

Five New Records of the Family Aspergillaceae in Korea, *Aspergillus europaeus*, *A. pragensis*, *A. tennesseensis*, *Penicillium fluviserpens*, and *P. scabrosum*

Thuong T. T. Nguyen, Monmi Pangging, Naila Khan Bangash and Hyang Burm Lee

Environmental Microbiology Lab, Department of Agricultural Biological Chemistry, College of Agriculture & Life Sciences, Chonnam National University, Gwangju, Korea

ABSTRACT

During an investigation of the fungi from the Aspergillaceae family obtained from different environmental sources in Korea, we isolated six strains, including CNUFC WJC9-1, CNUFC BPM36-33, CNUFC MSW6, CNUFC ESW1, CNUFC TM6-2, and CNUFC WD17-1. The morphology and phylogeny of these isolates were analyzed based on their partial β -tubulin (*BenA*) and calmodulin (*CaM*) gene sequences. Based on the morphological characteristics and sequence analyses, the isolates CNUFC WJC9-1, CNUFC BPM36-33, CNUFC TM6-2, and CNUFC WD17-1 were identified as *A. europaeus*, *A. pragensis*, *Penicillium fluviserpens*, and *P. scabrosum*, respectively, and isolates CNUFC MSW6 and CNUFC ESW1 were identified as *A. tennesseensis*. To the best of our knowledge, the species *A. europaeus*, *A. pragensis*, *A. tennesseensis*, *P. fluviserpens*, and *P. scabrosum* have not been previously reported in Korea.

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1. Introduction

Aspergillus and *Penicillium* are genera within the phylum Ascomycota (class: Eurotiomycetes; order: Eurotiales; family: Aspergillaceae). Species belonging to these two genera are mainly environmental saprobes, which act as decomposers of organic materials [1,2]. They can be found in water, soil, vegetation, fruits, foods, indoor environments, and air [2–4]. Several species are considered beneficial for their commercial, economic, or medical uses; they are used in enzyme production, and in the fermentation of foods such as soy sauce (e.g., *Aspergillus oryzae* and *A. sojae*), cheese (e.g., *Penicillium roqueforti*), and sausages (e.g., *P. nalgiovense*). These species also produce a wide range of secondary metabolites that can be used as drugs and antibiotics [5–7], while others can cause diseases in both humans and animals and can also act as plant pathogens [8–10].

The genus *Aspergillus* was first described by Micheli in 1729 [11] as asexual fungi whose conidiphores resemble an aspergillum. This genus consists of 339 species, which are classified into four subgenera (*Aspergillus*, *Circumdati*, *Fumigati*, and *Nidulantes*) and 20 sections [2,3,12,13]. Identification of *Aspergillus* species has been revised, and now relies on standardized methods based on morphological characteristics, extrolite characterization, and multi-

locus DNA sequence analyses. Molecular DNA markers used for *Aspergillus* involved sequencing of the internal transcribed spacer (ITS), calmodulin (*CaM*), β -tubulin (*BenA*), and the RNA polymerase II second largest subunit (*RPB2*) sequences. Due to the well-established *CaM* database, and the relative ease of locus amplification and adequate polymorphism, the *CaM* marker is being used for the identification of *Aspergillus* species [2,14]. About 56 species of *Aspergillus* have been reported from Korea [15]. Recently, a new *Aspergillus* species, *A. koreanus*, has been described [16]. Six more species were recorded recently from Korea, *A. allahabadii* and *A. caninus* from soil, *A. sojae* from meju, and *A. montenegroi*, *A. rhizopodus*, and *A. tabacinus* from tidal mudflats and sea sand [17–20].

The genus *Penicillium* was first described by Link in 1809 [21]. This genus is subdivided into two subgenera (*Aspergilloides* and *Penicillium*) and 26 sections [1,22]. Species of *Penicillium* can be isolated from different environmental sources including air, soil, indoor environments, and food products [1,23]. *Penicillium* species are also identified in a manner similar to *Aspergillus* species, through the use of morphological characteristics, multi-locus DNA sequencing, and extrolite analyses. The *BenA* marker appears to be suitable for their identification [2,14]. This genus includes 354 accepted species according

to Visagie et al. [24]. Approximately 100 *Penicillium* species have been reported from Korea [15,16,25–27]. Twelve species of *Penicillium* are currently reported as new from Korea (Source: www.indexfungorum.org as of July 2019).

The aims of this study were to identify five previously unrecorded fungal species in Korea, *A. europaeus*, *A. pragensis*, *A. tennesseensis*, *P. fluviserpens*, and *P. scabrosum* based on morphological and molecular analyses and to contribute to the knowledge about biodiversity in Korea.

2. Materials and methods

2.1. Sampling and isolation

Commercial corn grain was collected from Wanju, Korea in August 2016. Tomato (*Solanum lycopersicum* L.) fruits were purchased from markets in Gwangju, Korea in July 2017. Death moths (Lepidoptera; Sphingidae) were collected from a garden at Chonnam National University located in Gwangju, Korea in January 2018. By-products of rice bran were collected from Daejeon, Korea in August 2017. Water samples were collected from Eulsukdo Island located in Busan and from a reservoir at Wando island, Korea in August 2017 and 2018, respectively. The samples were collected in sterile plastic bags or sterile 50-mL Falcon tubes and transferred to the laboratory.

To isolate fungi from corn grain, 7–10 corn grains were plated directly onto malt extract agar (MEA) (Difco™, Sparks, MD) adjusted with NaCl, glycerol, or glucose to a water activity range of 0.9–0.85. The plates were incubated at 25 °C in the dark for 7–21 d. Hyphal tips were transferred to potato dextrose agar (PDA; Difco™, Sparks, MD) media using the tips of heat-stretched capillary tubes under a stereomicroscope.

For death moths and tomato fruits, samples were examined under a stereomicroscope to detect any fungal infection. Hyphal tips or spore were transferred to PDA media using the tips of heat-stretched capillary tubes. The plates were incubated at 25 °C in the dark for 7 d.

For by-products of rice bran and water samples, we used the serial dilution plating method as described by Nguyen and Lee [28] and Nguyen et al. [29]. Individual colonies with various morphologies were collected, transferred to PDA, and subcultured until pure mycelia were obtained.

For stock storage, pure isolates were maintained in PDA slant tubes in 20% glycerol at –80 °C at the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, Korea as CNUFC WJC9-1, CNUFC BPM36-33, CNUFC MSW6, CNUFC ESW1, CNUFC TM6-2, and CNUFC WD17-1. CNUFC WJC9-1, CNUFC BPM36-33, CNUFC MSW6, and CNUFC TM6-2 were also deposited at the Collection of National Institute of Biological Resources (NIBR), Incheon, Korea. CNUFC WD17-1 was deposited at the Culture Collection of the Nakdonggang National Institute of Biological Resources (NNIBR), Sangju, Korea. Information on all isolates used in this study was shown in Table 1.

2.2. Morphological studies

The strains were three-point inoculated onto Czapek yeast extract agar (CYA), MEA, yeast extract sucrose agar (YES), and PDA [25]. The plates were incubated at 25 °C in the dark for 7 d. Fragments of mycelia were removed from the cultures and placed on microscope slides with lactic acid (60%). An Olympus BX51 microscope with differential interference contrast optics (Olympus, Tokyo, Japan) was used to capture digital images. The size and shape of the microscopic features were recorded.

2.3. DNA extraction, PCR, and sequencing

Fungal isolates were cultured on PDA overlaid with cellophane at 25 °C for 5–7 d. Genomic DNA was extracted using the Solg™ Genomic DNA preparation Kit (Solgent Co. Ltd., Daejeon, Korea). The *BenA* was amplified using the primer pairs Bt2a/Bt2b, and T10/Bt2b [30]. *CaM* gene was amplified using the primer pairs CMD5/CMD6, and CF1/CF4 [31,32], respectively. PCR amplification was

Table 1. Information on all isolates used in this study.

Species	Culture no.	Substrate	Geographic origin
<i>A. europaeus</i>	CNUFC WJC9-1	Corn grain	Wanju, Korea (35°40'0.12"N 126°00'0.00"E)
<i>A. pragensis</i>	CNUFC BPM36-33	By-product rice bran	Daejeon, Korea (36°19'17.00" N 127°25' 10.99"E)
<i>A. tennesseensis</i>	CNUFC MSW6	Death moth	Gwangju, Korea (35°09'60.00"N 126°54' 59.99"E)
<i>A. tennesseensis</i>	CNUFC ESW1	Sea water	Eulsukdo, Busan, Korea (35°06'10.01"N 129°02'25.01"E)
<i>P. fluviserpens</i>	CNUFC TM6-2	Tomato fruit	Gwangju, Korea (35°09'60.00"N 126°54' 59.99"E)
<i>P. scabrosum</i>	CNUFC WD17-1	Freshwater	Wando, Korea (34°19'1.20" N 126°45'0.00" E)

Table 2. Accession numbers for fungal strains used for the phylogenetic analysis.

