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Research Article

Characterization of *Paecilomyces variotii* and *Talaromyces amestolkiae* in Korea Based on the Morphological Characteristics and Multigene Phylogenetic Analyses

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Abstract During fungal diversity surveys of the order Eurotiales in Korea, two fungal strains, EML-DG33-1 and EML-NCP50, were isolated from samples of rat dung and fig tree leaf collected at a garden located in Gwangju in 2014. To complete the National Species List of Korea, it is a prerequisite to verify whether many questionable species, which were previously recorded but not confirmed, indeed present in Korea. Herein, the isolates were confirmed as undescribed species, *Paecilomyces variotii* and *Talaromyces amestolkiae* based on the combination of morphological and phylogenetic analyses of multigenes including the rDNA internal transcribed spacer, β-tubulin, and RNA polymerase II subunit 2.

Keywords Fig tree leaf, Morphology, Multigene phylogenetic analysis, *Paecilomyces variotii*, Rat dung, *Talaromyces amestolkiae*

The order Eurotiales described by Beny and Kimbrough [1] consists of three families: Trichocomaceae, Thermoascaceae, and Eremomycetaceae. Especially, the Trichocomaceae contains species which are important to both industry and medicine and as mycotoxin producer on various foods [2]. The most well-known genera include *Aspergillus*, *Penicillium*, *Paecilomyces*, and *Talaromyces* [2-4].

The genus *Paecilomyces* (teleomorph, *Byssochlamys*) in the Eurotiales was originally described by Bainier [5], based on a single species, *Paecilomyces variotii*. This species was characterized by verticillate conidiophores with divergent whorls of phialides, which have a cylindrical or inflated base, tapering to a long and distinct neck. The genus was revised by Brown and Smith [6] and modified by Samson [7], who defined 31 species and divided the genus into two sections, *Paecilomyces* and *Isarioidea*. To date, more than 100 species of the genus *Paecilomyces* have been recognized [8]. The type species of *Paecilomyces*, *P. variotii*, has a sexual *Byssochlamys* state [9]. This species is frequently found in soils, animals, indoor environments, and food products [7, 10]. Some species of *Paecilomyces* have been isolated from insects, and some can even cause infections in humans [11-13]. Most members of the genus *Paecilomyces* have optimum growth temperatures ranging from 30~37°C [9]. Several studies have demonstrated the importance of *Paecilomyces* species in various biotechnological applications, including the production of tannase [6-10] and secondary metabolites, some of which have useful biological activities [14, 15].

On the other hand, the genus *Talaromyces* in the Eurotiales was described by Benjamin [16] with *Talaromyces vermiculatus* as the type species. This genus was characterized by soft cleistothecial ascomata with a wall of interwoven hyphae. It was re-defined and restricted to species producing asci in chains by Stolk and Samson [17]. Samson *et al.* [18] successfully transferred species of *Penicillium* subgenus *Biverticillium* to *Talaromyces* by following the one fungus one name concept. On the other hand, Yilmaz *et al.* [19] provided a monograph and accepted 88 species of

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Talaromyces. Based on internal transcribed spacer (ITS), β-tubulin, and RBP2 multigene phylogeny, Yilmaz *et al.* [19] divided those species in seven sections including: *Bacillispori*, *Helici*, *Islandici*, *Purpurei*, *Subinflati*, *Talaromyces*, and *Trachyspermi*. Especially, several species in *Talaromyces* such as *T. thermophilus*, *T. funiculosum*, and *T. marneffei* show biotechnological and medical important properties [20].

In Korea, seven species of *Paecilomyces* and seventeen species of *Talaromyces* have been reported in Korea, of which only three *Paecilomyces* and five *Talaromyces* species have been well described, excluding *P. variotii* and *T. amestolkiae* [21]. In the Korean fungal species list published by the National Institute of Biological Resources (NIBR), the *Paecilomyces variotii* species is listed as an undescribed record which does not match any references.

