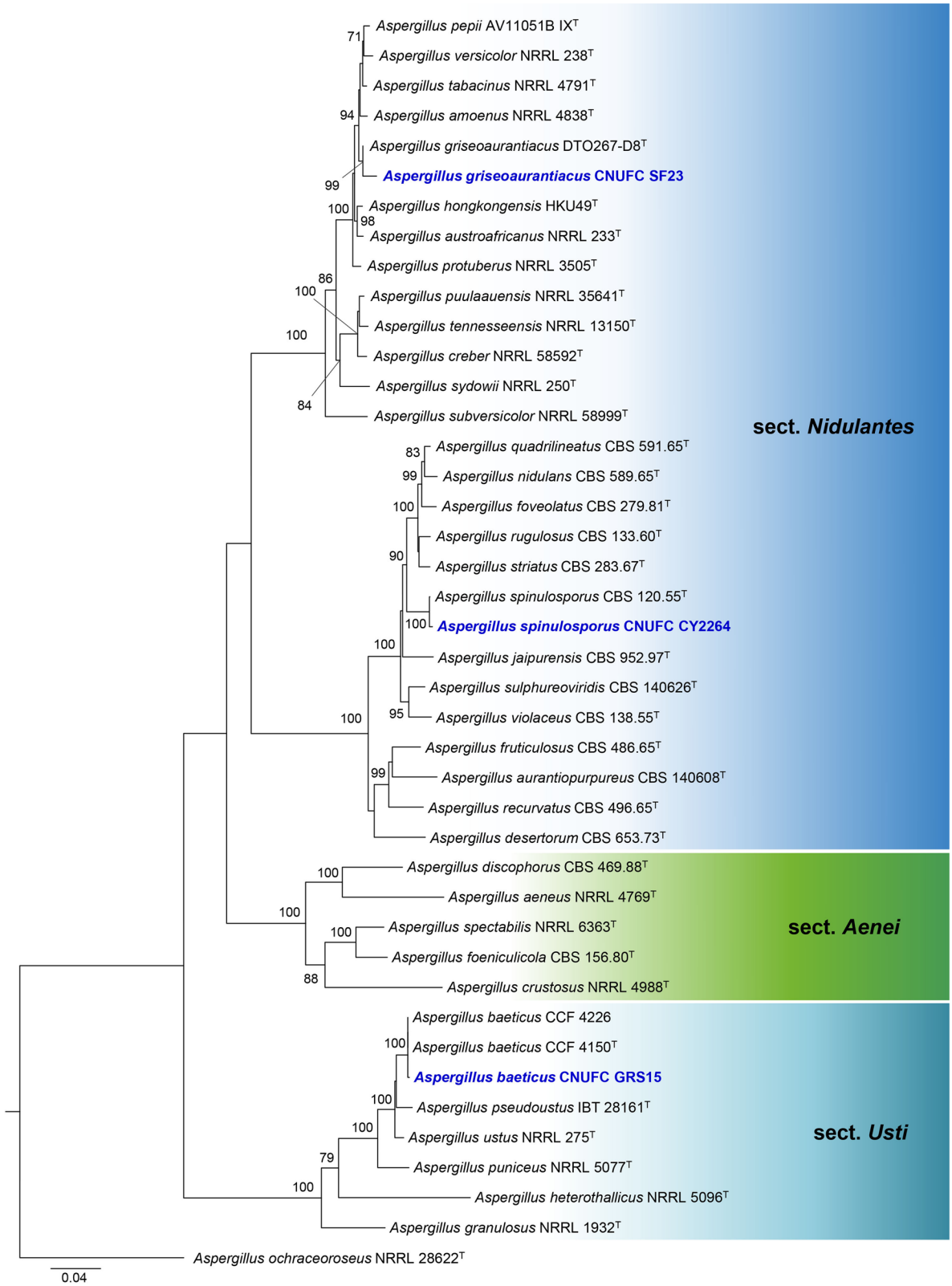

Figure 1. Maximum-likelihood (RAxML) analysis based on combined ITS, *BenA*, *CaM*, and *RPB2* sequence data showing the relationship of the isolates CNUFC SF23, CNUFC CY2264, and CNUFC GRS15 with related species in sections *Aenei*, *Nidulantes*, and *Usti* of the genus *Aspergillus*. The numbers above or below the branches represent maximum-likelihood bootstrap percentages. Bootstrap values equal to or greater than 70% are indicated above or below the branches. *Aspergillus ochraceoroseus* NRRL 28622 was used as the outgroup. The newly generated sequence is indicated in blue.

ML support, respectively. Isolates CNUFC SJ322 and CNUFC DW211 were grouped with the ex-type strain of *T. atroroseus*, and *T. georgiensis*, respectively, with strong statistical support (Figure 3).

3.2. Taxonomy

Aspergillus baeticus A. Novakova & Hubka, *Int. J. Syst. Evol. Microbiol.* 62 (Pt.2): 2783 (2012) [MB#564188] (Figure 4):