Species	Collection no.	GenBank accession no.	
		BenA	CaM
<i>Aspergillus amoenus</i>	NRRL 4838 (T)	EF652304	EF652392
<i>A. austroafricanus</i>	NRRL 233 (T)	JN853963	JN854025
<i>A. brunneo-uniseriatus</i>	NRRL 4273 (T)	EF652123	EF652138
<i>A. campestris</i>	CBS 348.81 (T)	EU014091	EF669535
<i>A. candidus</i>	CBS 566.65 (NT)	EU014089	EF669550
<i>A. chrysellus</i>	NRRL 5084 (T)	EF652109	EF652136
<i>A. creber</i>	NRRL 58592 (T)	JN853980	JN854043
<i>A. cvjetkovicii</i>	NRRL 227 (T)	EF652264	EF652352
<i>A. dimorphicus</i>	NRRL 3650 (T)	EF652111	EF652135
<i>A. dobrogensis</i>	CBS 143370 (T)	LT627027	LT558722
<i>A. europaeus</i>	CBS 140936	LN909018	LN909019
<i>A. europaeus</i>	CBS 134392	LN909004	LN909005
<i>A. europaeus</i>	NRRL 66252 (T)	LN909006	LN909007
<i>A. europaeus</i>	CNUFC WJC9-1	MN337608	MN894576
<i>A. europaeus</i>	CNUFC WJC9-2	MN337609	MN894577
<i>A. flaschentraegeri</i>	NRRL 5042 (T)	EF652113	EF652130
<i>A. flavus</i>	NRRL 1957 (T)	EF661485	EF661508
<i>A. fructus</i>	NRRL 239 (T)	EF652273	EF652361
<i>A. fruticans</i>	CBS 486.65 (T)	EF652307	EF652395
<i>A. griseoaurantiacus</i>	CBS 138191 (T)	KJ775086	KJ775357
<i>A. jensenii</i>	NRRL 58600	JN854007	JN854046
<i>A. penicillioides</i>	NRRL 4548 (T)	EF651928	EF652024
<i>A. pragensis</i>	CBS 135591 (T)	HE661604	FR751452
<i>A. pragensis</i>	CCF 4654	HG916673	HG916680
<i>A. pragensis</i>	CNUFC BPM36-33	MN337604	MN337610
<i>A. pragensis</i>	CNUFC BPM36-34	MN337605	MN337611
<i>A. protuberus</i>	NRRL 3505 (T)	EF652284	EF652372
<i>A. pseudonomius</i>	NRRL 3353	EF661495	EF661529
<i>A. pulvinus</i>	CBS 578.65 (T)	FJ531013	FJ531086
<i>A. puulaauensis</i>	NRRL 35641 (T)	JN853979	JN854034
<i>A. restrictus</i>	NRRL 154 (T)	EF651880	EF652029
<i>A. subalbidus</i>	CBS 567.65 (T)	KP987050	EF669551
<i>A. subversicolor</i>	NRRL 58999 (T)	JN853970	JN854010
<i>A. sydowii</i>	NRRL 250 (T)	EF652274	EF652362
<i>A. tabacinus</i>	NRRL 4791 (T)	EF652302	EF652390
<i>A. taichungensis</i>	IBT 19404 (T)	EU076297	HG916679
<i>A. tamarii</i>	NRRL 20818	EF661474	EF661526
<i>A. tritici</i>	CBS 266.81 (T)	EU076293	HG916678
<i>A. tennesseensis</i>	NRRL 13150 (T)	JN853976	JN854017
<i>A. tennesseensis</i>	LEMI875	KJ766999	KJ766995
<i>A. tennesseensis</i>	LEMI917	KJ766998	KJ766994
<i>A. tennesseensis</i>	CNUFC ESW1	MN337606	MN337612
<i>A. tennesseensis</i>	CNUFC MSW6	MN337607	MN337613
<i>A. venenatus</i>	NRRL 13147 (T)	JN854003	JN854014
<i>A. versicolor</i>	CBS 583.65 (T)	EF652266	EU076368
<i>A. wentii</i>	NRRL 375 (T)	EF652106	EF652131
<i>Penicillium alfredii</i>	CBS138224 (T)	KJ775177	KJ775411
<i>P. atrovenerum</i>	CBS241.56 (T)	JX140944	KJ867004
<i>P. astrolabium</i>	CBS122427 (T)	DQ645793	DQ645808
<i>P. brevicompactum</i>	NRRL 2011	DQ645784	AY484817
<i>P. cinnamomopurpureum</i>	NRRL162 (T)	EF626948	EF626949
<i>P. coleii</i>	NRRL13013 (T)	KF932926	KF932942
<i>P. coralligerum</i>	CBS123.65 (T)	KJ834444	KJ866994
<i>P. crystallinum</i>	CBS479.65 (T)	EF669682	FJ530973
<i>P. cvjetkovicii</i>	NRRL35841 (T)	KF932931	KF932948
<i>P. ellipsoideosporum</i>	CBS112493 (T)	JQ965104	AY678559
<i>P. fluviserpens</i>	NRRL35838 (T)	KF932929	KF932946
<i>P. fluviserpens</i>	NRRL35844	KF932933	KF932950
<i>P. fluviserpens</i>	CNUFC TM6-2	MN894578	MN317092
<i>P. fluviserpens</i>	CNUFC TM6-3	MN894579	MN317093
<i>P. gravinicasei</i>	NRRL66733 (T)	MG600565	MG600570
<i>P. idahoense</i>	NRRL5274 (T)	EF626953	EF626954
<i>P. incoloratum</i>	CBS101753 (T)	KJ834457	KJ866984
<i>P. jamesonlandense</i>	CBS102888 (T)	DQ309448	KJ866985
<i>P. janczewskii</i>	CBS521.28 (T)	KJ834460	KJ867001
<i>P. kojigenum</i>	CBS345.61 (T)	KJ834463	KJ867011
<i>P. lanosum</i>	CBS106.11 (T)	DQ285627	FJ530974
<i>P. lemhiflumine</i>	NRRL35843 (T)	KF932932	KF932949
<i>P. lentiscens</i>	CBS138215 (T)	KJ775168	KJ775404
<i>P. malacaense</i>	NRRL35754 (T)	EU427268	KJ866997
<i>P. mexicanum</i>	CBS138227 (T)	KJ775178	KJ775412
<i>P. monsgalana</i>	NRRL22302 (T)	KF932927	KF932943
<i>P. monsserratidens</i>	NRRL35884 (T)	KF932930	KF932947
<i>P. nodulum</i>	CBS227.89 (T)	KJ834475	KJ867003

(continued)

Table 2. Continued.

Species	Collection no.	GenBank accession no.	
		BenA	CaM
<i>P. novae-zeelandiae</i>	CBS137.41 (T)	KJ834477	KJ866996
<i>P. paradoxum</i>	NRRL2162 (T)	EF669683	EF669692
<i>P. parvulum</i>	NRRL35504 (T)	EF506218	EF506225
<i>P. pusillum</i>	NRRL2498 (T)	KF932925	KF932941
<i>P. raistrickii</i>	CBS261.33 (T)	KJ834485	KJ867006
<i>P. ribeum</i>	CBS127809 (T)	DQ285625	KJ866995
<i>P. sajarovii</i>	CBS277.83 (T)	KJ834489	KJ867007
<i>P. salmoniflumine</i>	NRRL35837 (T)	KF932928	KF932945
<i>P. scabrosum</i>	CBS683.89 (T)	DQ285610	FJ530987
<i>P. scabrosum</i>	CNUFC WD17-1	MN317088	MN317090
<i>P. scabrosum</i>	CNUFC WD17-2	MN317089	MN317091
<i>P. shennangianum</i>	CBS228.89 (T)	KJ834491	AY678561
<i>P. simile</i>	CBS129191 (T)	FJ376595	GQ979710
<i>P. soppii</i>	CBS226.28 (T)	DQ285616	KJ867002
<i>P. swiecickii</i>	CBS119391 (T)	KJ834494	KJ866993
<i>P. virgatum</i>	CBS114838 (T)	KJ834500	KJ866992
<i>Talaromyces flavus</i>	NRRL2098 (T)	EU021663	EU021694

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

CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CCF: Culture Collection of Fungi, Charles University, Prague, Czech Republic; CNUFC: Chonnam National University Fungal Collection (Gwangju, South Korea); NRRL: ARS culture collection, Peoria, IL, USA; T: ex-type strain; NT: neo-type.

performed according to the conditions described in Visagie et al. [24] and Yilmaz et al. [33]. PCR products were purified with an Accuprep PCR Purification Kit (Bioneer Corp., Daejeon, South Korea). Sequencing was performed using the same primers pairs and analyzed using the ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA).

