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RESEARCH ARTICLE

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Penicillium from Rhizosphere Soil in Terrestrial and Coastal Environments in South Korea

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ABSTRACT

Penicillium, the most common genus plays an important ecological role in various terrestrial and marine environments. However, only a few species have been reported from rhizosphere soil. As part of a project to excavate Korean indigenous fungi, we investigated rhizosphere soil of six plants in the forest (terrestrial habitat) and sand dunes (coastal habitat) and focused on discovering *Penicillium* species. A total of 64 strains were isolated and identified as 26 *Penicillium* species in nine sections based on morphological characteristics and the sequence analysis of β-tubulin and calmodulin. Although this is a small-scale study in a limited rhizosphere soil, eight unrecorded species and four potential new species have been identified. In addition, most *Penicillium* species from rhizosphere soil were unique to each plant. *Penicillium halotolerans*, *P. scabrosum*, *P. samsonianum*, *P. jejuense*, and *P. janczewskii* were commonly isolated from rhizosphere soil. Eight *Penicillium* species, *P. aurantioviolaceum, P. bissettii, P. cairnsense, P. halotolerans, P. kananaskense, P. ortum, P. radiatolobatum*, and *P. verhagenii* were recorded for the first time in Korea. Here, we provide the detailed morphological description of these unrecorded species.

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BenA; *CaM*; morphology; new records; phylogeny

1. Introduction

Fungi play an important role in rhizosphere soil. They affect plant fitness and adaptability in ecosystems through various biological processes [1,2]. The genus *Penicillium* is one of the most common fungi found in rhizosphere soil [3]. Some *Penicillium* species produce solubilized phosphorus, siderophore, and phytohormones such as indole acetic acid and gibberellic acid, which are important for plant health [4–6]. Although the importance of *Penicillium* isolated in rhizosphere soil has been reported, many *Penicillium* species were only identified at the genus level [7,8]. There are two possible reasons for this; one being that studies conducted previously have focused on simply screening for useful enzymes and bioactive compounds, and two that it is naturally difficult to identify *Penicillium* species.

The members of the *Penicillium* genus are easily identified at the genus level based on their microscopic features in culture condition, whereas they are difficult to identify at the species level due to their morphological plasticity under different growth conditions [9]. Recently, the identification of *Penicillium* species has become much easier since standardized methods including morphology and multigene phylogenetic analysis have been proposed [9]. To date, *Penicillium* has been reported in 25 sections of 345 species worldwide through the use of standardized methods [9]. In Korea, approximately 102 *Penicillium* species from terrestrial environments [10,11] and 100 *Pencillium* species from marine environments [12–17] have been reported. It was confirmed that many *Pencillium* species from marine environments are different from those found in terrestrial environments. So far, a total of 152 *Penicillium* species have been reported in Korea. However, there are only a few studies on *Penicillium* from rhizosphere soil [18,19], and since rhizosphere soil is unique for each plant species, it can be assumed that different *Penicillium* species may exist for each plant.

As part of the projects organized by the National Institute of Biological Resources (NIBR) and the Ministry of Ocean and Fisheries established the Marine Fungal Resource Bank (MFRB) to excavate Korean indigenous fungi, we investigated rhizosphere soil to discover *Penicillium* species. The main objective of this study was to isolate *Penicillium*

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Supplemental data for this article can be accessed here.

species from rhizosphere soil of six plants: *Rhododendron brachycarpum*, *Sorbus commixta,* and *Taxus cuspidate* from a forest (terrestrial habitat) and *Calystegia soldanella, Lathyrus japonicus,* and *Orobanche coerulescens* from a sand dune (coastal habitat). All *Penicillium* isolates were identified at the species level using the sequence analysis of the *β*-tubulin (*BenA*) and calmodulin (*CaM*) loci. We identified eight unrecorded species and four new species candidates of *Penicillium* in Korea.

2. Materials and methods

2.1. Sample collections and isolation

Rhizosphere soil from six plants were collected from six sites in Korea in 2019 (Figure 1, Table 1). *Rhododendron brachycarpum*, *Sorbus commixta,* and *Taxus cuspidate* were collected from a forest (terrestrial habitat) and *Calystegia soldanella, Lathyrus japonicus,* and *Orobanche coerulescens* were collected from a sand dune (coastal habitat). All samples were stored at 4 °C before fungal isolation. For each sample, 5 g of rhizosphere soil was diluted ten-fold with sterile water. Next, 100 μL of each dilution was plated on dichloran rose bengal chloramphenicol agar (Difco, Becton Dickinson, Sparks, MD). All plates were incubated at 25 °C for seven days. *Penicillium* strains were transferred to a potato dextrose agar (PDA; Difco, Becton Dickinson) plate. Each strain was then stored in 20% glycerol at −80 °C at the Seoul National University Fungus Collection (SFC) (Table 1).

2.2. DNA extraction, amplification, and sequencing

Genomic DNA was extracted from *Penicillium* using a modified cetyltrimethylammonium bromide extraction protocol [20] with respect to the amount of sample tissue. The primer sets, Bt2a/Bt2b [21] and CF1/CF4 [22] were used to amplify *BenA* and *CaM*, respectively. Each PCR was performed in a C1000 thermal cycler (Bio-Rad, Richmond, CA) using previously described methods [14]. The PCR products were purified with a Expin^{TM} PCR Purification Kit (GeneAll Biotechnology, Seoul, Korea), according to the manufacturer's instructions. DNA sequencing was performed on an ABI Prism 3700 genetic analyzer (Life Technologies, Gaithersburg, MD) at Macrogen (Seoul, Korea), in both directions using the PCR primers.