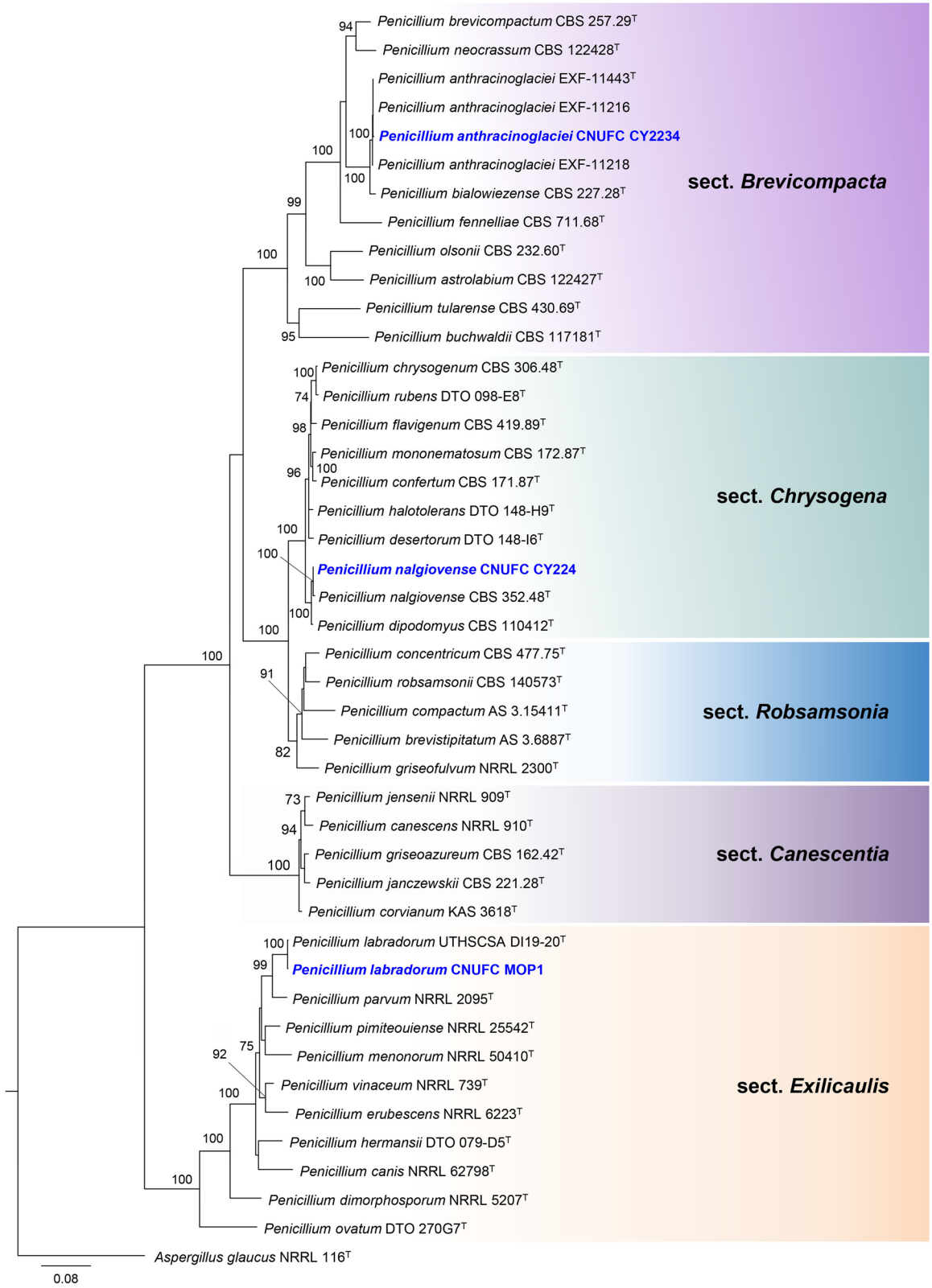

Figure 2. Maximum-likelihood (RAxML) analysis based on combined ITS, *BenA*, *CaM*, and *RPB2* sequence data showing the relationship of the isolates CNUFC CY2234, CNUFC MOP1, and CNUFC CY224 with related species in sections *Brevicompacta*, *Canescentia*, *Chrysogena*, *Exilicaulis*, and *Robsamsonia* of the genus *Aspergillus*. The numbers above or below the branches represent maximum-likelihood bootstrap percentages. Bootstrap values equal to or greater than 70% are indicated above or below the branches. *Aspergillus glaucus* NRRL 116 was used as the outgroup. The newly generated sequence is indicated in blue.

Description: On CYA at 25 °C, colonies slightly sulcate; mycelium white; texture floccose at center; sporulation sparse to moderate; no pigment or exudate produced; reverse yellow-brown at center to grayish yellow at margin, and reached 23–25 mm in diameter after seven days. On MEA at 25 °C, colonies raised at center, texture floccose; sporulation strong; no pigment or exudate produced; reverse

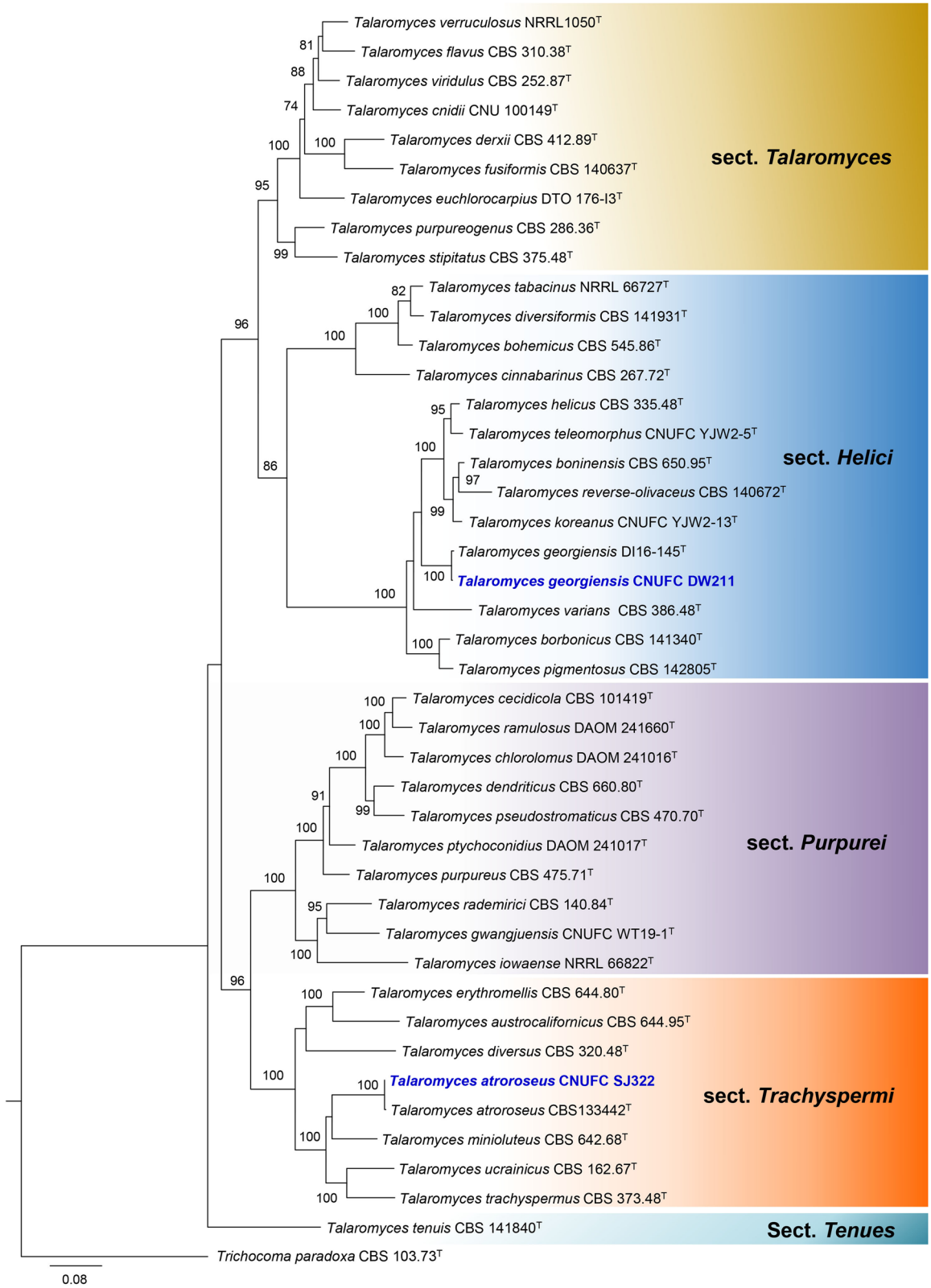

Figure 3. Maximum-likelihood (RAxML) analysis based on combined ITS, *BenA*, *CaM*, and *RPB2* sequence data showing the relationship of the isolates CNUFC SJ322, and CNUFC DW211 with species in sections *Helici*, *Purpurei*, *Talaromyces*, *Tenues*, and *Trachyspermi* of the genus *Talaromyces*. The numbers above or below the branches represent maximum-likelihood bootstrap percentages. Bootstrap values equal to or greater than 70% are indicated above or below the branches. *Trichocoma paradoxa* CBS 103.73 was used as the outgroup. The newly generated sequence is indicated in blue.

moderate yellow, and reached 36–40 mm in diameter after seven days. On YES at 25 °C, colonies slightly raised at center; radially sulcate; texture velutinous to slightly floccose; sporulation sparse to moderate; reverse vivid yellow and reached 34–37 mm in diameter after seven days.