2.4. Phylogenetic analysis

Sequences for selected strains were aligned with reference sequences obtained from GenBank using Clustal_X version 2.1 [34] and were edited manually with Bioedit version 7.2.6.0 [35]. Maximum likelihood (ML) phylogenies were constructed using MEGA version 6 [36]. The sequence of *Talaromyces flavus* was used as an out group. The sequences of the isolates in this study were deposited in the NCBI database under the accession numbers shown in Table 2.

3. Results

3.1. Phylogenetic analysis

A BLASTn search of the BenA regions of CNUFC WJC9-1, CNUFC BPM36-33, CNUFC ESW1, CNUFC TM6-2, and CNUFC WD17-1 showed similarities of 100% (405/405 bp), 100% (447/447 bp), 100% (399/399 bp), 99.6% (559/561 bp), and 99.6% (551/553 bp), with *A. europaeus* (LN909018), *A. pragensis* (HG916673), *A. tennesseensis* (KJ766999), *P. fluviserpens* (KF932929), and *P. scabrosum* (DQ285610), respectively. Similarly, CaM regions of

CNUFC WJC9-1, CNUFC BPM36-33, CNUFC ESW1, CNUFC TM6-2, and CNUFC WD17-1, showed similarities of 99.6% (507/509 bp), 100% (590/590 bp), 100% (642/642 bp), 100% (632/632 bp), and 99.3% (421/424 bp), with *A. europaeus* (LN909019), *A. pragensis* (FR751452), *A. tennesseensis* (KJ766995), *P. fluviserpens* (KF932946), and *P. scabrosum* (FJ530987), respectively. ML gene trees for BenA and CaM revealed that the strains, CNUFC WJC9-1, CNUFC BPM36-33, CNUFC ESW1, CNUFC TM6-2, and CNUFC WD17-1 were identical to *A. europaeus*, *A. pragensis*, *A. tennesseensis*, *P. fluviserpens*, and *P. scabrosum*, respectively (Figures 1 and 2).

3.2. Taxonomy

3.2.1. Taxonomy of CNUFC WJC9-1

A. europaeus Hubka, A. Nováková, Samson, Houbraeken, Frisvad, M. Kolařík, Plant Systematics and Evolution 302: 645 (2016) (Table 3; Figure 3).

Colony characteristics: Colonies on CYA were floccose, pale yellow to dark brown, with yellowish white mycelium, no soluble pigment, moderate sporulation, reverse yellow, and reached 11–13 mm in diameter after 7 d at 25 °C. On MEA, colonies were floccose, with a raised colony center, no soluble pigment, the reverse was pale yellow, and reached 20–22 mm in diameter after 7 d at 25 °C. On PDA, colonies were plane, floccose in the colony center, with strong sporulation, no soluble pigment, reverse light olive, and reached 21–23 mm in diameter after 7 d at 25 °C.

Micromorphology: Conidiophores were smooth-walled, 6.5–11.5 µm wide. Vesicles were pyriform or globose, and were 11–32 µm in diameter. Metulae were broadened toward the top, 6–13.5 × 3–4.5 µm. Phialides were ampulliform, 6–9.2 × 2.4–3.9 µm. Conidia were globose to subglobose, roughened, and yellow-brown to brown at maturity, 3.1–4.6 × 3.2–4.2 µm.

3.2.2. Taxonomy of CNUFC BPM36-33

A. pragensis V. Hubka, J. C. Frisvad & M. Kolařík, Medical Mycology 52: 565–576 (2014) (Table 4; Figure 4).

Colony characteristics: Colonies on CYA were white, with a floccose surface, reverse light brown, and no diffusible pigment, and reached 10–13 mm in diameter after 7 d at 25 °C. On MEA, colonies were pinkish white, with a floccose surface, slightly raised at the center, no diffusible pigment, the reverse was puff brown, and reached 5–8 mm in diameter after 7 d at 25 °C. On PDA, colonies were white, slightly raised at the center, no sporulation, and reached 4–6 mm in diameter after 7 d at 25 °C.

Micromorphology: Conidiophores were smooth-walled, and were 89–400 µm in diameter. Vesicles were pyriform or globose, and were 9–23 µm in diameter. Metulae were cylindrical or wedge-shaped, 3.8–11.4 × 2.6–3.3 µm. Phialides were ampulliform, 4–8 × 2.6–4.1 µm. Conidia were globose with rough echinulate walls, and were 2.6–3.4 µm in diameter.

3.2.3. Taxonomy of CNUFC ESW1

A. tennesseensis Jurjević, S.W. Peterson & B.W. Horn, IMA Fungus 3 (1): 73 (2012) (Table 5; Figure 5).

Colony characteristics: Colonies on CYA were radially sulcate, centrally raised, pea-green in color, with central sporulation, no soluble pigments, and exudates were observed in some isolates, the reverse was brown-yellow in color, and reached 19–22 mm in diameter after 7 d at 25 °C. On MEA, colonies were plane, mycelia green white at the margins to dark green color at the centers, no soluble pigments or exudates, reverse gray-green or pale lemon yellow, and reached 12–14 mm in diameter after 7 d at 25 °C. On YES, colonies were floccose, mycelial pale white at margins to gray-green at the center, centrally sparse sporulation, no soluble pigments, no exudates, reverse dull orange to pale brown, and reached 23–26 mm in diameter after 7 d at 25 °C.

Micromorphology: Conidial heads biserial, conidiophores greenish, smooth-walled stipes typically yellow to brown, sometimes hyaline to brownish shades, 18.5–413.5 × 4.2–7.3 µm. Vesicles were pyriform, 7.5–17.6 µm in diameter. Metulae were 4–7.1 × 2.4–4.1 µm. Phialides forming chains resembling penicillate fructifications, 5.5–11.4 × 2.1–3 µm. Conidia were globose, spherical, and finely roughened, and were 2.4–5.1 µm in diameter.

3.2.4. Taxonomy of CNUFC TM6-2

P. fluviserpens S. W. Peterson, Z. Jurjevic & J.C. Frisvad, PloS One 10: 1–28 (2015) (Table 6; Figure 6).

Colony characteristics: Colonies on CYA were velutinous, pale gray-green, radially sulcate at margins and sulcate to wrinkled centrally, white mycelium, abundant sporulation, no soluble pigment, reverse pale caramel brown, and reached 21–23 mm in diameter after 7 d at 25 °C. On MEA, colonies were calendine green, velutinous, lightly sulcate, with moderate sporulation, reverse was pale green, and reached 24–25 mm in diameter after 7 d at 25 °C. On PDA, colonies were moderately deep, with white mycelia, pale gray-green at the center, no sporulation, no soluble pigment, and reached 23–27 mm in diameter after 7 d at 25 °C.

Micromorphology: Conidiophores were monoverticillate, 17–110 µm. Phialides were ampulliform,