During fungal diversity surveys of the order Eurotiales in Korea, two fungal strains, EML-DG33-1 as a dung fungus and EML-NCP50 as an endophyte, were isolated from samples of rat dung and fig tree leaf collected at a garden located in Gwangju in 2014. The objective of the present study was to clarify the phylogenetic status of the polyphyletic species of *P. variotii* and *T. amestolkiae* and to confirm them as undescribed species in Korea based on the morphological and multigene phylogenetic analyses.

MATERIALS AND METHODS

Isolation of fungal strains from rat dung and fig tree leaves. Rat dung samples were collected using sterile forceps, from the garden of Chonnam National University, Gwangju, Korea, in 2014. The samples were transferred to the laboratory in plastic bags, placed on sterile moist Whatman's filter paper in Petri dishes, and incubated in a moist chamber at 25°C for 7 days. Hyphal tips were transferred to potato dextrose agar (PDA; Difco, Franklin Lakes, NJ, USA) plates using a stereomicroscope. To isolate pure cultures, individual colonies of varied morphologies were transferred to PDA plates. On the other hand, leaf samples of fig tree (Ficus sp.) were collected from the campus of Chonnam National University, Gwangju, Korea, in 2014. Collected samples were stored in sterile polyethylene bags where they were cleaned under running tap water to remove debris before use, air dried, and then processed for isolation of endophytic fungi. The leaves were cut into small, 1 cm long and 0.5 cm wide pieces. Tissue pieces were surface-sterilized with 2% sodium hypochlorite for 3 min and with 70% ethanol for 1 min, and then washed three times with sterile distilled water. The surface-sterilized samples were allowed to dry on sterile paper towel in a laminar airflow chamber. Ten fragments from each leaf were placed onto PDA and Rose Bengal chloramphenicol agar (Difco) supplemented with the antibiotic, streptomycin sulfate (0.4 mg/mL; Sigma-Aldrich, Munich, Germany), to suppress bacterial growth. After incubation at 25°C for 5 days, individual hyphal tips of the developing fungal colonies were removed and placed onto in PDA medium to be incubated for 5~10 days.

To isolate pure cultures, individual colonies of varied morphologies were transferred to PDA plates. Pure isolates of *P. variotii* (EML-DG33-1 and EML-DG33-2) and *T. amestolkiae* (EML-NCP50) were maintained on PDA slant tubes and also stored in 20% glycerol at −80°C at the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju. Dry cultures (EML-DG33-1 and EML-NCP50) were preserved at Chonnam National University Fungal Collection (CNUFC), Division of Food Technology, Biotechnology & Agrochemistry, College of Agriculture & Life Sciences, Gwangju. Duplicates of EML-DG33-1 and EML-NCP50 were also deposited as glycerol stock at −80°C at the Culture Collection of NIBR, Incheon, as KOSPFGC000002024 and KOSPFGC000002029, respectively.

Morphological studies. To obtain samples for microscopic examination and growth rate determination, EML-DG33-1 and EML-NCP50 were cultured on each of the three different media. The media used were malt extract agar (MEA; 30 g malt extract, 1 g peptone, 20 g glucose, 0.005 g $CuSO_4 \cdot 5H_2O$, 0.01 g $ZnSO_4 \cdot 7H_2O$, and 20 g agar, in 1 L of deionized water), Czapeck yeast autolysate agar (CYA; 3 g $NaNO_3$, 5 g yeast extract, 30 g sucrose, 1.3 g $K_2HPO_4 \cdot 3H_2O$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.005 g $CuSO_4 \cdot 7H_2O$, 0.01 g $ZnSO_4 \cdot 7H_2O$, and 20 g agar, in 1 L of deionized water), and yeast extract sucrose agar (YES; 20 g yeast extract, 150 g sucrose, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.005 g $CuSO_4 \cdot 7H_2O$, 0.01 g $ZnSO_4 \cdot 7H_2O$, and 20 g agar, in 1 L of deionized water). Plates were incubated at 20°C, 25°C, 30°C, 35°C, and 40°C in the dark for 7 days. Samples were mounted in lactophenol solution (Junsei Chemical Co. Ltd., Tokyo, Japan) and examined under a light microscope (DFC290; Leica Microsystems, Wetzlar, Germany). Fine fungal structures were observed using scanning electron microscopy (SEM, Hitachi S4700; Hitachi, Tokyo, Japan). Samples were cultured in PDA medium in the dark at 27°C for 7 days, fixed in 2.5% paraformaldehyde-glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 1 hr, and then washed with cacodylate buffer (Junsei Chemical Co. Ltd.). Cellular membranes were preserved by fixing the samples in 1% osmium tetroxide (Electron Microscopy Sciences, Hatfield, PA, USA) diluted with cacodylate buffer for 1 hr, washing again in cacodylate buffer, dehydrating in graded ethanol (Emsure, Darmstadt, Germany) and isoamyl acetate (Junsei Chemical Co. Ltd.), and drying 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.