2.3. Phylogenetic analysis

The sequences were assembled and proofread using MEGA5 [23] and were deposited in GenBank (accession Nos. are shown in Table 1). Molecular identification was performed in two steps. First, the sectional position of the strains was determined by comparison to the *BenA* sequences with database containing the sequence of the type strains. Next,

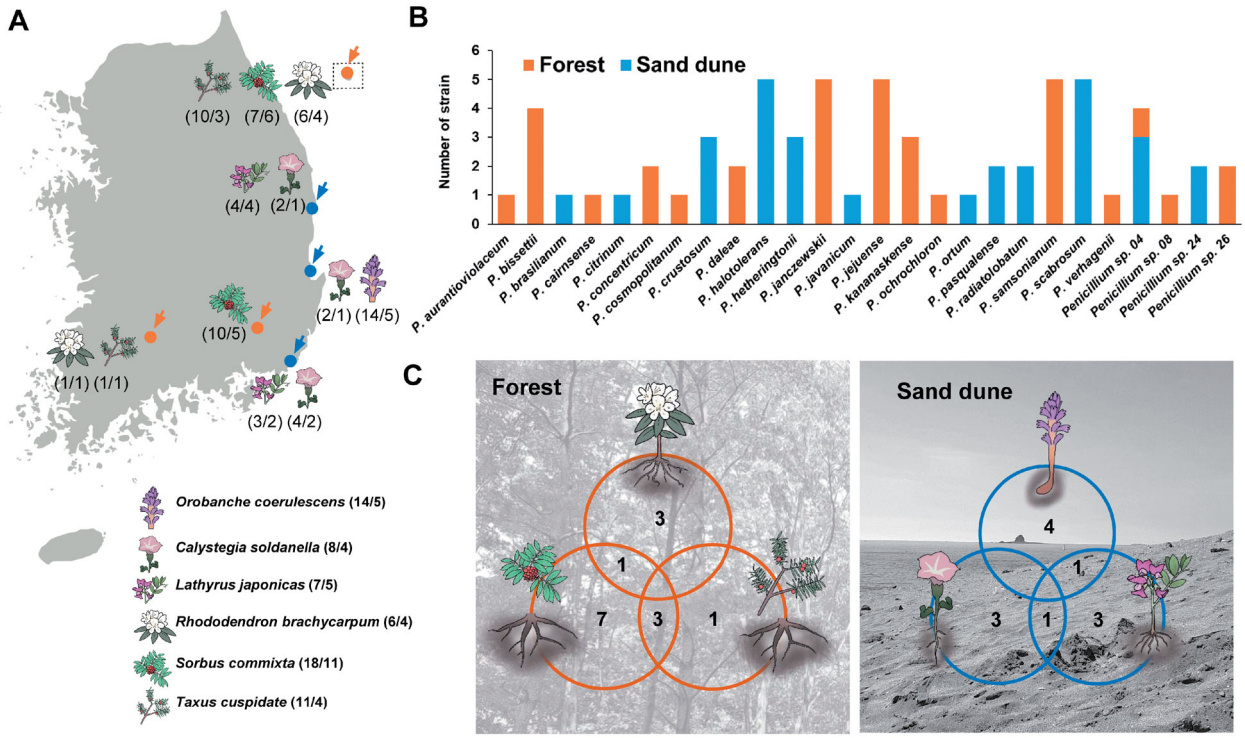

Figure 1. The number of *Penicillium* species from each sampling sites. (A) Map showing the number of (strains/species) at each location. (B) Total, unique, and shared *Penicillium* species from forest and sand dune. (C) Total, unique, and shared *Penicillium* species obtained from rhizosphere soil of six different plants.

Table 1. Summary and GenBank accession numbers for *Penicillium* strains isolated from the rhizosphere soil of six different plants. The unrecorded *Penicillium* species in Korea are represented in bold.

Species	Section	Strain	Location	Substrate	GenBank accession numbers	
					BenA	*CaM*
P. aurantioviolaceum	*Aspergilloides*	SFCP0220, ZRSGFG0000000005	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Sorbus commixta*	MT843816	MT843889
P. bissettii	*Lanata-Divaricata*	SFCP0218	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Sorbus commixta*	MT843814	MT843874
		SFCP0219	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Sorbus commixta*	MT843815	MT843875
		SFCP0229, ZRSGFG0000000006	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Rhododendron brachycarpum*	MT843824	MT843881
		SFCP0529	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Rhododendron brachycarpum*	MT843838	MT843888
P. brasilianum	*Lanata-Divaricata*	SFCP0198	Yongho-dong, Nam-gu, Busan	*Lathyrus japonicus*	MT843805	MT843866
P. cairnsense	*Citrina*	SFCP0168, NIBRFG0000506897	Sannae-myeon, Miryang-si, Gyeongsangnam-do	*Sorbus commixta*	MT843776	MT843845
P. citrinum	*Citrina*	SFCP0169	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843777	MT843846
P. concentricum	*Penicillium.*	SFCP0170	Sannae-myeon, Miryang-si, Gyeongsangnam-do	*Sorbus commixta*	MT843778	MT843847
		SFCP0231	Hwagae-myeon, Hadong-gun, Gyeongsangnam-do	*Sorbus commixta*	MT843826	
P. cosmopolitanum	*Citrina*	SFCP0236	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Rhododendron brachycarpum*	MT843831	MT843885
P. crustosum	*Fasciculata*	SFCP0215	Yongho-dong, Nam-gu, Busan	*Calystegia soldanella*	MT843811	MT843871
		SFCP0525	Yongho-dong, Nam-gu, Busan	*Calystegia soldanella*	MT843828	
		SFCP0233	Yongho-dong, Nam-gu, Busan	*Calystegia soldanella*	MT843834	
P. daleae	*Lanata-Divaricata*	SFCP0225	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Taxus cuspidata*	MT843821	MT843880
		SFCP0226	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Taxus cuspidata*	MT843822	
P. halotolerans	*Chrysogena*	SFCP0171, NIBRFG0000506904	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843779	MT843848
		SFCP0172	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843780	MT843849
		SFCP0173	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843781	MT843850
		SFCP0174	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843782	MT843851
		SFCP0175	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843783	MT843852
P. hetheringtonii	*Citrina*	SFCP0998	Yongho-dong, Nam-gu, Busan	*Lathyrus japonicus*	MT843804	MT843865
		SFCP0197	Yongho-dong, Nam-gu, Busan	*Lathyrus japonicus*	MT843807	
		SFCP0200	Jukbyeon-myeon, Uljin-gun, Gyeongsangbuk-do	*Lathyrus japonicus*	MT843839	
P. janczewskii	*Canescentia*	SFCP0221	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Sorbus commixta*	MT843817	MT843876
		SFCP0223	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Taxus cuspidata*	MT843819	MT843878
		SFCP0224	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Taxus cuspidata*	MT843820	MT843879
		SFCP0526	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Taxus cuspidata*	MT843830	MT843884
		SFCP0235	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Taxus cuspidata*	MT843835	
P. javanicum	*Lanata-Divaricata*	SFCP0214	Yongho-dong, Nam-gu, Busan	*Calystegia soldanella*	MT843810	MT843870
P. jejuense	*Aspergilloides*	SFCP0222	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Sorbus commixta*	MT843818	MT843877
		SFCP0227	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Taxus cuspidata*	MT843823	
		SFCP0234	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Taxus cuspidata*	MT843829	
		SFCP0527	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Taxus cuspidata*	MT843836	
		SFCP0528	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Taxus cuspidata*	MT843837	
P. kananaskense	*Aspergilloides*	SFCP0176	Sannae-myeon, Miryang-si, Gyeongsangnam-do	*Sorbus commixta*	MT843784	MT843853
		SFCP0177	Sannae-myeon, Miryang-si, Gyeongsangnam-do	*Sorbus commixta*	MT843785	MT843854
		SFCP0232, NIBRFG0000506913	Hwagae-myeon, Hadong-gun, Gyeongsangnam-do	*Taxus cuspidate*	MT843827	MT843883
P. ochrochloron	*Lanata-Divaricata*	SFCP0216	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Sorbus commixta*	MT843812	MT843872
P. ortum	*Lanata-Divaricata*	SFCP0199, NIBRFG0000506915	Jukbyeon-myeon, Uljin-gun, Gyeongsangbuk-do	*Lathyrus japonicus*	MT843806	MT843867
P. pasqualense	*Citrina*	SFCP0213	Jukbyeon-myeon, Uljin-gun, Gyeongsangbuk-do	*Lathyrus japonicus*	MT843786	MT843855
		SFCP0178	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843809	MT843869
P. radiatolobatum	*Canescentia*	SFCP0182, NIBRFG0000506918	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843789	MT843858
		SFCP0183	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843790	MT843859
P. samsonianum	*Osmophila*	SFCP0184	Sannae-myeon, Miryang-si, Gyeongsangnam-do	*Sorbus commixta*	MT843791	MT843860
		SFCP0185	Sannae-myeon, Miryang-si, Gyeongsangnam-do	*Sorbus commixta*	MT843792	