***Micromorphology*:** Stipes smooth walled, 105.5–491 (–601.5) × 5–7.5 µm. Vesicles broadly elliptical or

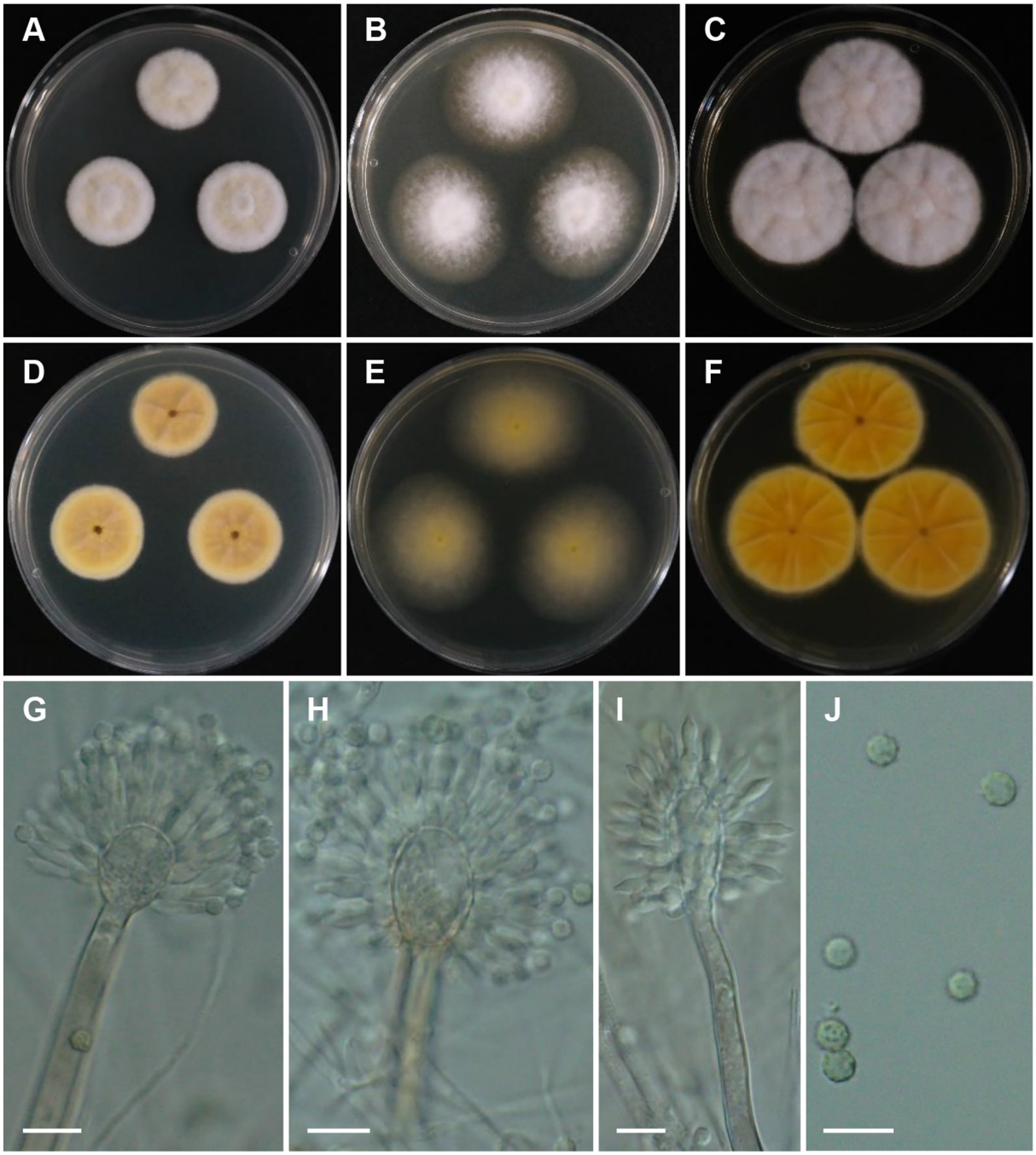

Figure 4. Morphology of *Aspergillus baeticus* CNUFC GRS15. (A, D) Colonies on Czapek yeast autolysate agar (CYA); (B, E) malt extract agar (MEA); (C, F) yeast extract sucrose agar (YES) (A–C: obverse view and D–F: reverse view); (G–I) Conidiophores; (J) Conidia. Scale bars = 10 µm.

elongated, (9.5–)11–18 × 13–20.5(–23.5) µm. Metulae 5–6.5 × (2.5–)3–4 µm. Phialides 6.5–8.5(–9) × 2–3.5 µm. Conidia globose to subglobose, finely roughened, (2.5–)3–4.5 µm.

***Material examined*:** Republic of Korea, Samgak-dong, Buk-gu, Gwangju (35°11′58.1″N 126°53′58.1″E), from soil, October 12, 2019 (culture CNUFC GRS15).

Notes: Our strain did not produce Hülle cells on CYA and grew slower than that of *A. baeticus* (ex-type strain) on CYA at 25 °C (23–25 mm vs. 35–38 mm) [47].

Aspergillus griseoaurantiacus Visagie, Hirooka & Samson, *Studies in Mycology* 78: 112 (2014) [MB#809197] (Figure 5):

***Description*:** On CYA at 25 °C, colonies radially sulcate; mycelium white; colony texture cottony at center; sporulation moderate; reverse moderate yellow and reached 26–28 mm in diameter after seven days. On MEA at 25 °C, colonies raised at center; radially sulcate; margins entire; texture velvety; sporulation moderate; reverse vivid yellow and reached 25–27 mm in diameter after seven days. On YES at 25 °C, colonies radially sulcate; mycelium white; colony texture

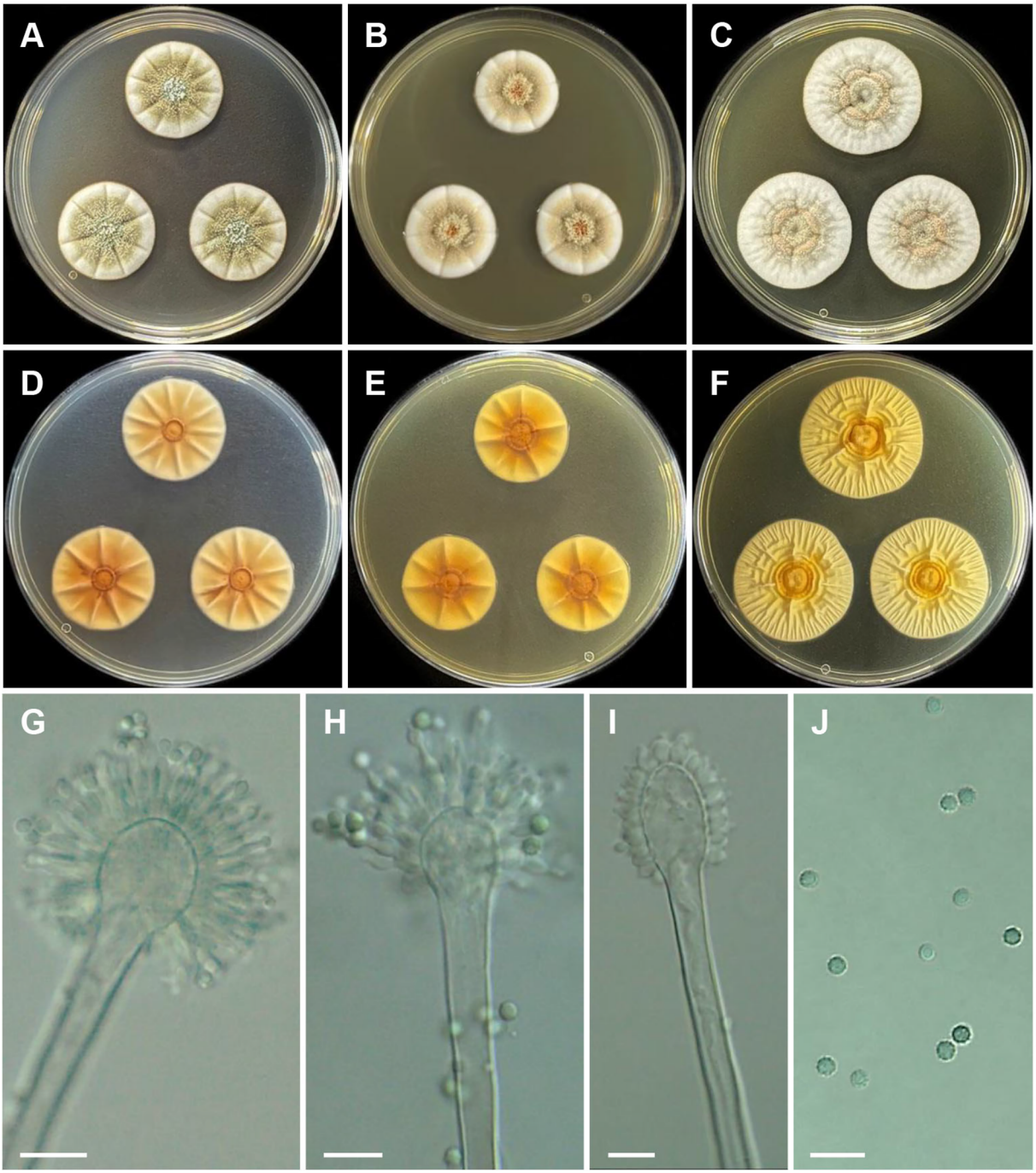

Figure 5. Morphology of *Aspergillus griseoaurantiacus* CNUFC SF23. (A, D) Colonies on Czapek yeast autolysate agar (CYA); (B, E) malt extract agar (MEA); (C, F) yeast extract sucrose agar (YES) (A–C: obverse view and D–F: reverse view); (G–I) Conidiophores; (J) Conidia. Scale bars = 10 µm.

floccose; sporulation strong at center and sparse at margins; reverse pale yellow and reached 32–38 mm in diameter after seven days.

