New Records of Four Species Belonging to Eurotiales from Soil and Freshwater in Korea

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ABSTRACT
Four strains of *Penicillium* and *Talaromyces* species are described and illustrated in an inventory of fungal species belonging to Eurotiales. The strains, CNUFC-DDS17-1, CNUFC-DDS27-1, CNUFC-PMT72-1, and CNUFC-YJW3-31, were isolated from soil and freshwater samples from South Korea. Based on their morphological characteristics and sequence analyses by the combined β-tubulin and calmodulin gene, the CNUFC-DDS17-1, CNUFC-DDS27-1, CNUFC-PMT72-1, and CNUFC-YJW3-31 isolates were identified as *Penicillium pasqualense*, *Penicillium sanguifluum*, *Talaromyces apiculatus*, and *Talaromyces liani*, respectively. The designated strains were found to represent a previously undescribed species of Korean fungal biota. In this study, detailed morphological descriptions and phylogenetic relationships of these species are provided.

1. Introduction
Soil and freshwater provide unique environments for the growth of fungi. Fungal communities perform essential functions in the biogeochemical cycles of these ecosystems. In soils, they act as agents governing soil carbon cycling, plant nutrition, and pathology [1]. Freshwater nourishes diverse habitats such as fallen leaves, plant litter, decaying wood, aquatic plants and insects, and soils for fungi. Fungi act in the energy flow, food-web, nutrient transformations, and self-purification in the freshwater ecosystem [2].

The genera *Penicillium* and *Talaromyces* (Trichocomaceae, Eurotiales), belonging to the Eurotiomycetes, are of high environmental and biotechnological relevance [3]. These genera are abundant in contaminated foods, fruits, indoor environments, air, soil, and water [4–6]. They also produce a variety of useful secondary metabolites including anticancer and antifungal compounds, extracellular enzymes, and harmful mycotoxins [7–9]. Previously, *Talaromyces* was thought to be related to teleomorphic *Penicillium* subgenus *Biverticillium* and other genera, but recently both have been classed as separate genera containing both sexual and asexual species with the introduction of single name nomenclature [10,11]. The species belonging to both these genera were identified on the recommendations of the polyphasic species concept, using morphological and molecular phylogenetic analyses [11,12].

The genus *Penicillium* was first described by Link and included asexual fungi bearing *Penicillium*-like fruiting bodies [13]. New species have since been added to the already 354 accepted *Penicillium* species [8,14,15]. Members of this genus were divided into 26 sections using the polyphasic approach [16]. To the best of our knowledge, over 100 *Penicillium* species have been reported from Korea [17,18]. Some of the new species discovered in Korea were isolated from various environmental samples such as *P. daejeonium* from grape and Schisandra fruit, *P. koreense* from soil, *P. samsonianum* from stems and leaves of *Viscum album* var. *coloratum*, *P. jejuense* from marine environments of Jeju Island, *P. punicae* from pomegranate (*Punica granatum*) fruit, *P. aquaticum* and *P. acidum* from freshwater [16,19–22]. Section *Citrina* represents one of these sections clade and are abundant worldwide [23]. Among the specified 39 species and 17 new species accepted in the section *Citrina*, only 11 species have been reported from Korea [24,25].

The genus *Talaromyces* was introduced by Benjamin for teleomorphic *Penicillium* species with *T. vermiculatus* as the type species [26]. These species were characterized taxonomically by their sexual morphology, having cleistothecial or gymnothecial ascomata, unitunicate 8-spored asci, and unicellular ascospores with or without equatorial crests.
Currently, the genus accepts 110 species which are divided into seven sections, i.e., *Bacillispori*, *Heici*, *Islandici*, *Purpurei*, *Subinflati*, *Talaromyces*, and *Trachyspermi* [6]. Recently, several new *Talaromyces* species were discovered from various environmental habitats [6,15,27,28]. To date, only 21 *Talaromyces* species have been reported from Korea [17,29–31]. Among the reported species, only six have been well described in Korea, these species are not reported before [30]. *Talaromyces apiculatus* and *Talaromyces liani* are classified in section *Talaromyces*. The diversity of the genus *Penicillium* and *Talaromyces* in Korea remains unknown using both molecular and morphological analyses.