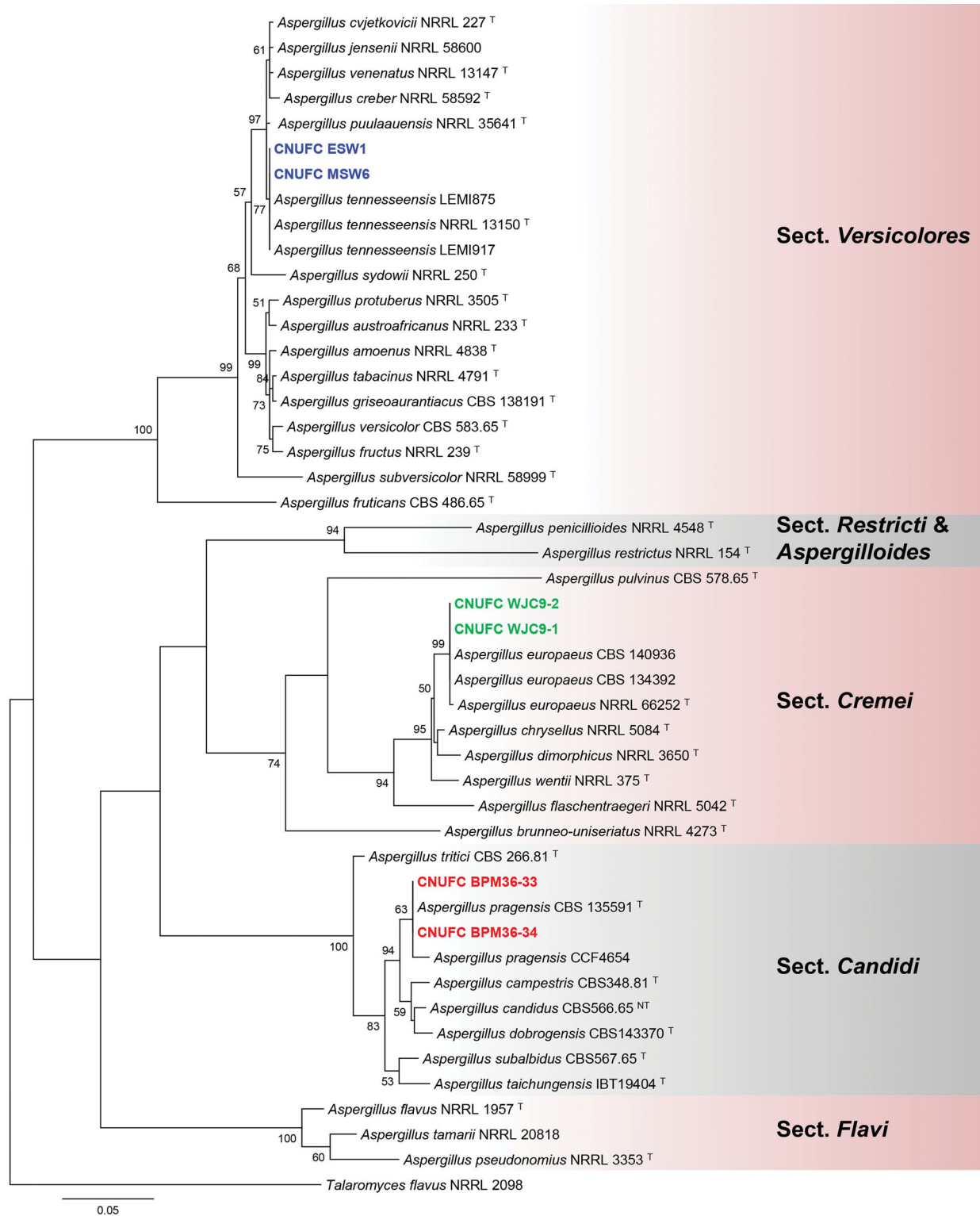


Figure 1. Phylogenetic tree of *Aspergillus europaeus* CNUFC WJC9-1 and CNUFC WJC9-2, *A. pragensis* CNUFC BPM36-33 and CNUFC BPM36-34, *A. tennesseensis* CNUFC ESW1 and CNUFC MSW6, and related species based on maximum likelihood analysis of the combined *BenA* and *CaM* sequences. The sequence of *Talaromyces flavus* was used as an out group. Numbers at the nodes indicate the bootstrap values (>50%) from 1000 replicates. The bar indicates the number of substitutions per nucleotide. The study isolates are shown in bold blue, green, and red.

5.5–9 × 2–3.2 µm. Conidia were sub-spherical to ellipsoidal, and were 2.5–3.3 µm in diameter.

3.2.5. Taxonomy of CNUFC WD17-1

P. scabrosum Frisvad, Samson & Stolk, Persoonia 14 (2): 177 (1990) (Table 7; Figure 7).

Colony characteristics: Colonies on CYA were plane, radially wrinkled, with good sporulation, white or green mycelium, green conidia, no soluble pigment, reverse bright yellow, and reached 13–16 mm in diameter after 7 d at 25 °C. On MEA, colonies were velutinous, with good sporulation,

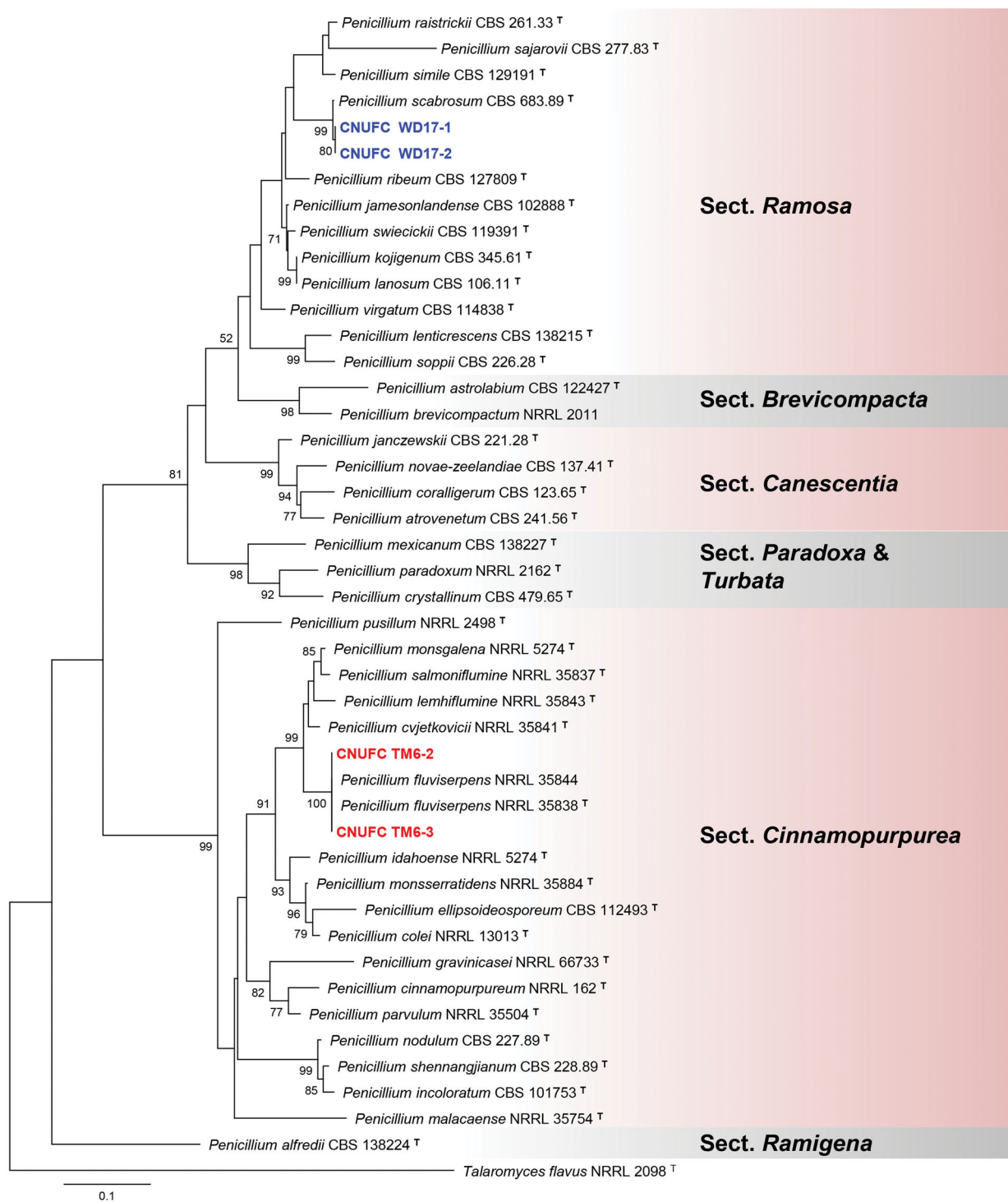


Figure 2. Phylogenetic tree of *Penicillium fluviserpens* CNUFC TM6-2 and CNUFC TM6-3, *P. scabrosum* CNUFC WD17-1 and CNUFC WD17-2 and related species, based on the maximum likelihood analysis of the combined *BenA* and *CaM* sequences. Sequence of *Talaromyces flavus* was used as an out group. Numbers at the nodes indicate the bootstrap values (>50%) from 1000 replicates. The bar indicates the number of substitutions per nucleotide. The study isolates are shown in bold blue and red.

green conidia, no soluble pigments, radially sulcate at margins, and the reverse was yellow, and reached 14–17 mm in diameter after 7 d at 25 °C. On YES, colonies were radially wrinkled, centrally floccose, no soluble pigment, reverse yellow, and reached 24–27 mm in diameter after 7 d at 25 °C.