DNA extraction, PCR, and sequencing. Total genomic DNA was extracted directly from the mycelia using the HiGene Genomic DNA Prep Kit (BIOFACT Corp., Daejeon, Korea). The gene sequences of EML-DG33-1 and EML-

NCP50-1, consisting of the ITS, Tub2, and RPB2 genes, were amplified with the primer pairs ITS1, ITS4 [22]; Bt2a, Bt2b [23]; and RPB2-5F, RPB2-7Cr [20], respectively. The PCR amplification mixture (total volume of 20 µL) contained 10 ng of fungal genomic DNA template, 5 pmol/µL of each primer, and Accupower PCR Premix (*Taq* DNA polymerase, dNTPs, buffer, and a tracking dye; Bioneer Corp., Daejeon, Korea). PCR products were purified using the Accuprep PCR Purification Kit (Bioneer Corp.) according to the manufacturer's instructions. DNA sequencing was performed on an ABI 3700 Automated DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA).

Phylogenetic analysis. Sequences (Table 1) were subjected to phylogenetic analysis using Clustal_X v.1.83 [24] and Bioedit v. 5.0.9.1 software [25]. Phylogenies were assessed using MEGA 6 software [26]. Maximum likelihood (ML) phylogenetic trees were constructed for combined datasets

Table 1. Sequences used in this study, including GenBank accession numbers

Taxon name	Collection No. (isolate No.)	GenBank accession No.		
		ITS	Tub2	RPB2
Aspergillus ochraceoroseus	CBS 101887	-	-	JN121416
A. sydowii	CBS 264.81	-	-	JN121476
A. versicolor	CBS 245.65	-	-	JN121468
Byssochlamys fulva	CBS 146.48	FJ389940	FJ389986	-
B. laguncullariae	CBS 373.70	FJ389944	FJ389995	-
B. nivea	CBS 100.11	FJ389934	FJ389999	JF417414
B. spectabilis	CBS 101075^{HT}	-	-	JF417446
B. verrucosa	CBS 604.74	DQ073329	DQ073328	-
B. zollerniae	CBS 374.70	FJ389933	FJ390008	-
Coccidioides immitis	RS	-	-	XM_001240649
Eupenicillium crustaceum	CBS 344.61^{IsoT}	-	-	JF417428
Geosmithia viridis	CBS 252.87^{HT}	-	-	JF417422
Hamigera avellanea	CBS 295.48^{IsoT}	-	-	JF417424
Paecilomyces brunneolus	CBS 370.70^{T}	EU037050	EU037068	-
P. divaricatus	CBS 284.48^{T}	FJ389931	FJ389992	-
P. formosus	CBS 990.73E	FJ389929	FJ389993	-
P. formosus	DTO 45H8	GU968650	GU968683	-
P. formosus	DTO 63F1	GU968670	GU968686	-
P. formosus	DTO 49D6	GU968655	GU968691	-
P. formosus	CBS 372.70	FJ389926	FJ389990	-
P. saturatus	CBS 323.34	FJ389947	FJ390005	-
P. variotii	DTO 63D7	GU968659	GU968693	-
P. variotii	CBS 102.74^{T}	EU037055	EU037073	-
P. variotii	DTO 63D9	GU968661	GU968695	-
P. variotii	CBS 101075	EU037051	EU037069	-
P. variotii	DTO 63E6	GU968667	GU968680	-
P. variotii	**EML-DG33-1**	**KX060750**	**KX060751**	**KY350194**
P. variotii	**EML-DG33-2**	**KX060753**	**KX060752**	**KY350195**