During investigation of the fungal species inhabiting soil and freshwater, two *Penicillium* and two *Talaromyces* species were identified. The objective of this study was to perform morphological and molecular analyses to characterize the undescribed *Penicillium* and *Talaromyces* species in Korea:

### Table 1. Taxa, collection numbers, sequences, and GenBank accession numbers used in this study.

| Taxon name                        | Collection no. (isolate no.) | GenBank accession no. |
|-----------------------------------|-------------------------------|-----------------------|
| *Penicillium aurantiacobrunneum*  | CBS 126228 (T)                | JN606702 JN606522     |
| *P. cairnsense*                   | CBS 124325 (T)                | JN606693 JN606512     |
| *P. chroasii*                     | CBS 217.28 (T)                | JN606758 JN606423     |
| *P. citrinum*                     | CBS 139.45 (T)                | GU944545 GU944638     |
| *P. cosmopolitanum*               | CBS 126995 (T)                | JN606733 JN606472     |
| *P. decaturense*                  | CBS 117509 (T)                | JN606685 JN606413     |
| *P. godlewskii*                   | CBS 215.28 (T)                | JN606768 JN606443     |
| *P. mangelii*                     | CBS 233.31 (T)                | JN606651 JN606381     |
| *P. miczynskii*                   | CBS 220.28 (T)                | JN606706 JN606526     |
| *P. neomiczynskii*                | CBS 126231 (T)                | JN606705 JN606523     |
| *P. nothofagii*                   | CBS 130383 (T)                | JN606732 JN606507     |
| *P. panosorii*                    | CBS 276.75 (T)                | JN606790 JN606446     |
| *P. pasqualense*                  | CBS 123427                    | JN606672 JN606392     |
| *P. pasqualense*                  | CBS 122402                    | JN606674 JN606393     |
| *P. pasqualense*                  | CNUFC-DDS17-1                | MK204815 MK204817     |
| *P. pasqualense*                  | CNUFC-DDS17-2                | MK204816 MK204818     |
| *P. quebecense*                   | CBS 101623 (T)                | JN606700 JN606509     |
| *P. roseopurpureum*               | CBS 101623 (T)                | JN606700 JN606509     |
| *P. rugosasporum*                 | CBS 269.29 (T)                | JN606890 JN606556     |
| *P. sanguiiium*                   | CBS 127032 (T)                | JN606819 JN606555     |
| *P. sanguiiium*                   | CBS 118024                    | JN606833 JN606537     |
| *P. sanguineum*                   | CBS 643.73                    | JN606853 JN606576     |
| *P. sanguineum*                   | CNUFC-DDS27-1                | MK204819 MK204821     |
| *P. sanguineum*                   | CNUFC-DDS27-2                | MK204820 MK204822     |
| *P. szaroae*                      | CBS 413.69 (T)                | GU944535 GU944618     |
| *P. succirioum*                   | CBS 135116 (T)                | JX141015 JX141506     |
| *P. sumatrare*                    | CBS 281.36 (T)                | JN606639 JN606368     |
| *P. ubiquesporum*                 | CBS 120477 (T)                | JN606800 JN606469     |
| *P. waksmanii*                    | CBS 230.28 (T)                | JN606779 JN606431     |
| *P. westlingii*                   | CBS 231.28 (T)                | JN606718 JN606500     |
| *Talaromyces aculeatus*           | CBS 289.48 (T)                | KF741929 KF741975     |
| *T. apiculatus*                   | CBS 101366                    | KF741910 KF741932     |
| *T. apiculatus*                   | CBS 312.59 (T)                | KF741916 KF741950     |
| *T. apiculatus*                   | CNUFC-PTM72-1                | MK204823 MK204825     |
| *T. apiculatus*                   | CNUFC-PTM72-2                | MK204824 MK204826     |
| *T. aurantacius*                  | CBS 314.59 (T)                | KF741917 KF741951     |
| *T. aurantiacus*                  | CBS 137102 (T)                | KF741922 KF741971     |
| *T. bacillisporus*                | CBS 296.48 (T)                | AY753368 KJ855262     |
| *T. dersii*                       | CBS 412.89 (T)                | JX94305 KF741959      |
| *T. duclauxii*                    | CBS 322.48 (T)                | JX091384 KF741955     |
| *T. flavoviiren*                  | CBS 102801 (T)                | JX091376 KF741933     |
| *T. francoae*                     | CBS 113344 (T)                | KX031489 KX031501     |
| *T. funiculosus*                  | CBS 272.86 (T)                | JX091383 KF741945     |
| *T. liani*                        | CBS 118434                    | KJ855262 KJ855262     |
| *T. liani*                        | CBS 225.66 (T)                | JX091380 KJ855262     |
| *T. liani*                        | CNUFC-YW3-31                 | MK204811 MK204813     |
| *T. liani*                        | CNUFC-YW3-32                 | MK204812 MK204814     |
| *T. kendrickii*                   | CBS 136666 (T)                | KF741921 KF741967     |
| *T. marneffei*                    | CBS 388.87 (T)                | JX091389 KF741958     |
| *T. purpureogenus*                | CBS 286.36 (T)                | JX315639 KF741947     |
| *T. stellenboschensis*            | CBS 135665 (T)                | JX091605 JX140683     |
| *T. stellii*                      | CBS 408.93 (T)                | JX315633 JX315646     |
| *T. verruculosus*                 | CBS 388.48 (T)                | KF741928 KF741974     |
| *T. viridis*                      | CBS 114.72 (T)                | JX94310 KF741935      |
| *T. viridulus*                    | CBS 252.87 (T)                | JX091385 KF741943     |