Micromorphology: Conidiophores were biverticillate, 2.9–4.5 µm. Metulae were 10.3–18.5 × 2.3–4.1 µm, 2–4. Phialides were ampulliform, 4–8 per metula, 8.6–11.8 × 2.1–3.1 µm. Conidia were globose to subglobose, and were 2.3–3.2 µm in diameter.

Table 3. Morphological characteristics of CNUFC WJC9-1 compared with those of the reference strain, *Aspergillus europaeus*.

Character	CNUFC WJC9-1	<i>Aspergillus europaeus</i> ^a
Conidiophores	Smooth-walled, 6.5–11.5 µm wide	300–750 × 7–13(–15) µm
Vesicle	Pyriiform or globose, 11–32 µm diam.,	Pyriiform or globose, 11–44 µm diam.,
Metulae	Broadening toward the top, 6–13.5 × 3–4.5 µm	Broadening toward the top, 6–25 × 5–9 µm
Phialides	Ampulliform, 6–9.2 × 2.4–3.9 µm	Ampulliform, 6–11.5 × 3–6 µm
Conidia	Globose to subglobose, 3.1–4.6 × 3.2–4.2 µm	Globose to subglobose, 3.5–5 × 3–4.5 µm

^aFrom the description by Hubka et al. [37].

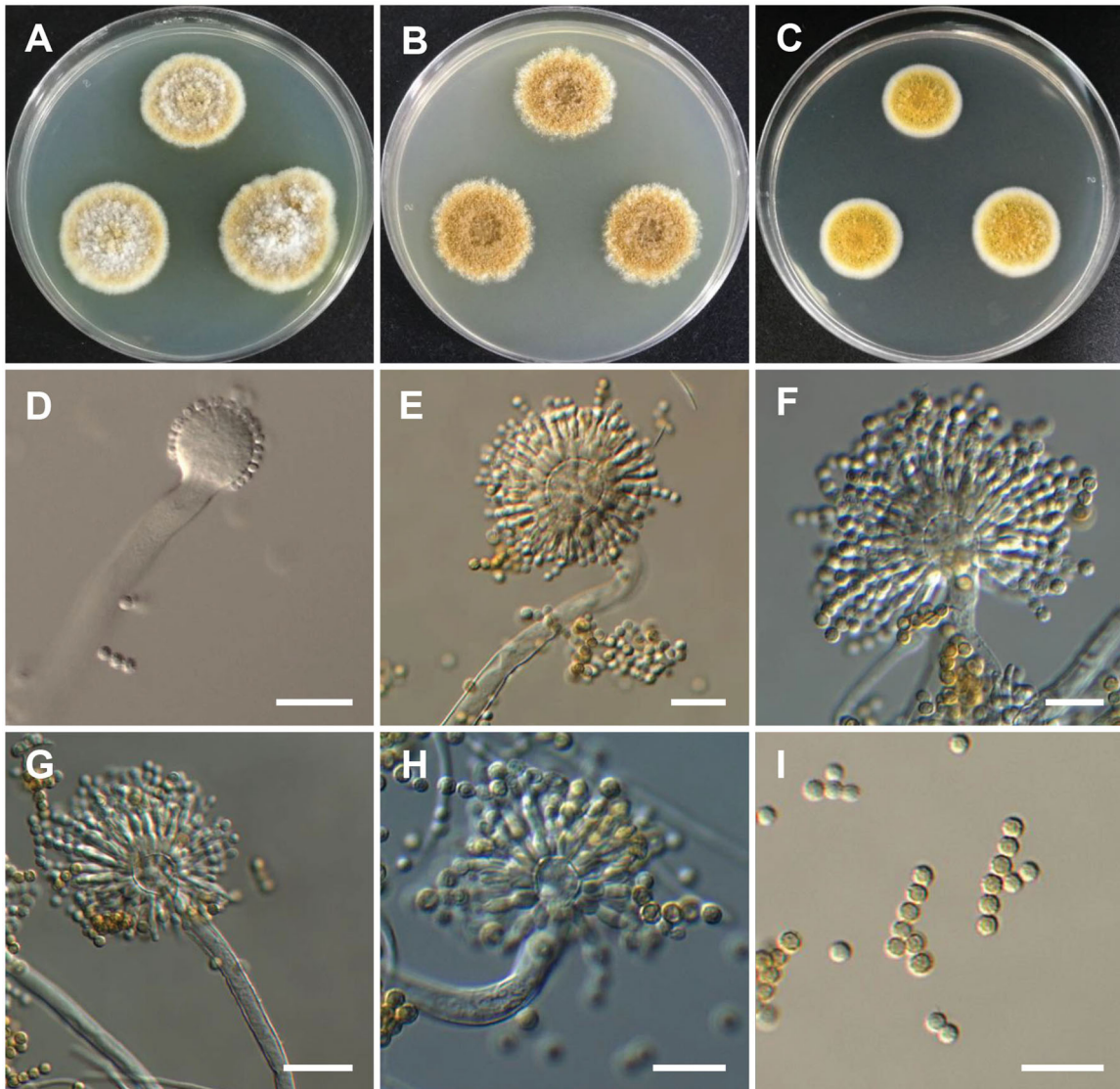


Figure 3. Morphology of *Aspergillus europaeus*. (A) Colonies on Potato dextrose agar (PDA); (B) Colonies on Blakeslee's malt extract agar (MEA); (C) Czapek yeast autolysate agar (CYA); (D–H) Conidiophores; (I) Conidia (scale bars: D–I = 20 µm).

4. Discussion

Species of *Aspergillus* and *Penicillium* belonging to the sections *Cremeri*, *Candidi*, *Versicolores*, *Cinnamopurpurea*, and *Ramosa*, were discovered during a survey on the biodiversity of Aspergillaceae inhabiting different substrates. Here, three *Aspergillus* and two *Penicillium* species in five different sections were identified and compared to their most closely related species.

Analysis of the combined *BenA* and *CaM* datasets showed that the strains CNUFC WJC9-1 and

CNUFC WJC9-2 were clustered within the same clade as *A. europaeus* NRRL 66252 (ex-type strain), belonging to the section *Cremeri* (Figure 1). The isolate CNUFC WJC9-1 is morphologically similar to *A. europaeus* previously described by Hubka et al. [37] with respect to producing globose to subglobose coarsely roughened conidia, with a yellow-brown to brown color at maturity. However, the sizes of metulae reported in the literature (6–25 × 5–9 µm) are bigger than those of our isolate. *Aspergillus* section *Cremeri* (known as the

Table 4. Morphological characteristics of CNUFC BPM36-33 compared with those of the reference strain *Aspergillus pragensis*.

Character	CNUFC BPM36-33	<i>Aspergillus pragensis</i> ^a
Conidiophores	Smooth-walled, 89–400 µm long	Hyaline, smooth-walled, usually 90–600 µm (but up to 1200 µm)
Vesicle	Pyriform or globose, 9–23 µm	Predominantly globose, 9–21 µm
Metulae	Cylindrical or wedge-shaped, measured 3.8–11.4 × 2.6–3.3 µm	Wedge-shaped or cylindrical, 4.5–10.5 × 3.5–5.5 µm
Phialides	Ampulliform, 4–8 × 2.6–4.1 µm	Ampulliform, 6–8.5 × 2.5–3.5 µm
Conidia	Globose with rough echinulate walls, 2.6–3.4 µm	Globose, 2.5–3.5 µm, smooth

^aFrom the description by Hubka et al. [41].