(*continued*)

Table 1. Continued.

Species	Section	Strain	Location	Substrate	GenBank accession numbers	
					BenA	*CaM*
		SFCP0186	Sannae-myeon, Miryang-si, Gyeongsangnam-do	*Sorbus commixta*	MT843793	
		SFCP0187	Sannae-myeon, Miryang-si, Gyeongsangnam-do	*Sorbus commixta*	MT843794	
		SFCP0188	Sannae-myeon, Miryang-si, Gyeongsangnam-do	*Sorbus commixta*	MT843795	
P. scabrosum	*Ramosa*	SFCP0189	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843796	MT843861
		SFCP0190	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843797	
		SFCP0191	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843798	
		SFCP0192	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843799	
		SFCP0193	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Orobanche coerulescens*	MT843800	
P. verhagenii	*Aspergilloides*	SFCP0194, ZRSGFG0000000007	Sannae-myeon, Miryang-si, Gyeongsangnam-do	*Sorbus commixta*	MT843801	MT843862
Penicillium sp. 04	*Canescentia*	SFCP0195	Jukbyeon-myeon, Uljin-gun, Gyeongsangbuk-do	*Calystegia soldanella*	MT843802	MT843863
		SFCP0196	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Rhododendron brachycarpum*	MT843803	MT843864
		SFCP0201	Jukbyeon-myeon, Uljin-gun, Gyeongsangbuk-do	*Lathyrus japonicus*	MT843808	MT843868
		SFCP0523	Jukbyeon-myeon, Uljin-gun, Gyeongsangbuk-do	*Calystegia soldanella*	MT843833	MT843887
Penicillium sp. 08	*Lanata-Divaricata*	SFCP0217	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Sorbus commixta*	MT843813	MT843873
Penicillium sp. 24	*Chrysogena*	SFCP0179	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Calystegia soldanella*	MT843787	MT843856
		SFCP0180	Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do	*Calystegia soldanella*	MT843788	MT843857
Penicillium sp. 26	*Citrina*	SFCP0230	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Rhododendron brachycarpum*	MT843825	MT843882
		SFCP0237	Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	*Rhododendron brachycarpum*	MT843832	MT843886

each strain was identified to the species level by analyzing the combined dataset of the two loci (*BenA* and *CaM*). *Talaromyces flavus* NRRL 2098 was used as the outgroup [24]. Multiple alignments were performed using the L-INS-i option of MAFFT v7 [25]. Maximum likelihood phylogenetic analyses were performed with RAxML [26] implemented on CIPRES web portal [27], using the GTR + G model with 1000 bootstrap replicates.

2.4. Morphological analysis

The morphology of eight unrecorded species was observed using previously described methods [9] on three different culture media: Czapek yeast autolysate agar (CYA; Difco, Sparks, MD), malt extract agar (MEA; Oxoid, Hampshire, UK), and yeast extract sucrose agar (YES; Difco). The Methuen Handbook of Color was used for the color names and alpha-numeric codes for macromorphological characteristics [28]. The microscopic features were observed under a light microscope (Eclipse 80i, Nikon, Tokyo, Japan) using colonies grown on MEA.

3. Results

3.1. Species identification

A total of 64 *Penicillium* strains were isolated from rhizosphere soil of six plants. They were clustered in 26 groups based on their *BenA* sequences (Supplemental Figure 1). For identification at the species level, one to six representative strains from each group were used for a phylogenetic analysis using the combined dataset from *BenA* and *CaM*. Finally, these were confirmed as 26 species in nine sections including eight unrecorded species and four potential new species (Table 1, Figure 2).

Section *Lanata-Divaricata* (7 *Penicillium* spp.) and *Citrina* (6) showed a relatively higher diversity compared to other sections (Table 1). Eight unrecorded species were detected in five sections. These were *Aspergilloides* (3 spp.), *Canescentia* (1 sp.), *Chrysogena* (1 sp.), *Citrina* (1 sp.), and *Lanata-Divaricata* (2 spp.). Four potential new species were identified in section *Canescentia*, *Chrysogena*, *Citrina*, and *Lanata-Divaricata* (Table 1). The detailed morphological descriptions for unrecorded species are presented in taxonomic part.