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

CBS: Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands); CNUFC: Chonnam National University Fungal Collection (Gwangju, South Korea); T: ex-type strain.
P. pasqualense, P. sanguifluum, T. apiculatus and T. liani belonging to the Eurotiales.

2. Materials and methods

2.1. Isolation of fungal strains from water and soil samples

Soil samples were collected from Dongdo (eastern islet) of Dokdo Island (37°14′21.3″ N, 131°52′04.4″ E) and under the rhizosphere of a pine tree located at Geumgol Mountain, Jin Island (Jindo). Freshwater samples were collected from Yeosu stream in a small falcon tube. Serial-dilution plating methods were employed using potato dextrose agar (PDA; Becton, Dickinson and Co., Sparks, MD) and malt extract agar (MEA; Becton). Pure isolates were obtained by picking individual colonies of varied morphologies and transferring them to PDA plates and subculturing until pure mycelia were obtained. All pure isolates were maintained in PDA slant tubes and in 20% glycerol at −80°C at the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, Korea.

P. pasqualense, P. sanguifluum, T. apiculatus, and T. liani strains isolated in our study were designated CNUFC-DDS17-1 and CNUFC-DDS17-2, CNUFC-DDS27-1 and CNUFC-DDS27-2, and CNUFC-PTM72-1 and CNUFC-PTM72-2, respectively. The isolates, CNUFC-DDS17-1, -DDS17-2, -DDS27-1, and -DDS27-2 were isolated from soil of Dokdo Island; CNUFC-PTM72-1 and -PTM72-1 from soil of Geumgol Mountain; CNUFC-YJW3-31 and -YJW3-32 from freshwater.

2.2. DNA extraction, PCR, and purification

Genomic DNA was extracted using the Solg TM Genomic DNA Prep Kit (Solgent Co. Ltd., Daejeon,
Korea). The internal transcribed spacer (ITS)-rDNA region, β-tubulin gene (BenA), and calmodulin (CaM) gene were amplified with the primer pairs ITS1/ITS4 [32], Bt2a/Bt2b [33], and Cmd5/Cmd6 [5], respectively. The PCR amplification reaction mixture was set up according to Nguyen et al. [30]. An Accuprep PCR Purification Kit (Bioneer Corp., Daejeon, Korea) was employed for PCR purification. DNA sequencing was performed using an ABI 3700 Automated DNA sequencer (Applied Biosystems Inc., Foster City, CA).