***Micromorphology*:** Stipes smooth walled, 241–449.5 × 4.5–6 µm. Vesicles spathulate or elongated, 8.5–17.5 µm wide. Metulae 3–5.5(–7) × 2.5–4(–5) µm. Phialides ampulliform, 3 per metula, 4–7.5(–8.5) × 2–3 µm. Conidia globose to subglobose, some ellipsoidal, finely roughened, 2.5–3.5 × 2–2.5 µm.

***Material examined*:** Republic of Korea, 54–30 Mukdong-gil, Cheongyang-eup, Cheongyang-gun, Chungcheongnam-do (33°14′52.7″N 126°24′48.3″E), from soil, February 2021, H.B. Lee (culture CNUFC SF23).

Notes: The size of metulae of our strain was slightly shorter than that of the ex-type of *A. griseoaurantiacus* (3–5.5 [–7] × 2.5–4 [–5] µm vs. 4–10 × 3–5.5 µm) [48]. In addition, the ex-type of *A. griseoaurantiacus* did not produce the globose and subglobose conidia observed in our strain [48].

Aspergillus spinulosporus Hubka, S.W. Peterson, M. Kolařík, *Plant Systematics and Evolution* 302 (9): 1290 (2016) (Figure 6):

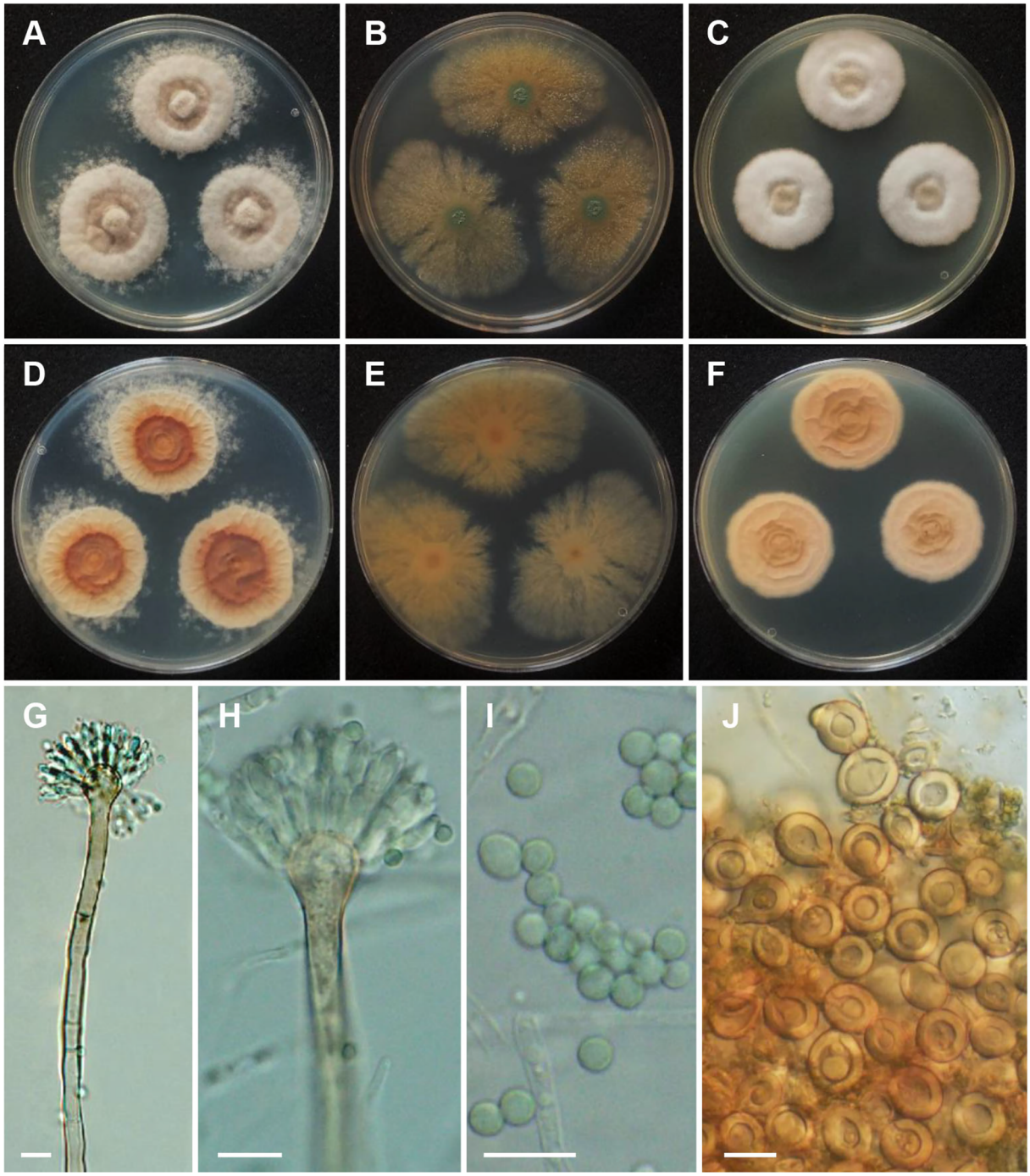

Figure 6. Morphology of *Aspergillus spinulosporus* CNUFC CY2264. (A, D) Colonies on Czapek yeast autolysate agar (CYA); (B, E) malt extract agar (MEA); (C, F) yeast extract sucrose agar (YES) (A–C: obverse view and D–F: reverse view); (G, H) Conidiophores; (I) Conidia; (J) Hülle cells. Scale bars: G–I = 10 µm, J = 20 µm.

***Description*:** On CYA at 25 °C, colonies raised at center; mycelium white; sporulation moderate; reverse deep orange at center and moderate orange yellow at margins and reached 29–33 mm in diameter after seven days. On MEA at 25 °C, colonies plane; poor sporulation; Hülle cells abundant; reverse pale yellow and reached 43–48 mm in diameter after seven days. On YES at 25 °C, colonies slightly raised at center; radially sulcate; mycelium white; colony texture floccose; sporulation absent; reverse light to moderate orange yellow and reached 25–30 mm in diameter after seven days.

***Micromorphology*:** Hülle cells globose, subglobose to ovoid, 15–21.5 µm wide. Conidiophores with smooth stipes, hyaline to light brown, 181–383 × 5.5–8.5 µm. Vesicles subclavate, 10.5–16 µm wide. Metulae hyaline, 7.5–10 × 3–4.5 µm. Phialides hyaline, flask-shaped, 6.5–10 × 3–4 µm. Conidia globose, some oval, smooth, 3–4 µm.

***Material examined*:** Republic of Korea, Kunryang-ri, Cheongyang-eup, Cheongyang, Chungnam Province (36°26′16.2″N 126°46′04.6″E), from soil, February 25, 2022, H.B. Lee (culture CNUFC CY2264).