For section *Aspergilloides*, 10 strains were identified as *P. aurantioviolaceum* (1 strain), *P. jejuense* (5), *P. kananaskense* (3), and *P. verhagenii* (1). Three species (*P. aurantioviolaceum, P. kananaskense,* and *P. verhagenii*) were confirmed as unrecorded species in Korea. For section *Canescentia*, 11 strains were confirmed as *P. janczewskii* (5), *P. radiatolobatum* (2), and *Penicillium* sp. 04 (4). *P.*

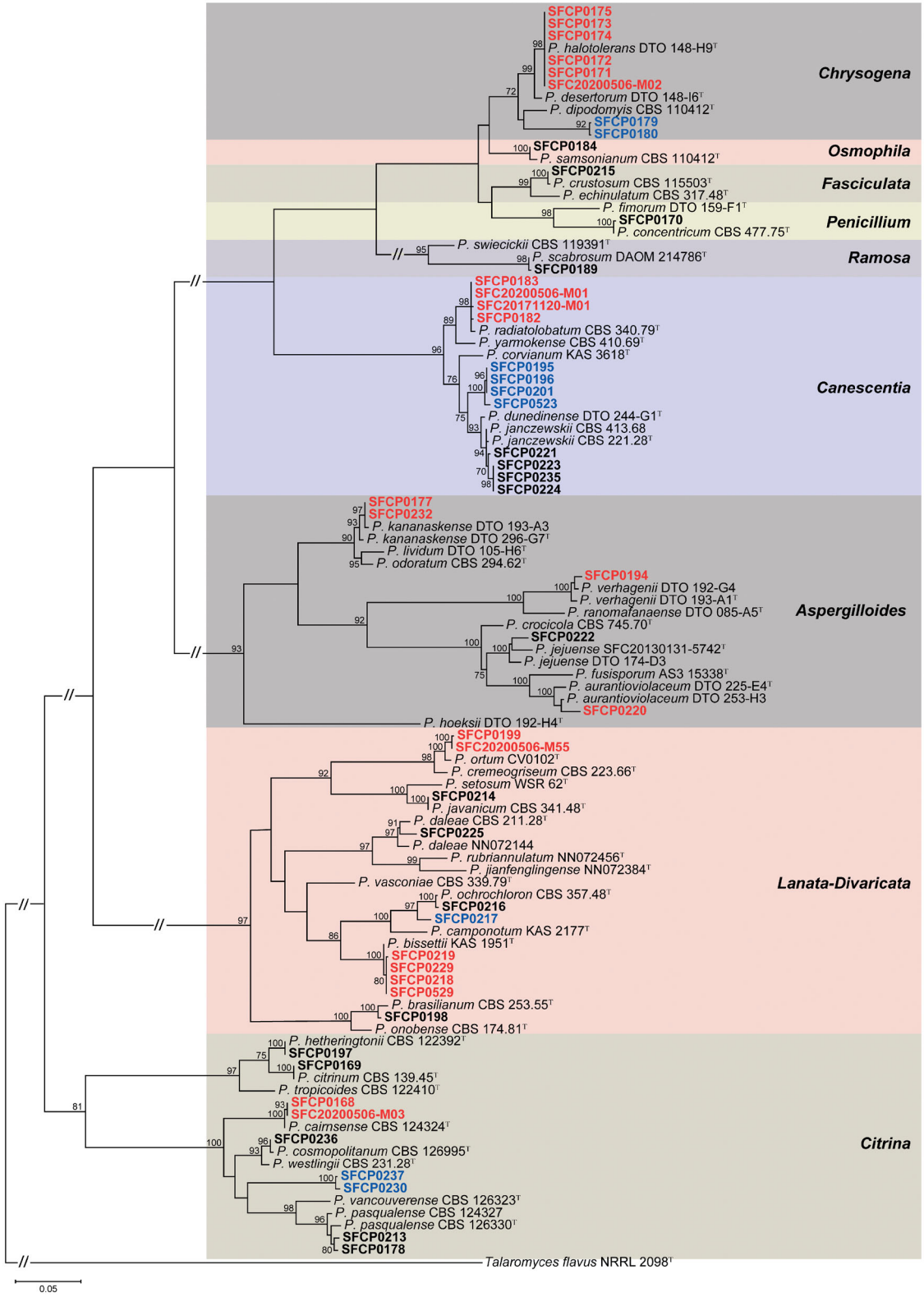

Figure 2. Maximum likelihood phylogenetic tree of the combined data set of *BenA* and *CaM* used to identify strains to the species level in *Penicillium* from rhizosphere soil. Bootstrap scores of >70 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site. "T" indicates the ex-type strains. Strains reported in the current study are represented in bold. The species labeled in red represent previously unrecorded species in Korea. The names in blue are potential new species.

radiatolobatum was an unrecorded species in Korea. *Penicillium* sp. 04 (SFCP0195, SFCP0196, SFCP0201, and SFCP523) formed a distinct clade from previously reported species. For section *Chrysogena*, seven strains were determined as *P. halotolerans* (5) and *Penicillium* sp. 24 (2). *P. halotolerans* was an unrecorded species in Korea. *Penicillium* sp. 24 was a potential new species. For section *Citrina*, 10 strains were identifed as *P. cairnsense* (1), *P. citrinum* (1), *P. cosmopolitanum* (1), *P. hetheringtonii* (3), *P. pasqualense* (2), and *Penicillium* sp. 26 (2). *P. cairnsense* was unrecorded species in Korea and

Penicillium sp. 26 was a potential new species. Section *Lanata-Divaricata* contained seven species (11 strains); *P. bissettii* (4), *P. brasilianum* (1), *P. daleae* (2), *P. javanicum* (1), *P. ochrochloron* (1), *P. ortum* (1), and *Penicillium* sp. 08 (1). Two species (*P. bissettii* and *P. ortum*) were new records to Korea. *Penicillium* sp. 08 (SFCP0217) was a potential new species. *P. crustosum* (3), *P. samsonianum* (5), *P. concentricum* (2), and *P. scabrosum* (5) included in Section *Fasciculata*, *Osmophila*, *Penicillium*, and *Ramosa* were identified from rhizosphere soil, respectively (Table 1, Figure 2).