2.3. Molecular analysis

Fungal sequences were aligned using Clustal_X version 2.1 [34] and edited with Bioedit version 7.2.6.0 [35]. Maximum likelihood (ML) was constructed using MEGA 6 software [36]. Sequences of CNUFC-DDS17-1, CNUFC-DDS17-2, CNUFC-DDS27-1, CNUFC-DDS27-2, CNUFC-PTM72-1, CNUFC-PTM72-2, CNUFC-YJW3-31, and CNUFC-YJW3-32 were deposited in the NCBI database under the accession numbers shown in Table 1.

2.4. Morphological studies

The isolated strains CNUFC-DDS17-1, CNUFC-DDS27-1, CNUFC-PTM72-1, and CNUFC-YJW3-31 were cultured on Czapek yeast autolysate agar (CYA), Blakeslee’s MEA, and yeast extract sucrose agar [5]. The plates were incubated at 25°C in the dark for one week. To observe fine fungal structures, the isolates were fixed in 2.5% paraformaldehyde-glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 2 h, and then washed with cacodylate buffer (Junsei Chemical Co. Ltd., Kyoto, Japan). Cellular membranes were preserved by fixing the samples in 2.5% paraformaldehyde-glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 2 h, and then washed with cacodylate buffer (pH 7.2) for 2 h, and then washed with cacodylate buffer (pH 7.2) for 2 h, and then washed with cacodylate buffer (pH 7.2) for 2 h, and then washed with cacodylate buffer (pH 7.2) for 2 h, and then washed with cacodylate buffer (pH 7.2) for 2 h, and then washed with cacodylate buffer (pH 7.2) for 2 h.
acetate (Junsei Chemical Co. Ltd.), and dried in a fume hood. Finally, samples were sputter-coated with gold and observed under a Hitachi S4700 field emission scanning electron microscope at the Korea Basic Science Institute, Gwangju, Korea. Samples were also observed under an Olympus BX51 microscope with DIC optics (Olympus, Tokyo, Japan) by mounting in a lactophenol solution (Junsei Chemical Co. Ltd.).

### 3. Results

#### 3.1. Molecular phylogenetic analysis

The results constructed by ML analyses based on combined BenA and CaM sequences analysis of the isolates respectively belonging to *Penicillium* and *Talaromyces* are shown in Figures 1 and 2. A BLASTn search was conducted using the ITS, BenA, and CaM sequences of CNUFC-DDS17-1, CNUFC-DDS27-1, CNUFC-PTM72-1, and CNUFC-YJW3-31 isolates. The ITS region of isolates CNUFC-DDS17-1, CNUFC-DDS27-1, CNUFC-PTM72-1, and CNUFC-YJW3-31 showed similarities of 99.3% (520/526 bp), 98.9% (348/353 bp), and 100% (537/537 bp) sequence similarities with *P. pasqualense* CBS 126330 (JN617676), *P. sanguifluum* CV1856 (JX140865), *T. apiculatus* CBS 101366 (KF741977), and *T. liani* CBS 225.66 (MH858781). BLASTn analysis of BenA of CNUFC-DDS17-1, CNUFC-DDS27-1, CNUFC-PTM72-1, and CNUFC-YJW3-31 showed similarities of 97.8% (402/411 bp), 100% (413/413 bp), 98.9% (373/377 bp), and 98.4% (358/364 bp), respectively, with *P. pasqualense* CBS122402 (JN686674), *P. sanguifluum* CBS 643.73 (JN606853), *T. apiculatus* CBS 312.59 (JX091378), and *T. liani* CBS 225.66 (JX091380). Similarly, BLASTn analysis of CaM of CNUFC-DDS17-1, CNUFC-DDS27-1, CNUFC-PTM72-1, and CNUFC-YJW3-31 showed similarities of 98.1% (418/426 bp), 99.8% (504/505 bp), 98.7% (441/447 bp), and 99.3% (466/449 bp) with *P. pasqualense* CBS 122402 (JN606393), *P. sanguifluum* CBS 118020 (JN606536), *T. apiculatus* CBS 101366 (KF741932), and *T. liani* CBS 225.56 (KJ885257), respectively.