Penicillium citrinum	CBS 139.45^{NT}	-	-	JN606604
P. expansum	CBS 325.48	-	-	JF417427
P. glabrum	CBS 125543^{NT}	-	-	JF417447
P. namyslowskii	CBS 353.48	-	-	JF417430
Sagenomella diversispora	CBS 398.69	-	-	JF417436
S. humicola	CBS 427.67^{IsoT}	-	-	JF417439
S. griseoviridis	CBS 426.67	-	-	JF417438
S. striatispora	CBS 429.67^{IsoT}	-	-	JF417440
Rasamsonia argillacea	CBS 101.69	-	-	JF417415
R. byssochlamydoides	CBS 413.71	-	-	JF417437
R. eburnea	CBS 100538	-	-	JN406532
Talaromyces amestolkiae	CBS 132696^{T}	JX315660	JX315623	JX315698
T. amestolkiae	CBS 263.93	JX315669	JX315625	JX315707
T. amestolkiae	CBS 252.31	JX315668	JX315624	JX315706
T. amestolkiae	**EML-NCP50**	**KU985307**	**KU985308**	**KX363885**
T. atroroseus	CBS 133442^{T}	KF114747	KF114789	KF114763
T. bacillisporus	CBS 296.48^{T}	JN899329	AY753368	JN121634

Table 1. Continued

Taxon name	Collection No. (isolate No.)	GenBank accession No.		
		ITS	Tub2	RPB2
T. coalescens	CBS 103.83^{T}	JN899366	JX091390	KM023277
T. derxii	CBS 412.89^{T}	JN899327	JX494306	KM023282
T. duclauxii	CBS 322.48^{T}	JN899342	JX091384	JN121491
T. emodensis	CBS 100536^{HT}	-	-	JF417445
T. flavus	CBS 310.38^{NT}	-	-	JF417426
T. helicus	CBS 335.48^{T}	JN899359	KJ865725	KM023273
T. leycettanus	CBS 398.68^{HT}	-	-	JF417435
T. luteus	CBS 371.87	-	-	JF417431
T. luteus	CBS 348.51^{NT}	-	-	JF417429
T. minioluteus	CBS 642.68^{NT}	-	-	JF417443
T. islandicus	CBS 338.48^{T}	KF984885	KF984655	KF985018
T. marneffei	CBS 388.87^{T}	JN899344	JX091389	KM023283
T. pseudostromaticus	CBS 470.70^{T}	JN899371	HQ156950	KM023298
T. purpureogenus	CBS 286.36^{T}	JX315671	JX315639	JX315709
T. purpureus	CBS 475.71^{T}	JN899328	GU385739	JN121522
T. ruber	CBS 132704^{T}	JX315662	JX315629	JX315700
T. rugulosus	CBS 371.48^{T}	KF984834	KF984575	KF984925
T. scorteus	CBS 340.34^{T}	KF984892	KF984684	KF984916
T. stollii	CBS 408.93^{T}	JX315674	JX315633	JX315712
T. subinflatus	CBS 652.95^{T}	JN899397	JX494288	KM023308
T. thailandensis	CBS 133147^{T}	JX898041	JX494294	KM023307
T. thermophilus	CBS 236.58^{HT}	-	-	JF417420
T. trachyspermus	CBS 373.48^{T}	JN899354	AY753371	JF417432
T. varians	CBS 386.48^{T}	JN899368	KJ865731	KM023274
T. wortmannii	CBS 391.48^{NT}	-	-	JF417433
Thermoascus aurantiacus	CBS 891.70	-	-	JF417444
T. crustaceus	CBS 181.67^{T}	-	-	JF417417
T. thermophilus	CBS 528.71^{NT}	-	-	JF417442
Thermomyces lanuginosus	CBS 218.34	-	-	JF417418
T. lanuginosus	CBS 224.63	-	-	JF417419
Trichocoma paradoxa	NRRL 28276	EU021600	EU021675	EU021633
T. paradoxa	CBS 247.57	-	-	JF417421
Warcupiella spinulosa	CBS 512.65^{NT}	-	-	JF417441