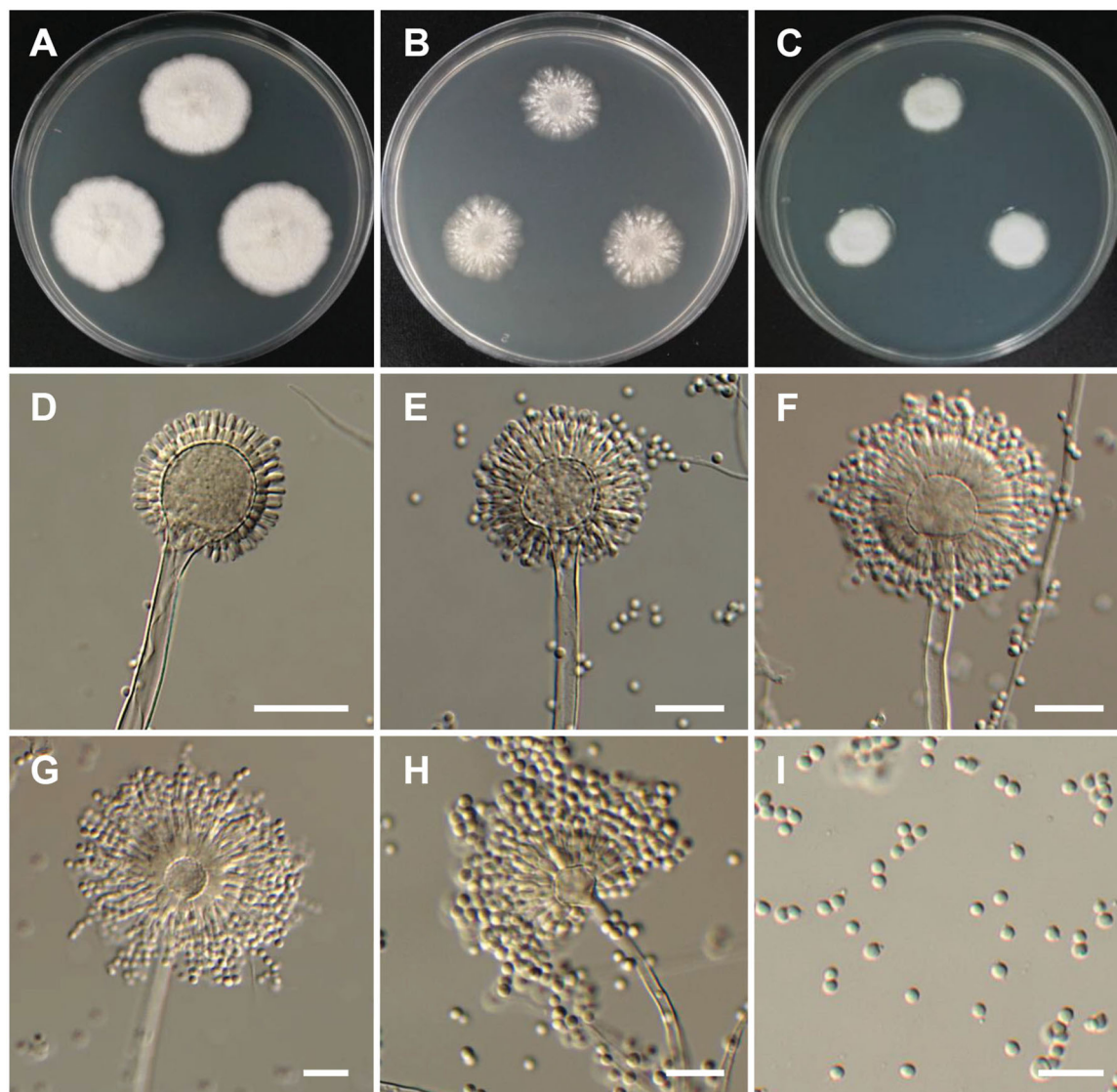


Figure 4. Morphology of *Aspergillus pragensis*. (A) Colonies on Czapek yeast autolysate agar (CYA); (B) Colonies on Blakeslee's malt extract agar (MEA); (C) Colonies on Potato dextrose agar (PDA); (D–H) Conidiophores; (I) Conidia (scale bars: D–I = 20 µm).

A. cremeus group) was first described by Raper and Fennell [38] and included five species, *A. itaconicus*, *A. flaschentraegeri*, *A. stromatoides*, *A. chrysellia*, and *A. cremea*. The species belonging to this section are characterized by their yellowish-brown to brown or gray-green colony color, with biseriate conidial heads and long conidiophores [2]. Several fungal species belonging to the section *Cremiti* are frequently found in soil and foods where they can cause spoilage of cereals and nuts; they are found less frequently in indoor environments or in clinical material [37,39]. *A. europaeus* was earlier reported

from soil samples in European caves and several steppe-like localities in the Czech Republic [37]. In this study, *A. europaeus* was isolated from corn grains. *A. europaeus* shares the production of 3-O-methylsulochrin and 3-O-demethylsulochrin with *A. wentii* [37,40].

The strains CNUFC BPM36-33 and CNUFC BPM36-34 reside in a well-supported clade with *A. pragensis* CBS 135591 (ex-type strain), belonging to the section *Candidi* (Figure 1). The morphological characteristics of the isolate *A. pragensis* in this study were similar to those previously described

Table 5. Morphological characteristics of CNUFC ESW1 compared with those of the reference strain *Aspergillus tennesseensis*.

Character	CNUFC ESW1	<i>Aspergillus tennesseensis</i> ^a
Conidiophores	Greenish smooth walled, yellow to brown, sometimes hyaline to brownish shades 18.5–413.5 × 4.2–7.3 μm	Smooth walled, hyaline to yellowish with brownish shades (35–)100–300(–400) × 4–7 μm
Vesicle	Pyriform, 7.5–17.6 μm	Pyriform (7–)10–16(–18) μm
Metulae	4–7.1 × 2.4–4.1 μm	(4–)6–(–8) × 2.5–4 μm
Phialides	Forming chains resembling penicillate fructifications, 5.5–11.4 × 2.1–3 μm	Fragmentary heads resembling penicillate fructifications, 5–8(–11) × 2–3 μm
Conidia	Conidia globose, spherical, finely roughened, 2.4–5.1 μm	Spherical to subspherical, ellipsoidal to pyriform (2.5–)3–4(–8) μm, roughened wall

^aFrom the description by Jurjevic et al. [50].

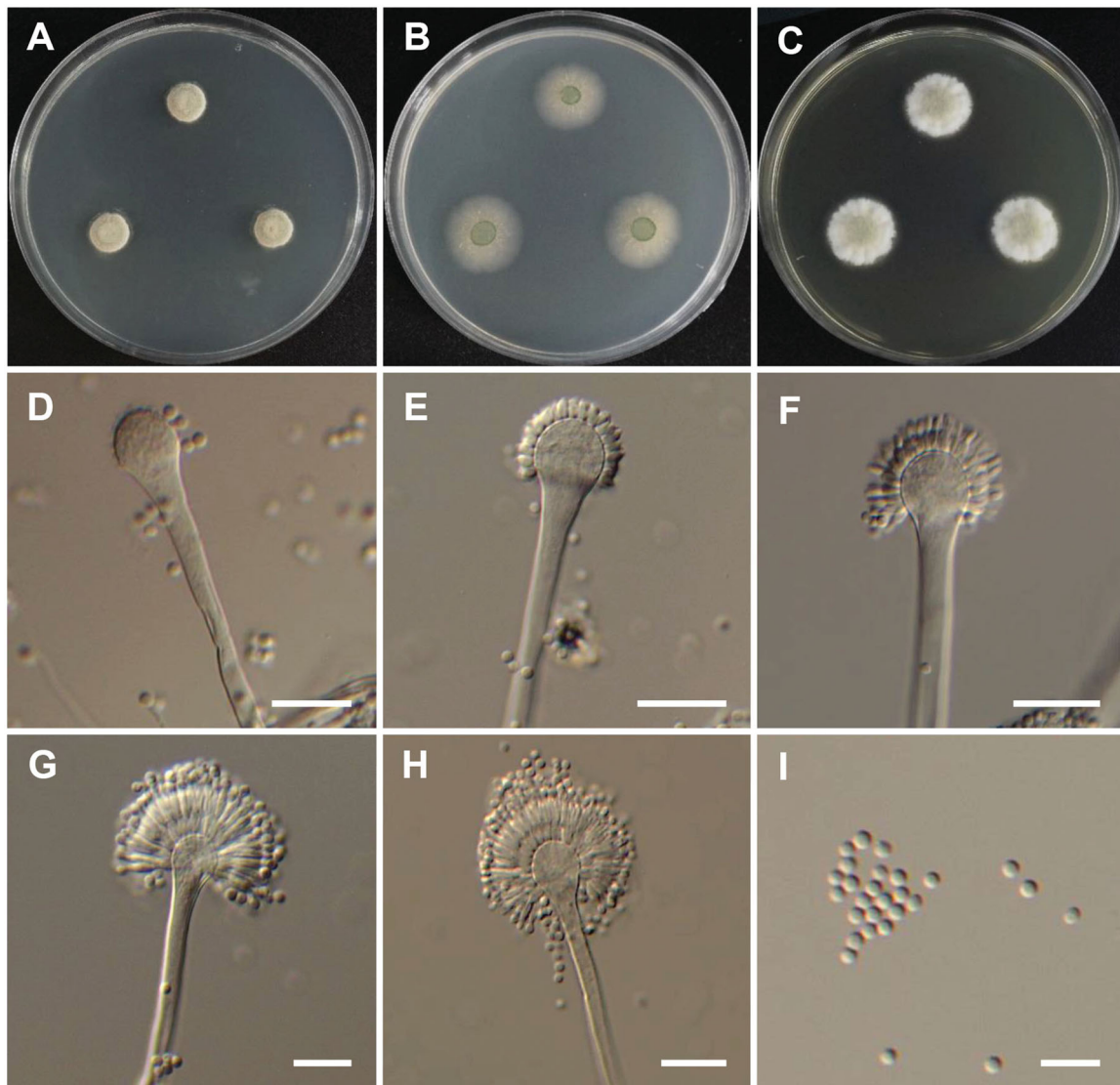


Figure 5. Morphology of *Aspergillus tennesseensis*. (A) Colonies on Czapek yeast autolysate agar (CYA); (B) Colonies on Blakeslee's malt extract agar (MEA); (C) Colonies on yeast malt extract agar (YES); (D–H) Conidiophores. (I) Conidia (scale bars: D–I = 20 μm).