In the combined BenA and CaM sequence tree, the isolates CNUFC-DDS17-1, CNUFC-DDS27-1, CNUFC-PTM72-1, and CNUFC-YJW3-31 were identical to *P. pasqualense*, *P. sanguifluum*, *T. apiculatus*, and *T. liani*, respectively (Figures 1 and 2).

#### 3.2. Taxonomy

### 3.2.1. Taxonomy of CNUFC-DDS17-1

*Penicillium pasqualense* Houbraken, Frisvad & Samson, Studies in Mycology 70: 108 (2011) (Table 2, Figure 3).

**Description:** Colonies on YES were dark beige with white mycelium, no soluble pigment, weak to moderate sporulation, and reached 20–26 mm in diameter after 7 d at 25 °C. Colonies on MEA were pale green, with good sporulation, and velvety to floccose texture. Colonies grew slow, reaching 25–35 mm in diameter after 7 d at 25 °C. Colonies were dark brown, with soluble pigment, entire margins, and floccose colony texture at the center on CYA, reaching 25–30 mm in diameter. There were no formations of asci or ascospores. The conidiophores were predominantly symmetrically biverticillate, triverticillate (few) with additional branches and divergent terminal verticals of metulae, measured 10–18.9 × 2.5–3.2 μm. Phialides were ampulliform and measured 5.9–10.2 × 2.5–3.5 μm in diameter. Conidia were dark green or dark-blue in color, globose to subglobose, and spinose and measured 2.3–3.1 μm in diameter.

### 3.2.2. Taxonomy of CNUFC-DDS27-1

*Penicillium sanguifluum* (Sopp) Biourge, La Cellule 33: 105 (1923) (Table 3, Figure 4).

≡*Citromyces sanguifluus* Sopp, Skrifter udgivne af Videnskabs-Selskabet i Christiania. Mathematisk-Naturvidenskabelig Klasse 11: 115 (1912).

**Description:** Colonies on YES were pale yellow with white mycelium, sparse sporulation, reaching 25–35 mm in diameter after 7 d at 25 °C. On MEA, colonies were muted greenish-blue at the center to white mycelium, colony texture floccose, sporulation sparse, and reaching 25–30 mm in diameter after

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**Table 2.** Morphological characteristics of CNUFC-DDS17-1 compared with the reference strain of *Penicillium pasqualense*.

| Character   | CNUFC-DDS17-1                                      | *P. pasqualense*   |
|-------------|----------------------------------------------------|--------------------|
| Conidiophores | Biverticillate, triverticillate conidiophores, measured 100–300 μm long, 4.5–5 μm wide | Predominantly symmetrically biverticillate, 200–400 μm, smooth, 2.5–3.0 μm wide |
| Metulae     | Few metulae, 10–18.9 × 2.3–3.2 μm                   | Metulae in a divergent vertical terminal, 11–17 × 2.5–3.5 μm, branches longer up to 25 μm |
| Phialides   | Ampulliform, 5.9–10.2 × 2.5–3.5 μm                   | Ampulliform, 7.5–10 × 2.5–3.5 μm |
| Conidia     | Globose to subglobose, 2.3–3.1 μm in diameter       | Globose to subglobose, spinose, 2.5–3.5 μm in diameter |
| Sclerotia   | Absent                                             | Orange or brown    |

*From the description by Houbraken et al. [24].
7 d at 25°C. Colonies on CYA grew slow, reaching 25–30 mm in diameter at 25°C, were pale beige to grey green at the center, had absent to moderate sporulation, and an entire or irregular margin. Sclerotia absent. Conidiophores were monovercillate with short stipes and measured to be 17–50 μm. Phialides were ampulliform and measured 5.5–8.3 × 2.0–3.0 μm. Conidia were grey-green in color, globose to subglobose and measured 2.0–2.5 μm in diameter.

3.2.3. Taxonomy of CNUFC-PTM72-1

*Talaromyces apiculatus* R.A. Samson, N. Yilmaz & J.C. Frisvad, Studies in Mycology 70: 174 (2011) (*Table 4, Figure 5*).

≡*Penicillium aculeatum* var. *apiculatum* Abe, S., J. Gen. Appl. Microbiol., Tokyo 2: 124 (1956).