Bold letters indicate accession numbers determined in our study.

ITS, internal transcribed spacer; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; HT, holotype; IsoT, isotype; T, ex-type strain; NT, neotype; EML, Environmental Microbiology Laboratory, Fungarium, Chonnam National University, Gwangju, South Korea; NRRL, ARS Culture Collection, Peoria, Illinois, USA.

of the ITS rDNA, Tub2, and RPB2 gene sequences. The nearest-neighbor-interchange was selected for the ML heuristic method, and the initial ML tree was set automatically. Sequences of *Byssochlamys verrucosa* and *Trichocoma paradoxa* and *Coccidioides immiti* were used as outgroups. The percentage of sequence identity was obtained using the basic local alignment search tool (BLAST) of the National Center for Biotechnology Information (NCBI) for each isolate.

RESULTS

Phylogenetic analysis. The groups of Eurotiales within ascomycete fungi has been known to be polyphyletic. In this study, the phylogenetic status of EML-DG33-1 and-2 isolates belonging to *Paecilomyces* & *Byssochlamys* clade and EML-NCP50 isolate belonging to *Talaromyces s. str.* and *Penicillium* subg. *Biverticillium* clade within Eurotiales are shown in the ML tree based on RPB2 sequence analysis (Fig. 1). The RPB2 sequence of EML-DG33-1 had 99.8% (971/973 bp) sequence similarity with the known sequence of a teleomorph of *Paecilomyces*, *Byssochlamys spectabilis* (GenBank accession No. JF417446).

A BLAST search of ITS sequences via the NCBI database indicated that the ITS sequence of EML-DG33-1 is most closest to *P. variotii* DTO 63E (GenBank accession No. GU968667), with 98.7% (473/479 bp) homology. The Tub2 sequence of *P. variotii* DTO 63D9 (GenBank accession No. GU968695) showed 99.1% (447/451 bp) homology to that of EML-DG33-1. Based on this multigene analysis, the isolate was identified as *P. variotii* (Fig. 2). The ITS, Tub2, and RPB2 sequences of EML-DG33-1 and EML-DG33-2