by Hubka et al. [41]. Colony diameter on CYA was similar to that of the previously described *A. pragensis* type species (CYA: 22–24 mm after 14 d); however, differences in colony diameter were observed on MEA (MEA: 16–18 mm after 14 d). No growth was observed for the isolate CNUFC BPM36-33 on MEA at 37 °C. The *Aspergillus* section *Candidi* was established by Gams et al. [42] for the previous *A. candidus* group based on the criterion proposed by

Thom and Raper [43]. Currently, this section includes seven species [41,44], isolated from dust, cave air, carpet, mouse dung, herbivore dung, cave sediment, bat droppings and guano, indoor environments, and clinical samples [44,45]. These are economically significant species, which are used in biotechnology sectors; these species are used as starter cultures for the production of food sauces, alcoholic beverages, production of extracellular

Table 6. Morphological characteristics of CNUFC TM6-2 compared with those of the reference strain *Penicillium fluviserpens*.

Character	CNUFC TM6-2	<i>Penicillium fluviserpens</i> ^a
Conidiophores	Monoverticillate, 17–110 µm	Smooth to finely roughened, monoverticillate (5–) 30–130 (–180) µm
Phialides	Ampulliform, 5.5–9 × 2–3.2 µm	Ampulliform (5–) 6–8 (–32) × 2–3.5 µm
Conidia	Ellipsoidal to sub-spherical, 2.5–3.3 µm in diameter	Ellipsoidal to sub-spherical 2.5–3.5(–7) µm

^aFrom the description by Peterson et al. [63].

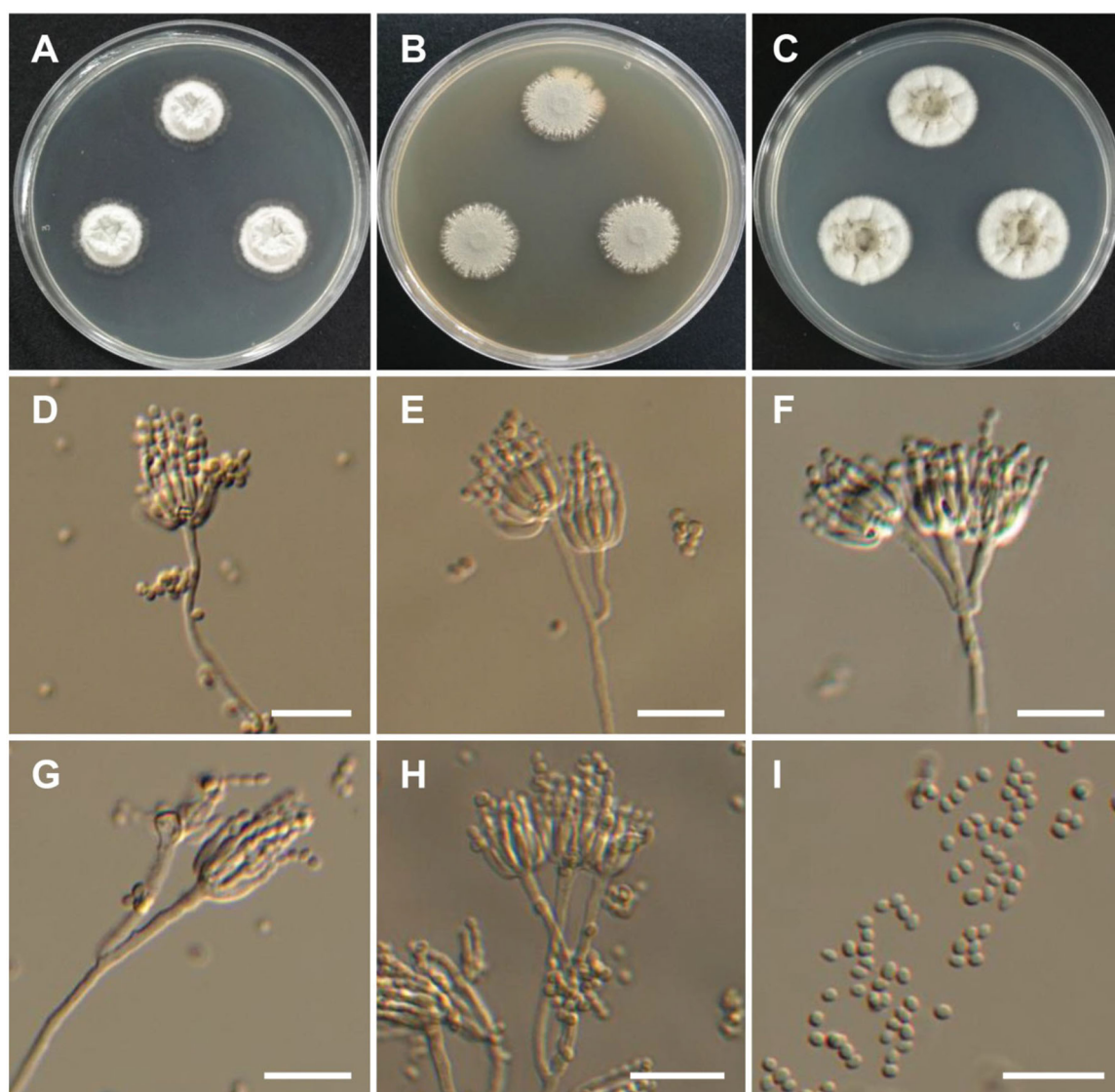


Figure 6. Morphology of *Penicillium fluviserpens*. (A) Colonies on Czapek yeast autolysate agar (CYA); (B) Colonies on Blakeslee's malt extract agar (MEA); (C) Colonies on Potato dextrose agar (PDA); (D–H) Conidiophores; (I) Conidia (scale bars: D–I = 20 µm).

enzymes, and waste degradation. They are also known to produce many bioactive compounds including antimicrobial, anti-oxidative, antitumor, and cytotoxic compounds [44]. In addition, these species are also known to cause human infection, namely, onychomycosis, invasive aspergillosis, otomycosis, and pulmonary aspergilloma [46,47]. Two species, *A. candidus* and *A. tritici* were isolated from Meju samples in Korea [48]. *A. pragensis* was recovered from human clinical material (nail) and was found to be responsible for causing onychomycosis in the Czech Republic [41]; it has also been isolated from rock samples from unnamed Karst caves in Suiyang located beside the Kuankuoshui National

Natural Reserve, China [49]. In this study, *A. pragensis* was isolated from a by-product of rice bran.

The strains CNUFC MSW6 and CNUFC ESW1 were clustered within the same clade as *A. tennesseensis* NRRL 13150 (ex-type strain) in the section *Versicolores* (Figure 1). The isolate CNUFC ESW1 was morphologically most similar to *A. tennesseensis* as described by Jurjevic et al. [50], although there were differences in the length and color of conidiophores. The conidiophores described by Jurjevic et al. [50] were (35–)100–300(–400) µm in length, while our isolates were 18.5–413.5 µm in length. The *Aspergillus* section *Versicolores* was first described by Thom and Church [51]. Members of this section are

Table 7. Morphological characteristics of CNUFC WD17-1 compared with those of the reference strain *Penicillium scabrosum*.

Character	CNUFC WD17-1	<i>Penicillium scabrosum</i> ^a
Conidiophores	Biverticillate, 2.9–4.5 μm	Biverticillate, 200–400 \times 3–4 μm
Metulae	2–4, 10.3–18.5 \times 2.3–4.1 μm	10–20 \times 2.5–4.0 μm
Phialides	Ampulliform, 4–8 per metula, 8.6–11.8 \times 2.1–3.1 μm .	5–12 per metula, 7–11 \times 2.0–2.5 μm
Conidia	Globose to subglobose, 2.3–3.2 μm	Globose to subglobose, 2.4–3.2 μm

^aFrom the description by Frisva et al. [67].