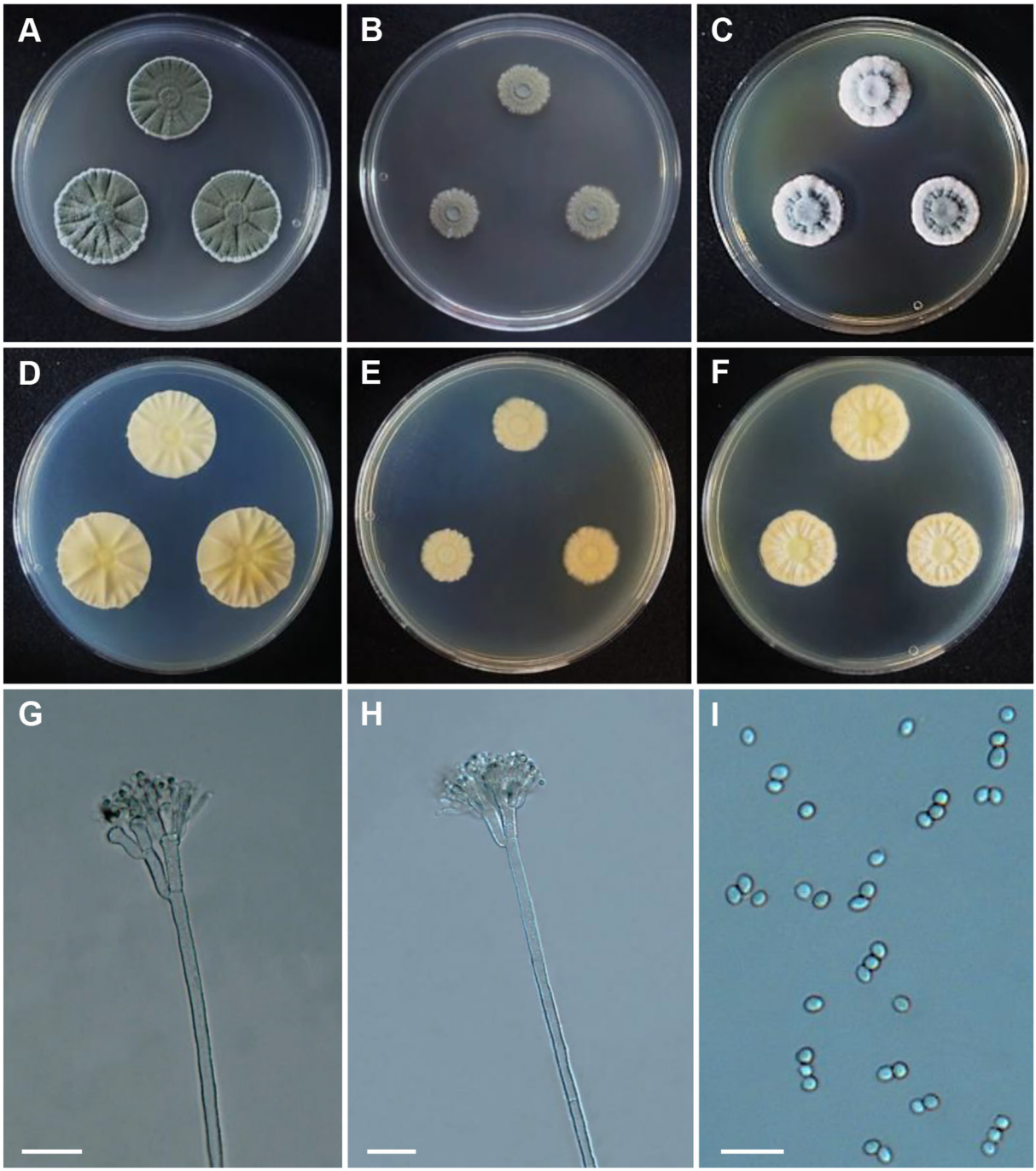

Figure 7. Morphology of *Penicillium anthracinoglaciei* CNUFC CY2234. (A, D) Colonies on Czapek yeast autolysate agar (CYA); (B, E) malt extract agar (MEA); (C, F) yeast extract sucrose agar (YES) (A–C: obverse view and D–F: reverse view); (G, H) Conidiophores; (I) Conidia. Scale bars= 20 μm.

***Notes*:** There were differences in the size of conidiophore and Hülle cell when compared with the ex-type strain of *A. spinulosporus* described in Chen et al. [49].

Penicillium anthracinoglaciei L. Perini, Frisvad & Zalar, *Microbial Ecology* 86, 282–296 (2022) [MB#835602] (Figure 7):

***Description*:** On CYA at 25 °C, colonies radially sulcate; mycelium white; colony texture velvety; sporulation strong; reverse moderate yellow and reached 28–30 mm in diameter after seven days. On MEA at 25 °C, mycelium white and green; texture velvety; sporulation moderate; reverse pale yellow and reached 16–18 mm in diameter after seven days. On YES at 25 °C, colonies raised at center, radially sulcate; mycelium white sometimes green; colony texture floccose; sporulation absent at center and sparse at margins; reverse pale yellow and reached 20–24 mm in diameter after seven days.

***Micromorphology*:** Conidiophores terverticillate. Stipes 85–215 × 3–4.5 μm. Metulae 6–11 × 3–4.5 μm. Phialides 5–13 × 2–4.5 μm. Conidia subglobose to ellipsoidal, 2.5–4 × 2–3.5 μm.

***Material examined*:** Republic of Korea, Kunryang-ri, Cheongyang-eup, Cheongyang, Chungnam Province (36°26′16.2″N 126°46′04.6″E), from dead bee in rainwater, July 2022, H.B. Lee (culture CNUFC CY2234). ***Notes*:** Compared with the ex-type strain of *P. anthracinoglaciei*, our strain grew faster on CYA (28–30 mm vs. 13–20 mm) [50].

Penicillium labradorum Gibas, Wiederh, C. Sanders, Rothacker, E.R. Rogers & Fales, *Medical Mycology*, 58(8): 1061 (2020) (Figure 8):

***Description*:** On CYA at 25 °C, colonies wrinkled; mycelia white; sporulation poor; soluble pigments absent, exudates vivid yellow droplets; reverse moderate to strong yellow and reached 19–22 mm in diameter after seven days. On MEA at 25 °C, colonies plane; mycelia white, sporulation absent; reverse pale yellow and reached 17–22 mm in diameter after seven days. On YES at 25 °C, colonies sulcate; texture floccose, slightly raised at center, sporulation absent; reverse light to moderate yellowish brown and reached 13–21 mm in diameter after seven days.