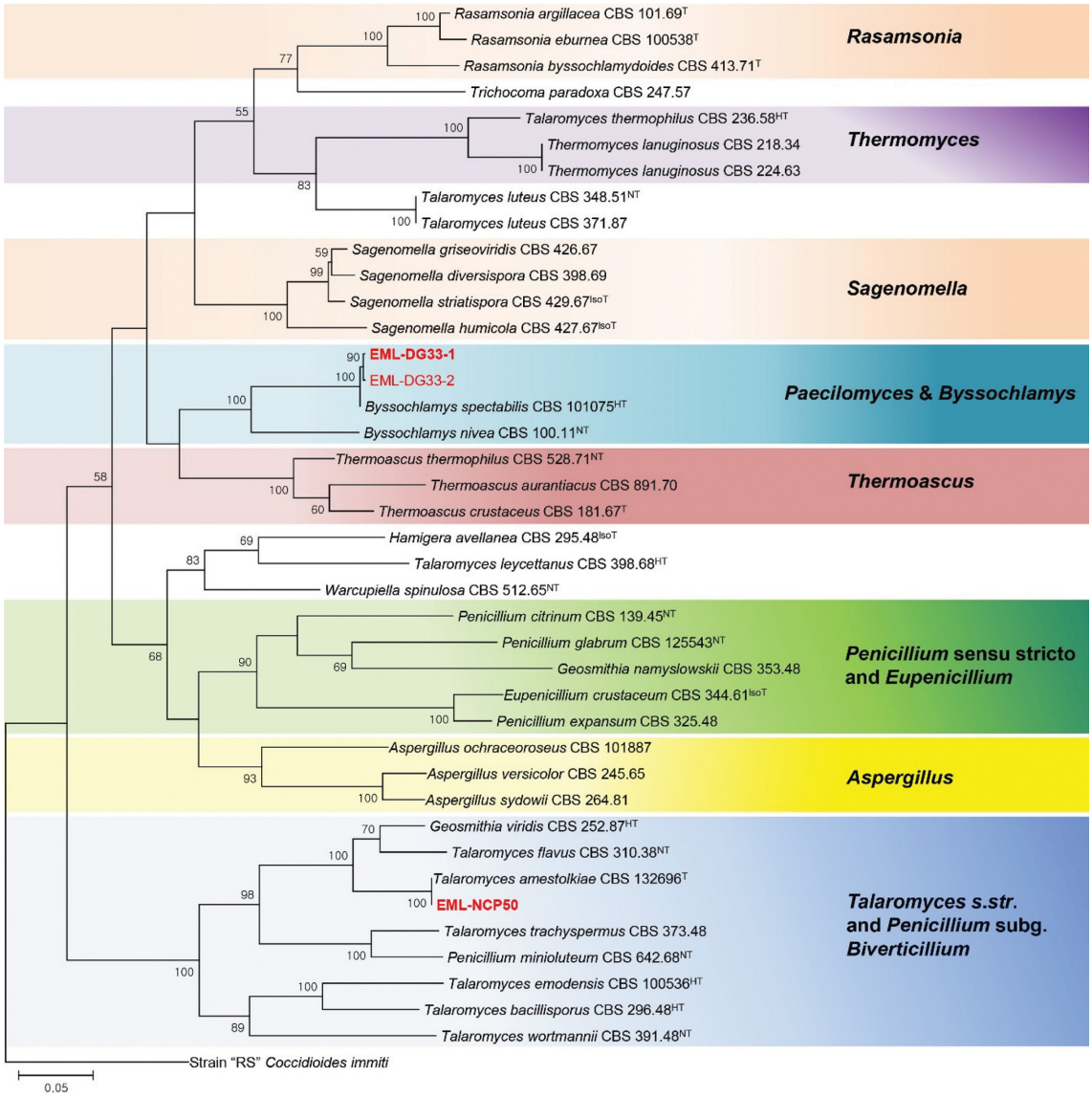

Fig. 1. Phylogenetic relationships within genera *Paecilomyces* (teleomorph, *Byssochlamys*) and *Talaromyces* of the Trichocomaceae (Eurotiales) based on Maximum likelihood analysis of RPB2 sequence data. The corresponding sequence of *Coccidioides immiti* was used as the outgroup. Bootstrap support values > 50% are indicated at the nodes. The bar indicates the number of substitutions per position. Classification system presented by Houbraken *et al.* [3].

were deposited in the NCBI database. The accession numbers for ITS, Tub2 and RPB2 sequences were KX060750, KX060751 and KY350194 for EML-DG33-1 isolate, and KX060753, KX060752, and KY350195 for EML-DG33-2 isolate, respectively. Analysis of the multigene genes placed the strains of EML-DG33-1 and EML-DG33-2 within the *variotii* group (Fig. 2).