3.2. Penicillium composition

Penicillium halotolerans, *P. scabrosum*, *P. samsonianum*, *P. jejuense*, and *P. janczewskii* were the dominant species in rhizosphere soil (Table 1). A different number of species were found in the forest (15 spp.) and sand dune (12 spp.). *Penicillium* sp. 04 was the only species shared across both habitats. Most species were detected in their own sites: forest (14 species) and sand dune (11 species) (Figure 1(B)).

The different numbers (4–11 *Penicillium* spp.) of *Penicillium* species were found depending on the plant surveyed. *Penicillium* diversity was the highest in *Sorbus commixta* (11 spp.), followed by *Orobanche coerulescens* (5) and *Lathyrus japonicus* (5) (Figure 1(C)). There were only a few *Penicillium* species commonly found between the plant hosts on the sand dune, whereas more species were found to overlap in the forest (Figure 1(C)).

4. Taxonomy

***Penicillium aurantioviolaceum* Biourge (1923)**

Description: Colony diam, 7 d, in mm: CYA 60–65; CYA 15 °C 28–33; CYA 30 °C 28–33; CYA 37 °C no growth; MEA 50–56; YES 60–65 (Figure 3(A)).

Colony characters: CYA, 25 °C, 7d: Colonies low, radially sulcate; margins low to moderately deep, wide, entire; mycelia white; texture velvety; sporulation dense; conidia dull green (25D3); exudates clear; soluble pigments absent; reverse color pastel yellow (3A4). MEA, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, wide, entire; mycelia white; texture velvety; sporulation dense; conidia dull green (25D3); exudates clear; soluble pigments absent; reverse color deep yellow (4A8). YES, 25 °C, 7d: Colonies deep, randomly furrowed; margins low, narrow, entire; mycelia white; texture velvety, slightly fasciculate at center; sporulation dense; conidia dull green (25D3); exudates absent; soluble pigments absent; reverse color grayish yellow with light yellow (3A5) at margin.

Conidiophores monoverticillate, rough walls, 2.4–4.0 μm wide, phialides ampulliform, 8.0–11.0 × 2.5–3.0 μm. Conidia smooth walls, ellipsoidal, 3.0–3.7 × 2.0–2.8 μm; Sclerotia white when young becoming orange (6B7) at age; Asci and ascospores not observed.

Strain examined: SFCP0220 and SFC20190612-M12

Note: *Penicillium aurantioviolaceum* is phylogenetically similar to *P. fusisporum*. The former species can be distinguished from the latter by the shape of the conidia and growth rate on CYA at 25 °C. *P. aurantioviolaceum* is characterized by ellipsoidal spores, whereas *P. fusisporum* has fusiform to oblong conidia. *P. aurantioviolaceum* grows faster than *P. fusisporum* on CYA at 25 °C (60–65 vs. 50–53) [29].

***Penicillium bissettii* Visagie & Seifert (2016)**

Description: Colony diam, 7 d, in mm: CYA 45–55; CYA 15 °C 25–30; CYA 30 °C 35–40; CYA 37 °C no growth; MEA 50–60; YES 45–55 (Figure 3(B)).

Colony characters: CYA, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, wide, entire; mycelia white; texture floccose; sporulation absent to sparse; exudates clear; soluble pigments absent; reverse color grayish yellow (3B5) with pale yellow (3A3) at margin. MEA, 25 °C, 7d: Colonies low, radially sulcate; margins low, wide, entire; mycelia white; texture floccose; sporulation absent to sparse; conidia grayish green (25B2); exudates clear; soluble pigments absent; reverse color deep yellow (4A8). YES, 25 °C, 7d: Colonies low to moderately deep, randomly furrowed; margins low, narrow, entire; mycelia white; texture floccose; sporulation dense; conidia grayish green (25B2); exudates absent; soluble pigments absent; reverse color grayish yellow (4B4).

Conidiophores biverticillate; stipes rough walls, 2.0–3.0 μm wide, phialides ampulliform, 7.0–11.0 × 2.5–3.5 μm. Conidia rough walls, globose to subglobose, 2.5–3.5 μm; Sclerotia absent; Asci and ascospores not observed.

Strain examined: SFCP0218, SFCP0219, SFCP0229, and SFCP0529

Note: *Penicillium bissettii* is similar to *P. vasconiae* and *P. annulatum*. This species is characterized by the roughened conidiophores and no growth at 37 °C, whereas *P. desertorum* has smooth walled conidiophores. *Penicillium annulatum* can be distinguished from *P. bissettii* by good growth on CYA at 37 °C [30]. *Penicillium bissettii* in Korea grows faster than type strain of *Penicillium bissettii* on CYA at 25 °C [30]. The type strain of *Penicillium bissettii* showed pinkish red mycelia on MEA, whereas the Korean strains have white mycelia.

***Penicillium cairnsense* Houbraken, Frisvad & Samson (2011)**

Description: Colony diam, 7 d, in mm: CYA 36–38; CYA 15 °C 17–19; CYA 30 °C 9–10; CYA 37 °C no growth; MEA 30–33; YES 42–47 (Figure 3(C)).

Colony characters: CYA, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety; sporulation dense; conidia dull green (27E3); exudates clear; soluble pigments absent; reverse color light yellow (4A4). MEA, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety; sporulation dense; conidia dull green (27E3); exudates absent; soluble pigments absent; reverse color light brown (7D5) at center, orange white (5A2) elsewhere. YES, 25 °C, 7d: Colonies low to moderately deep, radially sulcate, randomly furrowed as well, sunken in at center; margins low, narrow, entire; mycelia white;