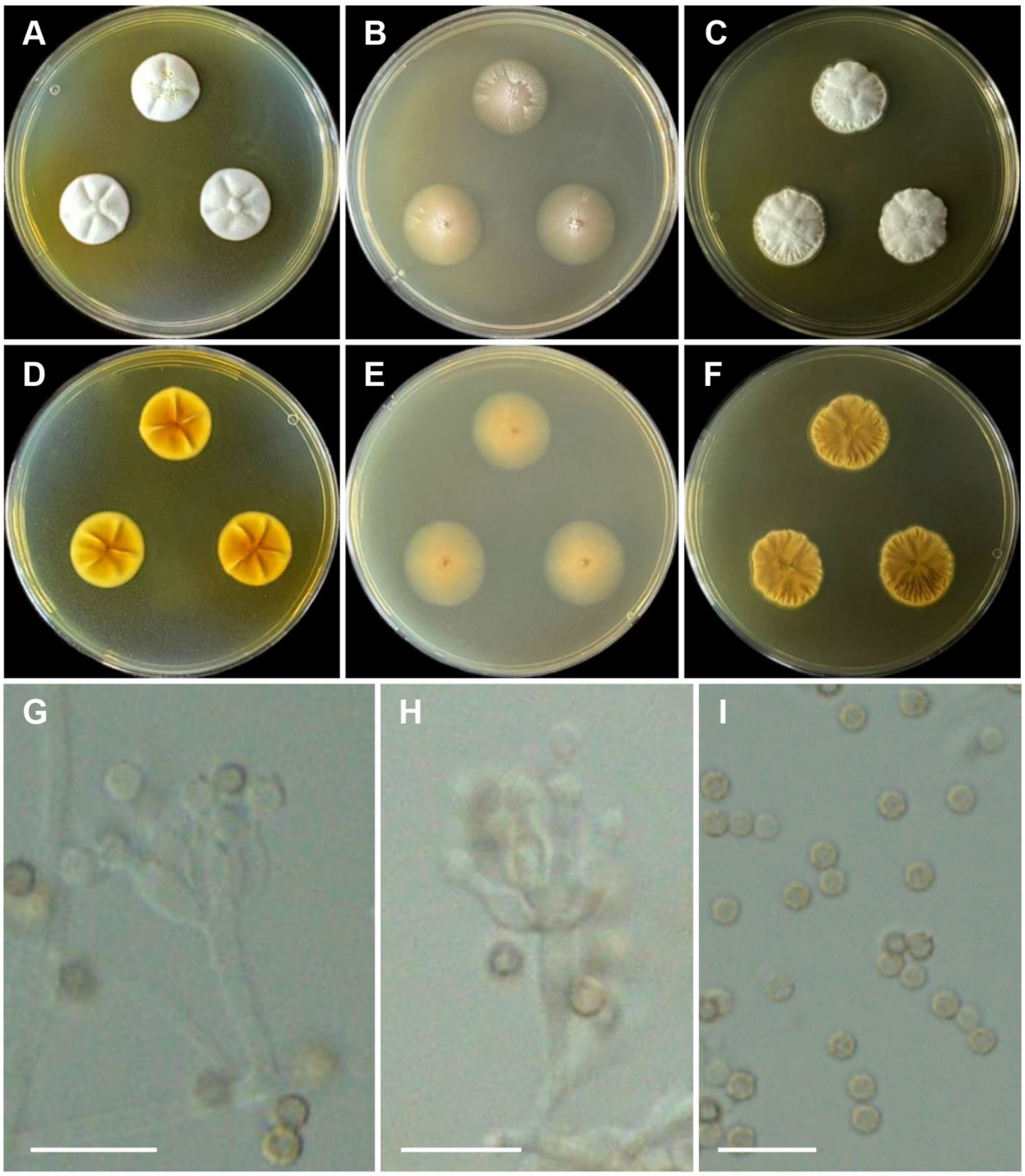

Figure 8. Morphology of *Penicillium labradorum* CNUFC MOP1. (A, D) Colonies on Czapek yeast autolysate agar (CYA); (B, E) malt extract agar (MEA); (C, F) yeast extract sucrose agar (YES) (A–C: obverse view and D–F: reverse view); (G, H) Conidiophores; (I) Conidia. Scale bars = 10 μm.

***Micromorphology*:** Conidiophores monoverticillate. Stipes 5.5–17.5 µm. Phialides smooth, ampulliform, 2–4 per stipe, 5–6.5(–7) × 2–3 µm. Conidia globose to subglobose, finely roughened, 2–2.5 µm.
***Material examined*:** Republic of Korea, Gwangju, Buk-gu, Chonnam National University (35°10′34.0″N 126°54′21.0″E), from an indoor air sample, April 9, 2020 (culture CNUFC MOP1).
***Notes*:** Compared with the ex-type strain of *P. labradorum*, there was a slight difference. Our strain grew faster on CYA (19–22 mm vs. 10–11 mm) and MEA (17–22 mm vs. 16–17 mm) [39].

Penicillium nalgiovense Laxa, Zentralbl. Bakteriol. 2. Abt. 86 (5–7): 160 (1932) [MB#114239] (Figure 9):
***Description*:** On CYA at 25 °C, colonies radially sulcate; margins entire; mycelium white and greenish grey; colony texture velvety to floccose; sporulation moderate to strong; reverse grayish yellow and reached 29–30 mm in diameter after seven days. On

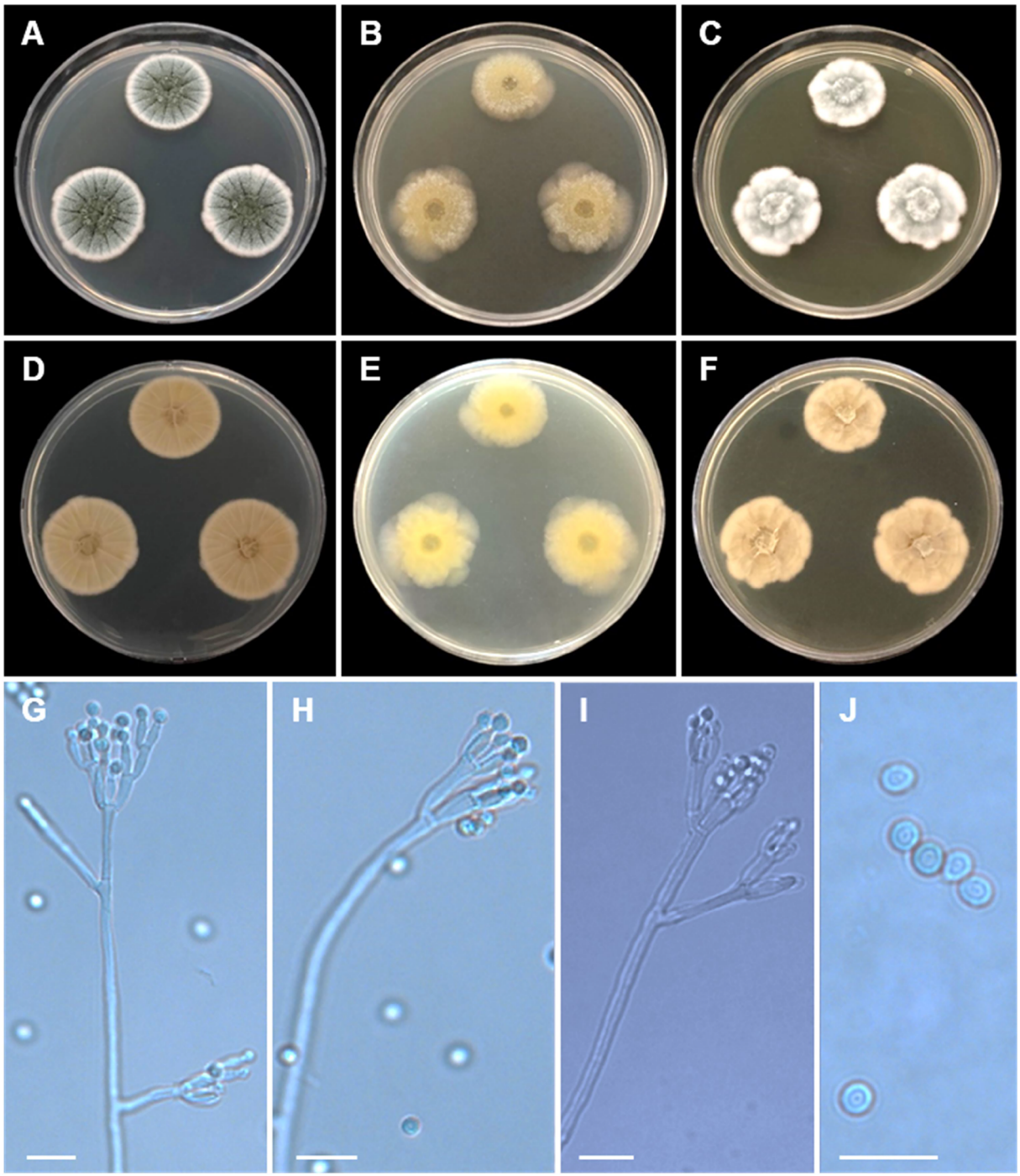

Figure 9. Morphology of *Penicillium nalgiovense* CNUFC CY224. (A, D) Colonies on Czapek yeast autolysate agar (CYA); (B, E) malt extract agar (MEA); (C, F) yeast extract sucrose agar (YES) (A–C: obverse view and D–F: reverse view); (G–I) Conidiophores; (J) Conidia. Scale bars = 10 µm.

MEA at 25 °C, colonies texture floccose, mycelia white; sporulation poor or absent; soluble pigments absent; reverse moderate to strong yellow and reached 26–30 mm in diameter after seven days. On YES at 25 °C, colonies raised at center; radially and concentrically sulcate; margins irregular; mycelia white; sporulation moderate; reverse grayish yellow and reached 26–29 mm in diameter after seven days.
***Micromorphology*:** Conidiophores biverticillate, terverticillate, and quaterverticillate. Metulae 6–15 × 2–3 µm. Phialides flask-shaped, 4.5–12 × 2–2.5 µm. Conidia globose to subglobose, smooth, 2.5–3.5 µm in diam.
***Material examined*:** Republic of Korea, Kunryang-ri, Cheongyang-eup, Cheongyang, Chungnam Province (36°26′16.2″N 126°46′04.7″E), from soil, May 15, 2022, H.B. Lee (culture CNUFC CY224).
***Notes*:** The morphological characters, such as biverticillate, terverticillate, and quaterverticillate conidiophores, flask-shaped phialides, and globose to subglobose conidia, of our strain were similar to the ex-type strain of *P. nalgiovense* [51].