**Description:** Colonies on CYA were moderately deep, had radial, low margins, white mycelia, floccose texture, absent to sparse sporulation, no soluble
pigment, and reached 22–23 mm in diameter after 7 d at 25°C. On MEA, colonies were moderately deep, slightly raised at the center, had white mycelia, floccose texture, no soluble pigment, their reverse was brownish orange, and they reached 24–25 mm in diameter. On YES, colonies were moderately deep, low margins, white mycelia, no sporulation, no soluble pigment, and reaching 30–32 mm in diameter. Conidiophores were monoverticillate and measured to be 61–141 × 2.3–3.5 μm. Phialides were flask-shaped and measured 7.5–10 × 2.3–3.6 μm. Conidia were globose with rough echinulate walls and measured 3.3–4.6 × 3.3–4.3 μm in diameter.

3.2.4. Taxonomy of CNUFC-YJW3-31

_Talaromyces liani_ (Kamyschko) N. Yilmaz, J.C. Frisvad & R.A. Samson, Studies in Mycology 78: 266 (2014) (Table 5, Figure 6).
Colonies were light yellow, with sparse sporulation, no soluble pigment, and their reverse was pastel yellow on CYA after 7 d at 25°C. On MEA, colonies formed abundant yellow ascomata, had sparse sporulation, no soluble pigments, and their reverse was greyish yellow. Colonies were deep yellow on their reverse, had poor sporulation, white mycelium, and no soluble pigments on YES after 7 d at 25°C. Conidiophores were monoverticillate and biverticillate, 2.0–4.5 μm wide. Phialides were acerose, three to six per metula, and measured 10–17.5 × 2–3.5 μm. Conidia were globose to ellipsoidal and measured 2.5–4.0 × 2–3.5 μm.

**Table 5.** Morphological characteristics of CNUFC-YJW3-31 compared with reference strain of Talaromyces liani.

| Character       | CNUFC-YJW3-31                                      | Talaromyces liani* |
|-----------------|----------------------------------------------------|--------------------|
| Conidiophores   | Monoverticillate and biverticillate                 | Monoverticillate and biverticillate |
| Phialides       | Acerose, three to six per metula, 10–17.5 × 2–3.5 μm | Acerose, three to six per metulae, 9–20 × 2–3.5 μm |
| Conidia         | Globose to ellipsoidal, 2.5–4.0 × 2–3.5 μm.         | Ellipsoidal, 2.5–4–4.5) × 2–3.5 μm. |
| Ascomata        | Yellow, globose to subglobose, 110–429.5 × 109–428.5 μm | Yellow to orange red, globose to subglobose, 150–550 × 150–545 μm. |
| Asci            | Globose to subglobose or slightly ellipsoidal, 9.5–11.5 × 8.0–10.0 μm | 9–13 × 7.5–11 μm |
| Ascospores      | Ellipsoidal, spiny, 3.5–5.5 × 3.0–4.5 μm            | Ellipsoidal, spiny, 4–6 × 2.5–4 μm |

*From the description by Yilmaz et al. [11].

**Penicillium liani** Kamyschko, Not. Syst. Crypt. Inst. Bot. Acad. Sci. USSR 86: 115 (1962).

**Description:** Colonies were light yellow, with sparse sporulation, no soluble pigment, and their reverse was pastel yellow on CYA after 7 d at 25°C. On MEA, colonies formed abundant yellow ascomata, had sparse sporulation, no soluble pigments, and their reverse was greyish yellow. Colonies were deep yellow on their reverse, had poor sporulation, white mycelium, and no soluble pigments on YES after 7 d at 25°C. Conidiophores were monoverticillate and biverticillate, 2.0–4.5 μm wide. Phialides were acerose, three to six per metula, and measured 10–17.5 × 2–3.5 μm. Conidia were globose to ellipsoidal and measured 2.5–4.0 × 2–3.5 μm. Ascomata occurred within 7 d on MEA at 25°C, were yellow in color and from globose to subglobose shape, and measured 110–429.5 × 109–428.5 μm. Asci were
globose to subglobose or slightly ellipsoidal and measured 9.5–11.5 × 8.0–10.0 μm. Ascospores were ellipsoidal, spiny, and measured 3.5–5.5 × 3.0–4.5 μm.