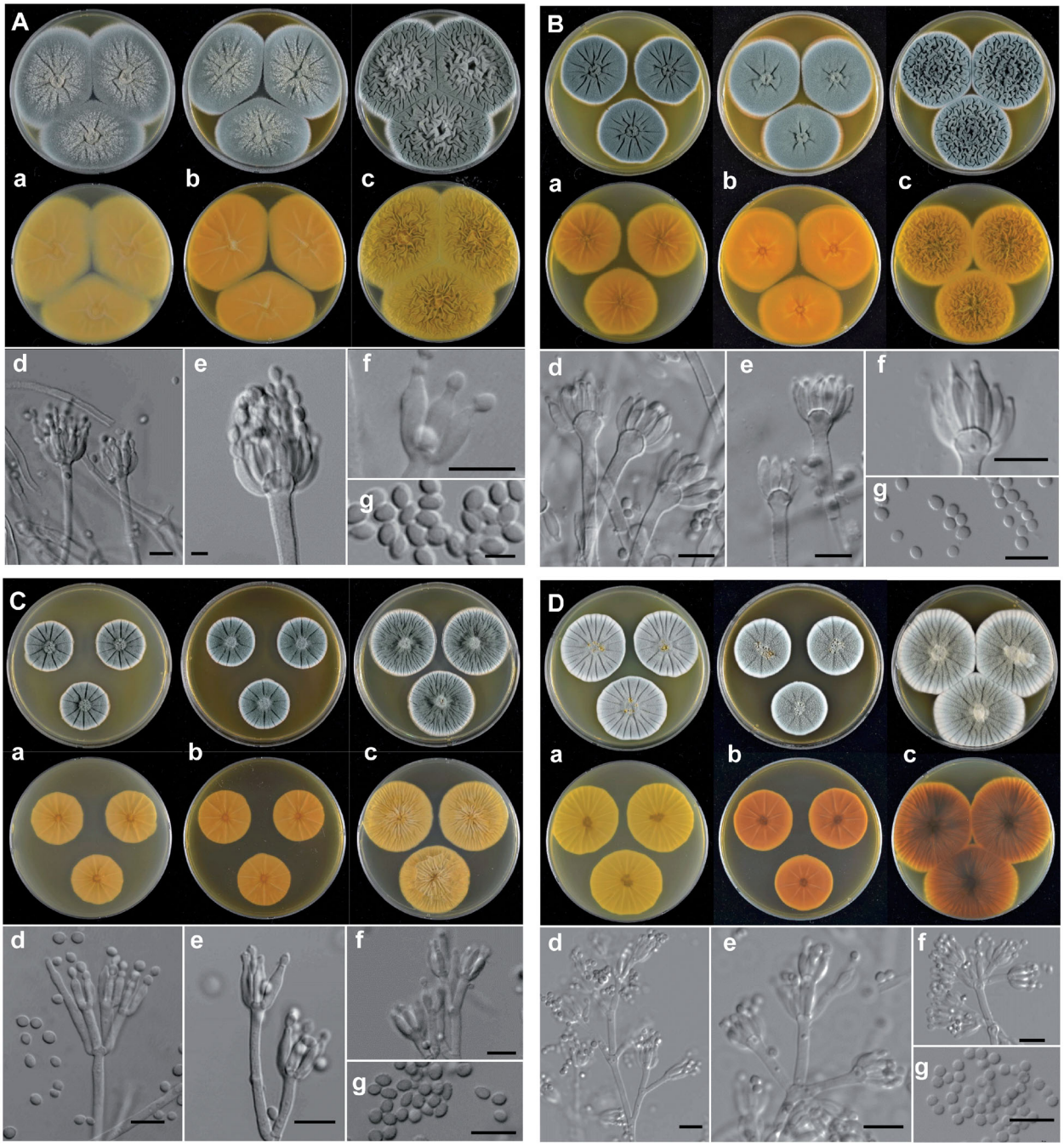

Figure 3. The unrecorded *Penicillium* species in Korea. (A) *P. aurantioviolaceum* (SFCP0220), (B) *P. bissettii* (SFCP0229), (C) *P. cairnsense* (SFCP0168), (D) *P. halotolerants* (SFCP0171), (E) *P. kananaskense* (SFCP0232), (F) *P. ortum* (SFCP0199), (G) *P. radiatolobatum* (SFCP0182), (H) *P. verhagenii* (SFCP0194). (a–c) Colonies grown on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and yeast extract sucrose agar (YES) from left to right (top = obverse, bottom = reverse). (d–f) Conidiophores; (g) Conidia (scale bar: d–*g* = 10 μm).