Talaromyces atroroseus N. Yilmaz, Frisvad, Houbraken & Samson, *PLoS One* 8(12): e84102,8 (2013) (Figure 10):
Description: On CYA at 25 °C, colonies texture floccose and velvety; mycelia white and olive green; sporulation strong; soluble pigments red; reverse dark red and reached 31–34 mm in diameter after seven days. On MEA at 25 °C, colonies texture floccose, mycelia white to grayish olive green; sporulation strong; soluble pigments absent; reverse pale greenish yellow and reached 32–38 mm in diameter after seven days. On YES at 25 °C, colonies slightly raised at center, texture velvety; mycelia white; sporulation poor; no soluble pigments; reverse vivid reddish orange and reached 29–37 mm in diameter after seven days.
***Micromorphology*:** Conidiophores biverticillate. Stipes (83.5–)94–137.5(–154) × 2–3.5 µm. Metulae 8.5–12 × 2–3.5 µm. Phialides acerose, 8–11.5 × 2–3 µm. Conidia finely rough to rough, ellipsoidal, 2.5–3 × 2–2.5 µm.
***Material examined*:** Republic of Korea, Namseong-dong, Sangju-si, Gyeongsangbuk-do (36.4109°N, 128.1591°E), from soil, July 31, 2019 (culture CNUFC SJ322).
***Notes*:** The morphological characters of our strain were similar to the ex-type strain of *T. atroroseus* [52].

Talaromyces georgiensis M. Guevara-Suarez, D.A. Sutton & N. Wiederhold, *Mycoses* 60 (10): 656 (2017) [MB#820460] (Figure 11):
***Description*:** On CYA at 25 °C, colonies raised at center; texture velvety; mycelium white; sporulation sparse; reverse yellowish white and reached 22–23 mm in diameter after seven days. On MEA at 25 °C, colonies cottony; mycelium white becoming greenish grey; sporulation moderate; reverse grayish yellow, and reached 31–33 mm in diameter after seven days. On YES at 25 °C, colonies cottony; mycelium white; margins entire; sporulation absent; exudates and soluble pigments absent; reverse pale yellow, and reached 23–26 mm in diameter after seven days.
***Micromorphology*:** Conidiophores monoverticillate. Stipes rough-walled, 12.5–35.5(–37.5) x 2–3 µm. Phialides acerose, 2–4 per stipe, 7–13.5 × 2–3 µm. Conidia smooth to finely rough-walled, globose to subglobose, 2–3(–3.5) µm.
***Material examined*:** Republic of Korea, Mannyeon-dong, Seo-gu, Daejeon East Dongsan of Hanbat Arboretum (36°22′06.4″N 127°23′24.9″E), from freshwater, February 15, 2020 (culture CNUFC DW211).
***Notes*:** Compared with the ex-type strain of *T. georgiensis*, our strain grew slower on CYA (22–23 mm vs. 29–31 mm) and MEA (23–26 mm vs. 28–30 mm) [26].

4. Discussion

Aspergillus, *Penicillium*, and *Talaromyces* species have been frequently isolated from various environments such as soil, water, air, seed, and food [1,8,18,19,22]. However, isolation of fungi from insects is still limited. In this study, *P. anthracinoglaciei* species was isolated from insect sample (dead bee). They are of great interest to pharmacologists due to their ability to produce a wide range of biological activities, including anti-inflammatory, antibacterial, antioxidant, and anti-cancer properties. More than 315 bioactive metabolites of the genus *Aspergillus* and 221 compounds of the genus *Talaromyces* have been documented [53,54].

Eight isolates from different niches were identified as *A. baeticus*, *A. griseoaurantiacus*, *A. spinulosporus*, *P. anthracinoglaciei*, *P. labradorum*, *P. nalgiovense*, *T. atroroseus*, and *T. georgiensis* belonging to sections *Usti*, *Nidulantes*, *Brevicompacta*, *Exilicaulis*, *Chrysogena*, *Trachyspermi*, and *Helici*, respectively (Figures 1–3).

Aspergillus spinulosporus acts as a biocontrol agent against *Xanthomonas oryzae* and produces signals that upregulate superoxide dismutase to protect plants. It also exhibits chitinolytic and amylolytic activities [55]. Additionally, it causes infections in the central nervous system and is associated with unique microbiomes found in tumors [56,57]. *Aspergillus spinulosporus* has been isolated from patients with aspergillosis, birds, clinical samples, brain tissue, sputum, BAL samples, undetermined sources, and asthmatic patients [56–61] and from

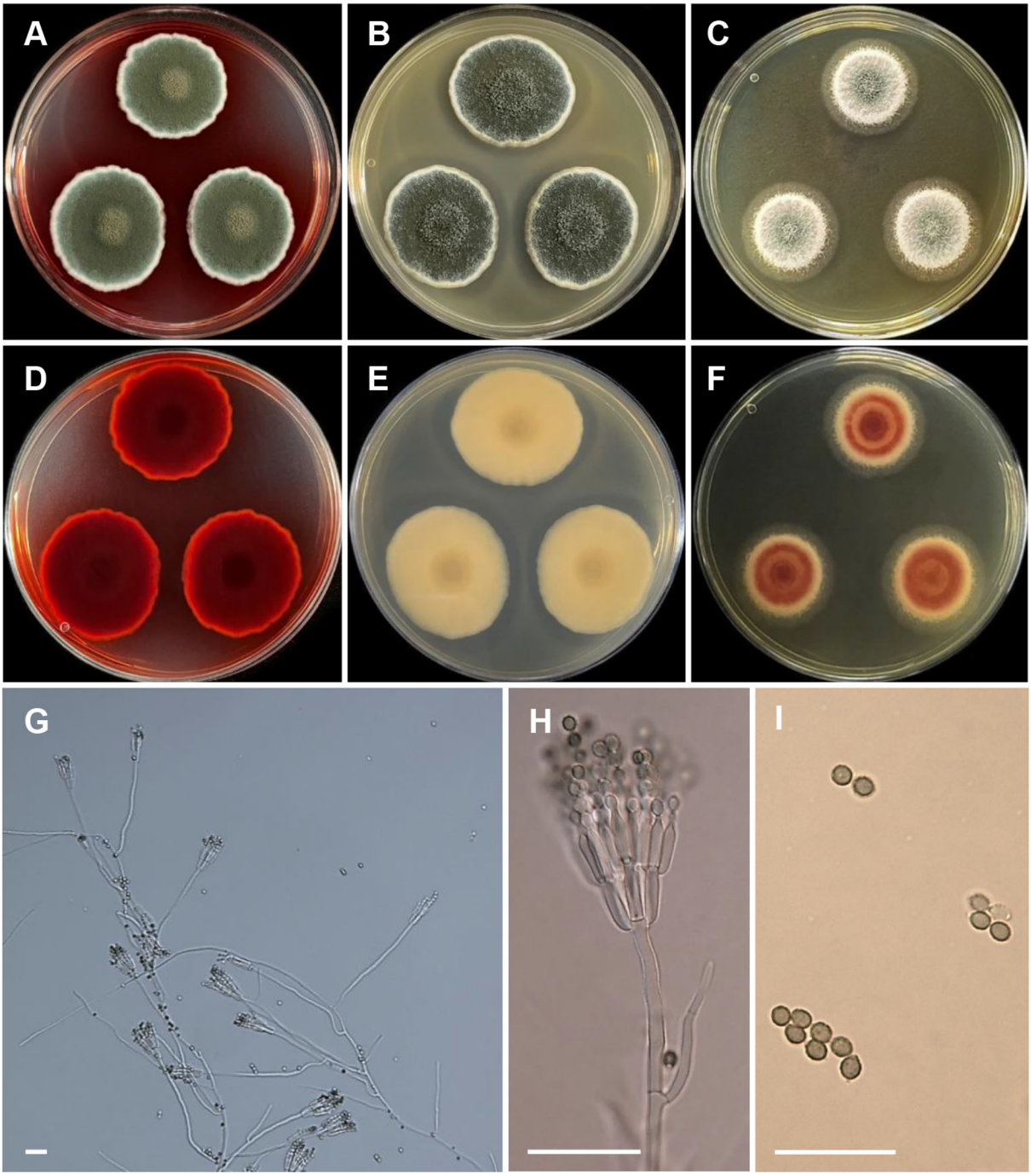

Figure 10. Morphology of *Talaromyces atroroseus* CNUFC SJ322. (A, D) Colonies on Czapek yeast autolysate agar (CYA); (B, E) malt extract agar (MEA); (C, F) yeast extract sucrose agar (YES) (A–C: obverse view and D–F: reverse view); (G, H) Conidiophores; (I) Conidia. Scale bars: G = 20 μm, H, I = 10 μm.