4. Discussion

The use of molecular study based on DNA sequences has dramatically increased the identification of fungal species [37]. DNA sequences, especially those of ITS-5.8S rDNA, have become important features for the rapid identification of fungi [38]. Although ITS sequences are well-accepted barcodes and place *Penicillium* species into their respective sections, more than 50% of the species could be erroneously linked as many species share the same ITS sequences. This led to the use of secondary markers, such as BenA and CaM, for the correct identification of *Penicillium* species in section *Citrina*. These markers act as a hub covering larger databases for the diverse genus *Penicillium*. Similarly, phylogenetic analyses of BenA genes are imperative for identification of *Talaromyces* species [11]. Here, we discuss the phylogeny and morphological characteristics of *Penicillium* and *Talaromyces* species and compare them to the most closely related species.

There were differences observed in the colony diameter for *P. pasqualense* and *P. sanguifluum* in comparison to previous descriptions. Colony diameter on MEA and YES media was shown to be different from the previously described *P. pasqualense* species (YES: 25–35 mm; MEA: 25–30) and (YES: 40–45 mm; MEA: 30–40) [8,24]. However, cultures on the other media were similar to the described species. In comparison to *P. sanguifluum* described by Visagie et al. [8], colony growth was shown to be restricted on CYA to 15–26 (vs. 7–16) mm at 25°C. Also, no sclerotia production was observed for *P. pasqualense* [8,24]. These differences could be
attributed to different soil conditions, seasons of sample collection, or different climate conditions. Another reason could be that the present isolated strain is a sub-cultured strain, because most species produce sclerotia in their first isolated strains [24].

Species of section Citrina have a cosmopolitan distribution and are commonly isolated from soil, but are also isolated from foods, leaf litter, and indoor air. Previously P. pasqualense and P. sanguifluum were reported to be isolated from various habitats; soils, indoor air environments, ants, and chestnuts [8,24]. Members belonging to Penicillium section Citrina are valuable owing to their ability to produce a variety of metabolites including citrinins and citreoviridins [23]. Many specific extrolites were extracted and identified from P. pasqualense and P. sanguifluum such as pyrenocines, indol alkaloids, PAS, bisanthenrs, roseopurpurin, β-hydroxycurvularin, dehydrocurvularin, curvularin, FOSI, FYKS, SNIT, TIDL, and VERN [24]. The members of section Citrina can grow at temperatures ranging from 23 to 26°C [24]. In this study, the two isolates had slow growth at lower temperatures (5–10°C) and optimum growth at 25–30°C, whereas growth was restricted at higher temperatures 35–37°C for P. pasqualense and P. sanguifluum.

In comparison to T. apiculatus [11], the present isolate CNUFC-PTM72-1 shows similar morphological characteristics with small length conidio- phores. The morphological characteristics of the T. liani isolate studied were generally similar to those previously described by Yilmaz et al. [11]. However, our observations showed that the size of asc and ascomata of isolate CNUFC-YJW3-31 were smaller than previously reported. Sexual reproduction structures were only observed on MEA. There were previously only reports of T. apiculatus and T. liani from soil samples [11,39]. This is the first isolation of T. liani from freshwater. T. apiculatus produces various bioactive extrolites such as macrocyclic polyla- lactones (apiculides) NG-011, NG-012 (potentiators of nerve growth factors); BK223-A (=NG-012), BK-223B, BK-223C, 15G256B, and 15G256a-2 (act as antifungal) and bioxanthracene B, whereas no available information on the metabolites produced by T. liani yet [11].

Four species were identified, namely P. pasqua- lense, P. sanguifluum, T. apiculatus, and T. liani, successfully combining molecular and morphological analyses in this study. The main focus was to describe the diversity of Penicillium and Talaromyces species from soil and freshwater in Korea. The soil ecosystem has a tremendous impact on environmental sustainability in forestry, agriculture, and horticulture. Therefore, it is necessary to identify fungal species from soil and to characterize their role in the environment. Thus, our future studies would include studying the species ecological roles, such as the production of extracellular enzyme activity, antibacterial and antifungal activities, as well as the production of secondary metabolites.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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