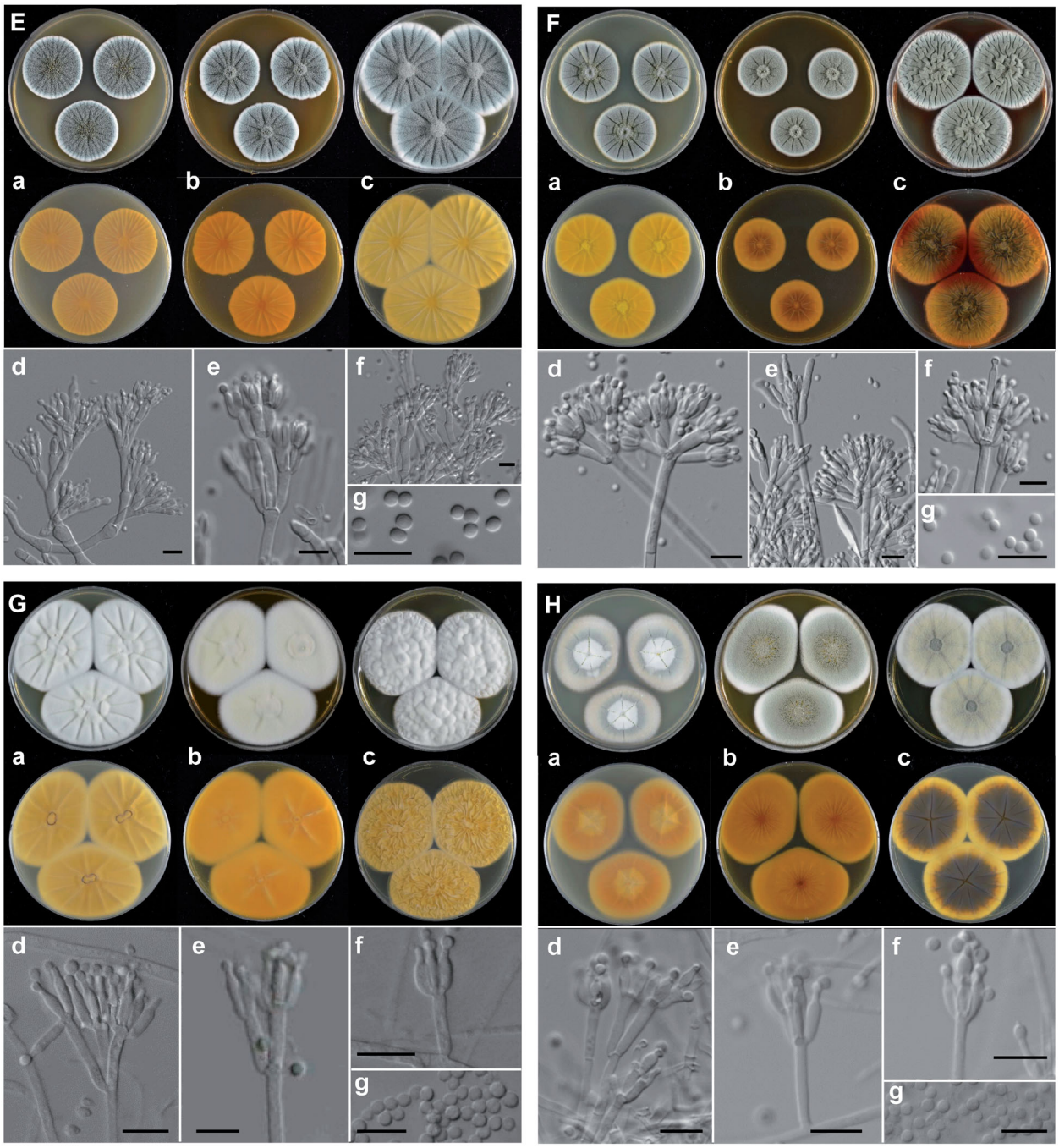

Figure 3. Continued

texture velvety; sporulation dense; conidia dull green (27E3); exudates absent; soluble pigments brownish red (9C6); reverse color brownish gray (11E2) with grayish red (8B63) area present.

Conidiophores mostly biverticillate; stipes smooth walls, 2.7–3.7 μm wide, phialides ampulliform, 7.0–10 × 2.5–3.5 μm. Conidia smooth walls, subglobose to broadly ellipsoidal, 2.5–3.5 × 2.5–3.0 μm; Sclerotia absent; Asci and ascospores not observed.

Strain examined: SFCP0168 and SFC20200506-M03

Note: *Penicillium cairnsense* is morphologically similar to *P. quebecense*. This species (9–10 mm in length) can be distinguished from *P. quebecense* (16–20 mm) by slower growth on CYA at 30 °C [31].

***Penicillium halotolerans* Frisvad, Houbraken & Samson (2012)**

Description: Colony diam, 7 d, in mm: CYA 30–35; CYA 15 °C 20–25; CYA 30 °C 30–35; CYA 37 °C 10–13; MEA 34–36; YES 50–54 (Figure 3(D)).

Colony characters: CYA, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety; sporulation dense; conidia grayish turquoise (24D3); exudates clear to pale yellow (3A3); soluble pigments absent; reverse color yellowish orange (4B7). MEA, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety, slightly floccose in center;

sporulation moderate; conidia grayish turquoise (24D3); exudates clear to pale yellow (3A3) at center; soluble pigments absent; reverse color brownish orange (5C6). YES, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture floccose; sporulation moderate; conidia grayish turquoise (24D3), pale turquoise (24A3) at margin; exudates absent; soluble absent; reverse color light yellow (4A4).

Conidiophores terverticillate; stipes smooth walls, 3.0–4.0 μm wide, phialides ampulliform, 6.0–11.0 × 2.5–3.5 μm. Conidia smooth walls, globose, 2.5–3.5 μm; Sclerotia absent; Asci and ascospores not observed.

Strain examined: SFCP0171, SFCP0174, and SFC20200506-M02

Note: *Penicillium cairnsense* is phylogenetically similar to *P. desertorum*. This species is characterized by radially sulcate colonies on YES, whereas *P. desertorum* has randomly furrowed colonies on YES. *Penicillium halotolerans* in Korea grows faster than the type strain of *P. halotolerans* on CYA at 30 and 37 °C. (30–35 vs. 20–25 at 30 °C and 10–13 vs. 0–2 at 37 °C) [32].

Penicillium kananaskense **Seifert, Frisvad & McLean (1994)**

Description: Colony diam, 7 d, in mm: CYA 38–42; CYA 15 °C 24–26; CYA 30 °C 14–16; CYA 37 °C no growth; MEA 42–47; YES 45–48 (Figure 3(E)).

Colony characters: CYA, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety; sporulation dense; conidia dull green (25E3); exudates absent; soluble pigments absent; reverse color golden yellow (5B7), deep yellow (4A8) at margin. MEA, 25 °C, 7d: Colonies low, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety; sporulation dense; conidia dull green (25D3); exudates absent; soluble pigments absent; reverse color deep orange (5A8). YES, 25 °C, 7d: Colonies moderately deep, randomly furrowed; margins low, narrow, entire; mycelia white; texture velvety; sporulation dense; conidia dull green (25D3); exudates absent; soluble pigments absent; reverse color dark yellow (4C8) with orange yellow (4A7) at margin.

Conidiophores monoverticillate; stipes smooth walls or rough walls, 3.0–4.5 μm wide, phialides ampulliform, 8.0–13.0 × 3.0–4.0 μm. Conidia rough walls, broadly ellipsoidal to ellipsoidal, 3.0–3.5 × 2.5–3.0 μm; Sclerotia absent; Asci and ascospores not observed.

Strain examined: SFCP0176, SFCP0177, and SFCP0232

Note: *Penicillium kananaskense* is phylogenetically similar to *P. lividum* and *P. odoratum*. This species is characterized by velvety colonies on MEA and slower growth on CYA at 30 °C [33].

Penicillium ortum **Visagie & K. Jacobs (2015)**

Description: Colony diam, 7 d, in mm: CYA 39–46; CYA 15 °C 20–23; CYA 30 °C 38–44; CYA 37 °C 24–32; MEA 50–55; YES 45–50 (Figure 3(F)).

Colony characters: CYA, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white at center, orange white (6A2) at margin; texture floccose; sporulation absent to sparse; conidia greenish gray (26E2); exudates clear; soluble pigments absent; reverse color pale orange (5A3) at center, fading into brownish orange to pale yellow (4A3). MEA, 25 °C, 7d: Colonies low, plane; margins low, narrow, entire; mycelia white to light yellow; texture velvety to floccose; sporulation moderate; conidia greenish gray (26E2); exudates absent; soluble pigments absent; reverse color brown (6D7) at center, brownish orange (5C6) elsewhere. YES, 25 °C, 7d: Colonies moderately deep, radially sulcate; margins low, narrow, entire; mycelia white at margin, light yellow elsewhere; texture floccose; sporulation sparse; conidia greenish gray (26E2); exudates absent; soluble pigments absent; reverse color pale yellow at margin, grayish brown (6E3) elsewhere.