BLAST search analysis indicated that the ITS (accession No. KU985307), Tub2 (accession No. KU985308), and RPB2 (accession No. KX363885) sequences of EML-NCP50 had 99.8% (524/525 bp), 100% (427/427 bp), and 100% (1,038/1,038 bp) sequence similarity, respectively, with the known sequences of *T. amestolkiae* DTO179F5 (GenBank accession Nos. JX315660, JX315623, and JX315698, respectively). Analysis of the multigene genes placed the EML-NCP50 strain within the section *Talaromyces* (Fig. 3).

Taxonomy of EML-DG33-1.

Paecilomyces variotii Bainier, Bull. Soc. Mycol. Fr. 23:27 (1907) (Table 2, Fig. 4)

≡ *Penicillium variotii* (Bainier) Sacc., Syll. Fung. 22:1273 (1913)

= *Penicillium aureocinnamomeum* Biourge, La Cellule 33:213 (1923)

Description: The colonies of EML-DG33-1 exhibited fast growth on MEA, covering the Petri dish within 7 days at 25°C. The color of the colonies was pale yellow and white at the margins. The color of the colony reverse was greenish

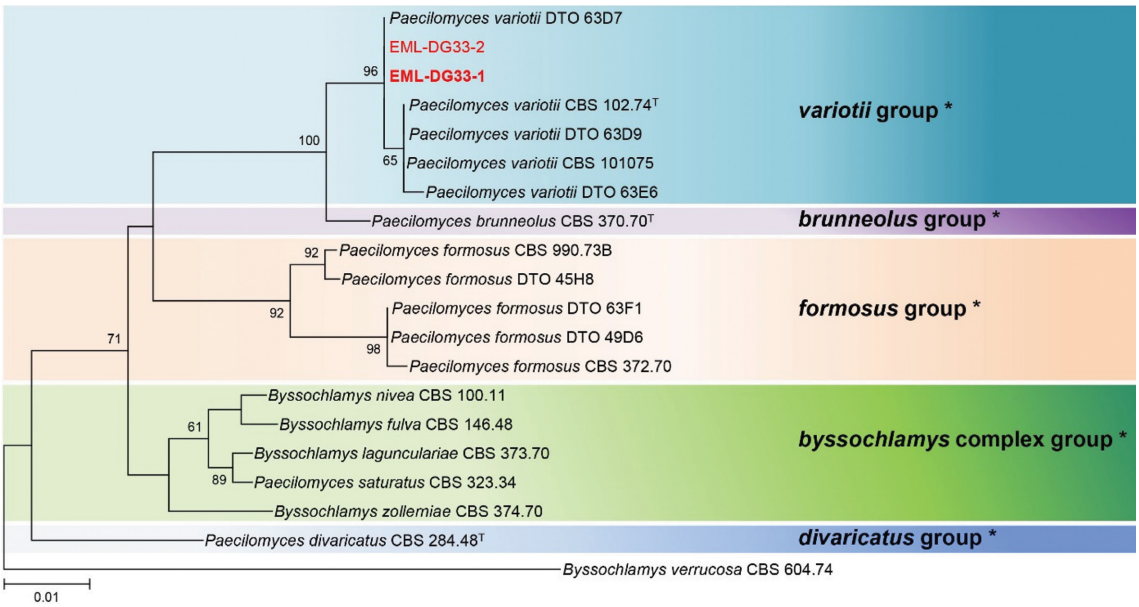

Fig. 2. Phylogenetic tree of the *Paecilomyces variotii* EML-DG33-1 and EML-DG33-2 within the *variotii* group, based on maximum likelihood analysis of combined datasets for internal transcribed spacer rDNA, and Tub2. The corresponding sequence of *Byssochlamys verrucosa* was used as the outgroup. Bootstrap support values > 50% are indicated at the nodes. The bar indicates the number of substitutions per position. Classification presented by Houbraken *et al.* [27]. *Asterisk indicates classification system suggested by authors.