soil in the current study. *Aspergillus griseoaurantiacus* exhibits the highest chitinase and chitosanase activities when cultured under solid-state fermentation of potato shell [62]. Chitinase shows antifungal activity against the pathogenic fungus, *Fusarium solani* [62]. Chitosan oligosaccharides, which are the degraded products of chitosan obtained through chitosanase, exhibit good antibacterial and antioxidant activities [62]. *Aspergillus griseoaurantiacus* was isolated from house dust collected from Thailand, Mexico, and Yela of Kosrae Island [48], as well as from a soil sample in this study. *Aspergillus baeticus* has been isolated from cave sediment, cave air and bat cadaver in Spain [47,63], and from soil environment in this study.

Penicillium anthracinoglaciei acts as decomposers or parasites of glacier ice algae by utilizing and converting the pigment purpurogallin carboxylic acid-6-O-β-D-glucopyranoside into purpurogallin carboxylic acid [50]. The species was isolated from the surface of the Greenland Ice Sheet [50] and from dead bee in rainwater in this study. *Penicillium labradorum* isolated from a Labrador Retriever with disseminated fungal disease shows some clinical

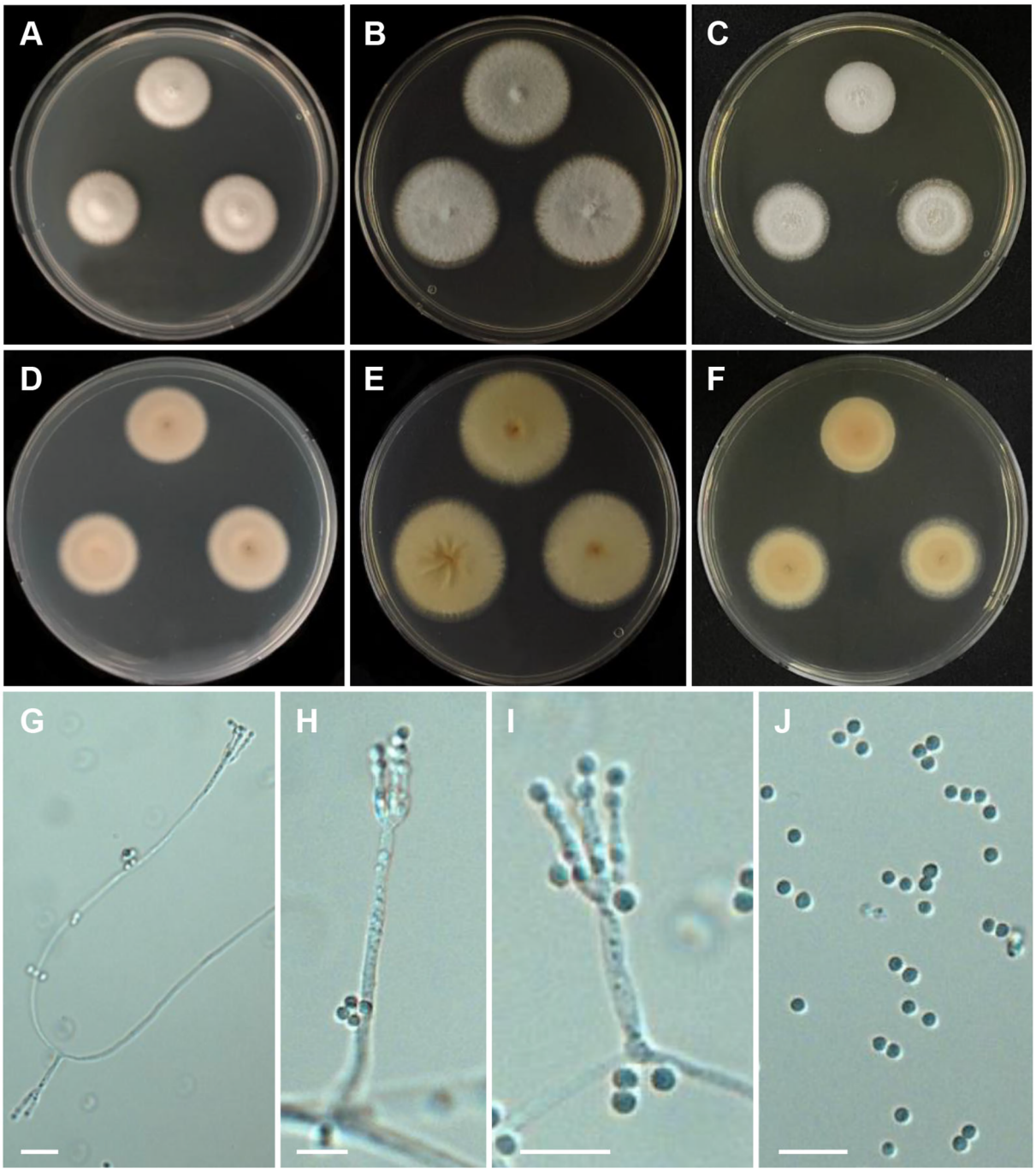

Figure 11. Morphology of *Talaromyces georgiensis* CNUFC DW211. (A, D) Colonies on Czapek yeast autolysate agar (CYA); (B, E) malt extract agar (MEA); (C, F) yeast extract sucrose agar (YES) (A–C: obverse view and D–F: reverse view); (G–I) Conidiophores; (J) Conidia. Scale bars = 10 μm.

signs, including lethargy, lymphadenopathy, tachypnea, moderate pitting edema, and non-weight bearing lameness in the right hind limb [39]. Additionally, it was isolated from the bone tissue of a 6-year-old female Beagle dog [64] and was identified as an indoor contaminant in this study. *Penicillium nalgiovense* has various applications in medicinal chemistry and organic synthesis. It is capable of producing isocoumarins such as dichlorodiaportin, diaportinol, and diaportinic acid [65]. In addition, it is involved in coprogen, amphotericin B, and penicillin production, protease production, fermentation of salchichon, and serves as a starter culture in dry fermentation in the food industry [66–70]. *Penicillium nalgiovense* was isolated from the soil from an abandoned penguin's nest, from Ellischauer cheese [51,68], and from the soil in this study.

Talaromyces georgiensis has been isolated from clinical samples [26], from animal joint fluid [71], and a 4-year-old male Schnauzer dog [64]. Our new isolate, *T. georgiensis* CNUFC DW211, was isolated from a freshwater habitat in Korea. *Talaromyces atroroseus* has been reported in respiratory specimens of patients with pulmonary disorders, house dust (from

Mexico, Thailand, and South Africa), coprophilous, red sweet bell pepper, a contaminated Petri dish, mouse dung, soil, and as a parasite in *Aspergillus niger* culture [8,52,72]. *Talaromyces atroroseus* from this study was isolated from soil sample. *Talaromyces atroroseus* secretes large amounts of red pigments that have great potential for coloring foods [52] and many other compounds such as glauconic acid, glaucanic acid, Monascus red pigments, purpactins AC, purpuride, purpurogenone, N-glutarylmonascorubramine, monascorubrin, monascorubramine, rubropunctatin and ZG-1494α [8,52]. Therefore, future studies are required for the isolation and identification of compounds from *Talaromyces atroroseus* in Korea.

In this study, eight new records of *Eurotiales* were identified from different niches in Korea. Our results suggest that there are numerous undescribed species awaiting discovery in Korea. Further exploration and sampling could result in the discovery of fungal groups that could be utilized for various taxonomic and ecological research.

Disclosure statement

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

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