Conidiophores mostly biverticillate, sometimes monoverticillate; stipes smooth walls, 2.0–3.2 μm wide, phialides ampulliform, 6.5–9.5 × 2.2–3.2 μm. Conidia smooth walls, globose to subglobose, 2.5–3.3 × 2.4–3.1 μm; Sclerotia absent; Asci and ascospores not observed.

Strain examined: SFCP0199 and SFC20200506-M55

Note: *Penicillium ortum* is phylogenetically similar to *P. cremeogriseum*. The former species can be distinguished from the latter by relatively fast growth on CYA at 30 °C (38–44 mm) [34].

Penicillium radiatolobatum **Lörinczi (1972)**

Description: Colony diam, 7 d, in mm: CYA 35–40; CYA 15 °C 18–21; CYA 30 °C 28–33; CYA 37 °C 10–13; MEA 30–35; YES 45–51 (Figure 3(G)).

Colony characters: CYA, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety, slightly floccose at central; sporulation moderate; conidia greenish gray (25D2); exudates pale yellow (2A3) droplets at central; soluble pigments absent; reverse color dull yellow (3B3) to olive brown (4B3)

at margin. MEA, 25 °C, 7d: Colonies low, radially sulcate; margins low, narrow, entire; mycelia white; texture floccose; sporulation moderate; conidia dull green (28E3); exudates hyaline to orange white (5A2) droplets in central areas; soluble pigments absent; reverse color light brown (6D5). YES, 25 °C, 7d: Colonies low, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety to floccose; sporulation moderate; conidia greenish gray (25D2); exudates absent; soluble pigments absent; reverse color brown (7E7).

Conidiophores biverticillate; stipes smooth walls, 2.8–4.0 μm wide, phialides ampulliform, 5.5–9.0 × 2.5–3.0 μm. Conidia smooth walls, globose to subglobose, 2.8–3.2 × 2.8–3.1 μm; Sclerotia absent; Asci and ascospores not observed.

Strain examined: SFCP0182, SFCP0183, SFC20171120-M01, and SFC20200506-M01

Note: *Penicillium radiatolobatum* is phylogenetically similar to *P. canescens*. This species can be distinguished from *P. canescens* by faster growth on CYA and MEA at 25 °C [30,35].

***Penicillium verhagenii* Houbraken (2014)**

Description: Colony diam, 7 d, in mm: CYA 27–30; CYA 15 °C 24–26; CYA 30 °C no growth; CYA 37 °C no growth; MEA 28–31; YES 34–40 (Figure 3(H)).

Colony characters: CYA, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety to floccose; sporulation moderate; conidia grayish green (27C3); exudates absent; soluble pigments absent; reverse color pale yellow (4A3) to grayish yellow (4B5). MEA, 25 °C, 7d: Colonies low to moderately deep, radially sulcate; margins low, narrow, entire; mycelia white; texture velvety to floccose; sporulation moderate; conidia dull green (25E4); exudates absent; soluble pigments absent; reverse color pale brownish orange (5C4). YES, 25 °C, 7d: Colonies low to moderately deep, radially sulcate, random furrows also present; margins low, narrow, entire; mycelia white; texture velvety to floccose; sporulation moderate; conidia dull green (27E3) at the center of colony, but greenish gray (27B2) at margin; exudates absent; soluble pigments absent; reverse color grayish yellow (4B3).

Conidiophores biverticillate; stipes finely roughoth walls, 2.8–3.8 μm wide, phialides ampulliform, 8.0–11.0 × 2.8–3.5 μm. Conidia roughened walls, broadly ellipsoidal to ellipsoidal, 3.0–4.0 × 2.5–3.5 μm; Sclerotia absent; Asci and ascospores not observed.

Strain examined: SFCP0194

Note: *Penicillium verhagenii* is phylogenetically similar to *P. ranomafanaense*. *P. verhagenii* is characterized by yellow reverse colors on CYA and YES, whereas *P. ranomafanaense* has orange or reddish [33].

5. Discussion

The rhizosphere is known as the most dynamic environment that provides a close association between plant root and fungi [1]. We isolated 64 *Penicillium* strains from rhizosphere soil of six plants. They were identified accurately by sequence-based identification as 26 species in nine sections. Four species could not be identified due to an unclear phylogenetic relationship. They were designated as *Penicillium* sp. We might be able to report them as new species in the future after a more detailed morphological comparison with phylogenetically similar species.

Eight species were records for the first time in Korea. These were *P. aurantioviolaceum, P. bissettii, P. cairnsense, P. halotolerans, P. kananaskense, P. ortum, P. radiatolobatum*, and *P. verhagenii*. The morphological characteristics of the unrecorded species were consistent with those of the respective type species, except for *P. bissettii* and *P. halotolerans*. The strains isolated from Korea of these two species grow faster compared to the type strains. Some fungi exhibit different growth rates or metabolism as they adapt to different environments [36,37]. *P. bissettii* and *P. halotolerans* were isolated from sand dune in Korea, whereas their type cultures were obtained from forest soil and salt marsh, respectively [30,32]. Although this is a small-scale study in a limited habitat, many unrecorded species and potential new species have been found. By analyzing a larger variety of hosts, it would be possible to discover more species.

Fungal diversity and composition were significantly correlated with habitat and plant communities [38,39]. Fungal diversity was significantly higher in terrestrial habitats than freshwater and mangrove habitats [38]. Similarly, the diversity of *Penicillium* species in rhizosphere soil was relatively higher in terrestrial habitats than coastal habitats. Most *Penicillium* species from rhizosphere soil were unique to each plant. The composition of the *Penicillium* species did not only differ by habitats but also by the plant host species. The *Penicillium* composition, in particular, is relatively much affected by plants in the marine environment. Despite the two host plants, *Calystegia soldanella* and *Lathyrus japonicus* having very similar environments, such as poor nutrient and abiotic stresses, fungal diversity varied depending on plants [40]. Recently, various *Penicillium* species have been reported by NGS-based studies in rhizosphere soil

[41,42]. Although the role of these *Penicillium* in rhizosphere soil is unclear, they might have important effects on plants. Further studies are required to investigate the function of *Penicillium* in rhizosphere soil.

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

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