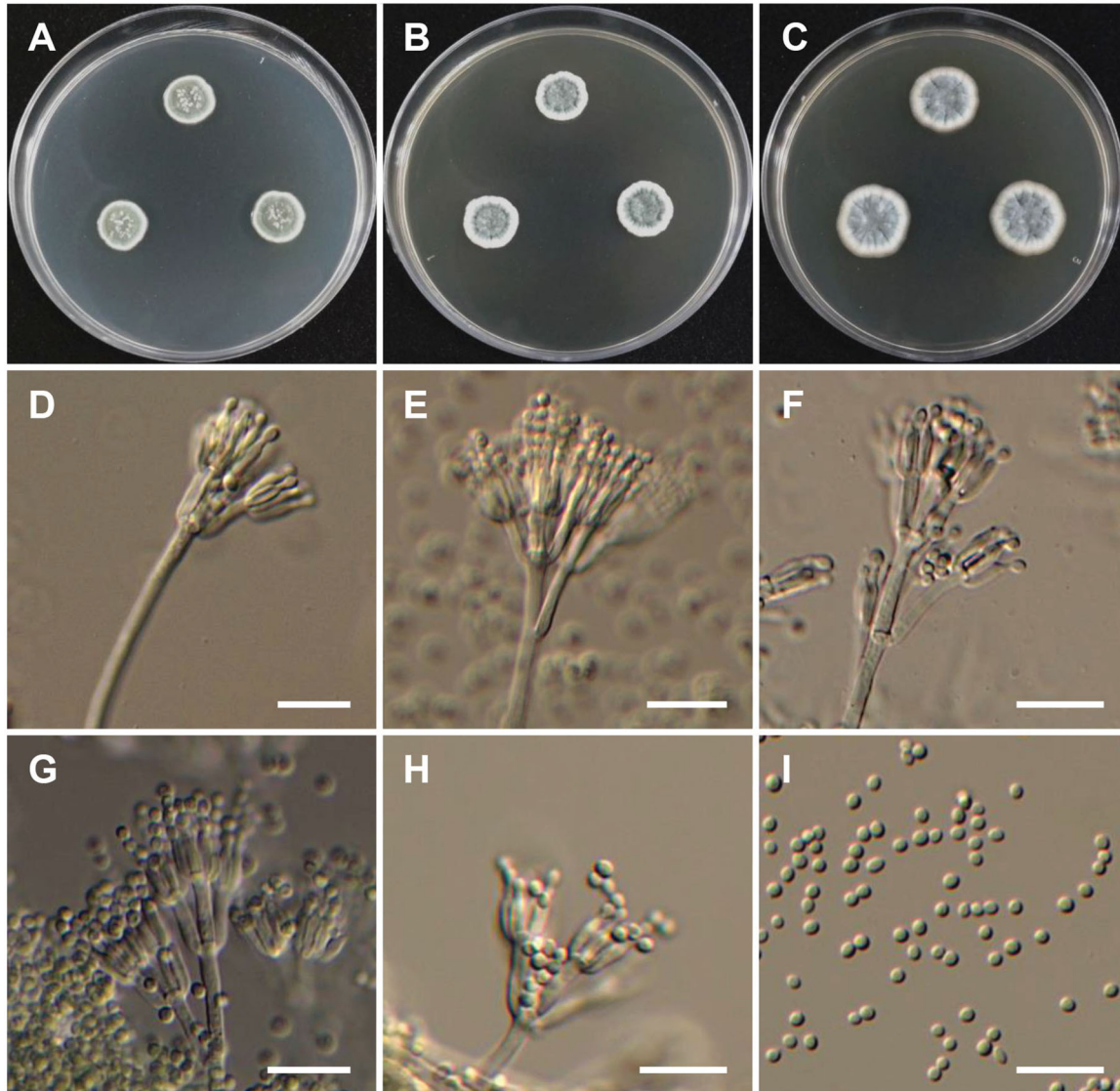


Figure 7. Morphology of *Penicillium scabrosum*. (A) Colonies on Czapek yeast autolysate agar (CYA); (B) Colonies on Blakeslee's malt extract agar (MEA); (C) Colonies on yeast malt extract agar (YES); (D–H) Conidiophores; (I) Conidia (scale bars: D–I = 20 μm).

found in soil, foods items [45], toxic dairy feed [50], and indoor environments [4,52,53], and can cause diseases in humans and animals [54,55]. Some species of this section produce kipukasins, nucleoside derivatives, and the mycotoxin, sterigmatocystin [56–58]. *A. tennesseensis* has been reported to produce various compounds such as versicoamides F–H, prenylated indole alkaloids, diorcinol L, and (*R*)-diorcinol B [59,60]. In Korea, there are only six species reported to belong to the section *Versicolores*, including *A. creber*, *A. jensenii*, *A. nidulans*, *A. sydowii*, *A. tabacinus*, and *A. versicolor*.

These were isolated from different sources including meju [48], chronic granulomatous patient [61], poultry farming soil [62], tidal mudflats, and sea sand [17]. In this study, *A. tennesseensis* was isolated from sea water and dead moths.

The strains CNUFC TM6-2 and CNUFC TM6-3 are well placed with other species in the *Penicillium* section *Cinnamopurpurea* as shown in Figure 2. The morphological features of our isolates were in line with the description of *P. fluviserpens* by Peterson et al. [63]. However, the isolate CNUFC TM6-2 exhibited a colony measurement which differed

from that of the description of *P. fluviserpens* on CYA (10–12 mm), MEA (8–11 mm), and PDA (10–12 mm) by Peterson et al. [63]. Members of this section are slow-growing, often with brown reverse on some media and mostly produce colonies with similar morphologies, subglobose to ellipsoidal, smooth to finely roughened spores, and have monoverticillate to divaricate biverticillate smooth-walled conidiophores. This section contains about 16 species [24,63,64]. Only two species, *P. chermesinum* (recently found to have phylogeny similar to *P. cvjetkovicii*) and *P. malacaense* were reported from meju samples in Korea with no detailed description [65]. Species in this section are known to produce the human lung tumor inhibitor compound, citreoviridin, and are commonly isolated from pecans, moldy nuts, air samples and hospital environments [63]. *P. fluviserpens* was previously isolated from air sampler from different locations, USA, California, Pennsylvania [63] and as endophytes from coffee plants in Colombia [66]. Interestingly, the present isolates in our study are from a tomato sample.

The strains CNUFC WD17-1 and CNUFC WD17-2 were grouped with *P. scabrosum* CBS 683.89 (ex-type strain) in the phylogenetic analysis of *BenA* and *CaM* sequences, and belong to the section *Ramosa* (Figure 2). There were differences observed with respect to the number of phialides per metula and colony diameter for the isolate *P. scabrosum* in comparison to previous descriptions by Frisvad et al. [67]. However, the size and shape of phialides and conidia were similar to those of the described species. *Penicillium* species in this section are characterized by biverticillate or terverticillate conidiophores [1]. They are commonly isolated from soil [67,68], but it is also found in sea water, fruiting bodies of *Chroogomphus rutilus*, and Tunisian orchard apples [69–71]. *P. scabrosum* has been reported to produce cyclopenin, cyclophenol, viridicatin, fumagillin, as well as a large number of unknown metabolites [67]. Larsen et al. [72] found two metabolites produced by *P. scabrosum* to be penigequinolone A and B. To the best of our knowledge, this is the first report of isolation of *P. scabrosum* from a freshwater sample.

Different species of the genus *Penicillium* have been reported to produce a variety of bioactive extrolites, including mycotoxins citrinin and patulin. Andersen and Frisvad [73] showed that *P. tularense* isolated from tomato fruit could produce janthitrems, paspalinine, paxilline, and 3-O-acetoxypaxilline. Harwig et al. [74] have reported that *P. expansum* is capable of producing patulin and citrinin in tomato fruit. Our strain *P. fluviserpens* was also isolated from tomato fruit. Therefore, it suggested that the strain may also produce mycotoxins

as well as secondary metabolites. Interestingly, in this study, strains of *A. tennesseensis* found on the moths may be a potential as new biopesticide. Isolation and descriptions of new record from specific substrates and habitats, like freshwater, sea water, and dead moths, will be added to our knowledge on fungal diversity. Further studies are needed to better understand the ecological roles of both *Aspergillus* and *Penicillium* on different substrates. More studies on extrolites production and their ecological roles, the production of extracellular enzymes and antimicrobial compounds are needed.

Disclosure statement

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

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