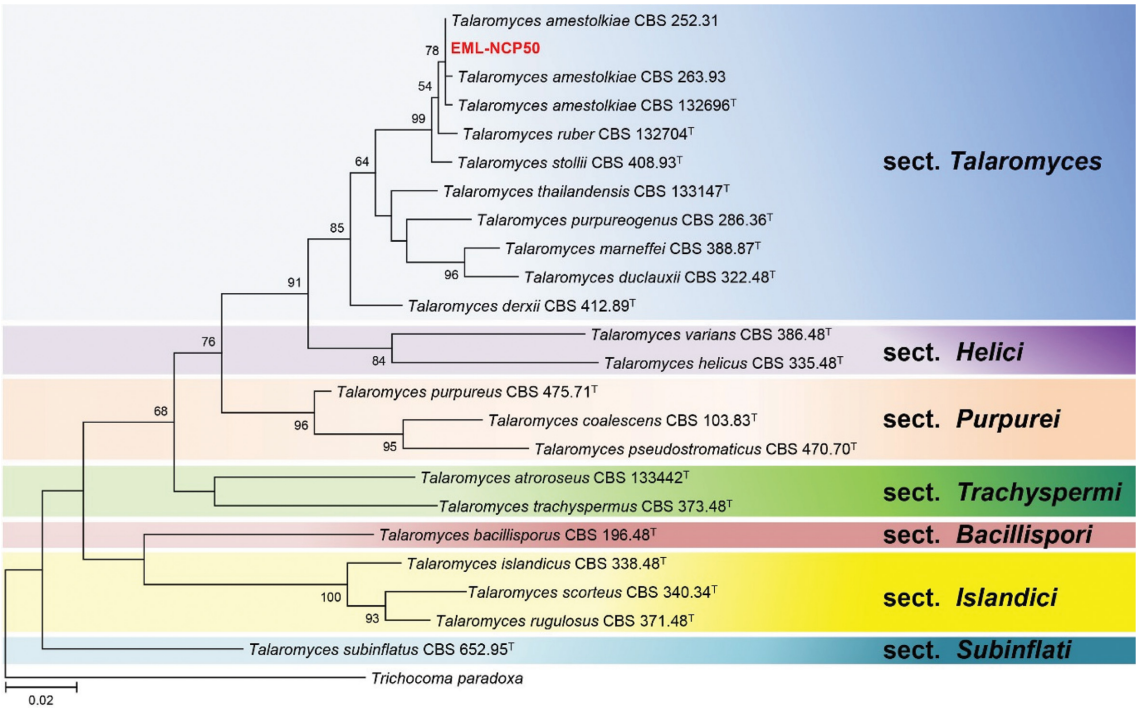

Fig. 3. Phylogenetic tree of the *Talaromyces amestolkiae* EML-NCP50 within the Sect. *Talaromyces* based on maximum likelihood analysis of combined datasets for internal transcribed spacer rDNA, Tub2, and RPB2. The corresponding sequence of *Trichocoma paradoxa* was used as the outgroup. Bootstrap support values > 50% are indicated at the nodes. The bar indicates the number of substitutions per position.

yellow (Fig. 4A and 4D). Mycelial growth on MEA was sparse; however, sporulation was extensive. Conidiophores were mostly short and irregularly branched (Fig. 4G and 4I). Phialides were club-shaped with a long neck, with up

Table 2. Morphological comparison of the isolate EML-DG33-1 and closely related species

Characteristic	Present isolate	*Paecilomyces variotii*[a]
Colony	Fast-growing, covering the Petri dish within 7 days at 25°C, pale yellow; reverse: greenish yellow	Fast-growing, attaining a diameter of 40 mm after 7 days, light yellowish olive; reverse: yellow to yellow-brown
Phialide	8.5~20.5 µm long, club-shaped showing long neck, up to 7 in a whorl	12~20 × 2.5~5.0 µm, cylindrical to ellipsoidal
Conidia	4.0~6.0 × 2.5~4.5 µm, ellipsoidal to cylindrical in chains, light yellow	3.2~5.0 × 2.0~4.0 µm, ellipsoidal to cylindrical, hyaline to yellow

[a]From the description by Samson [7].

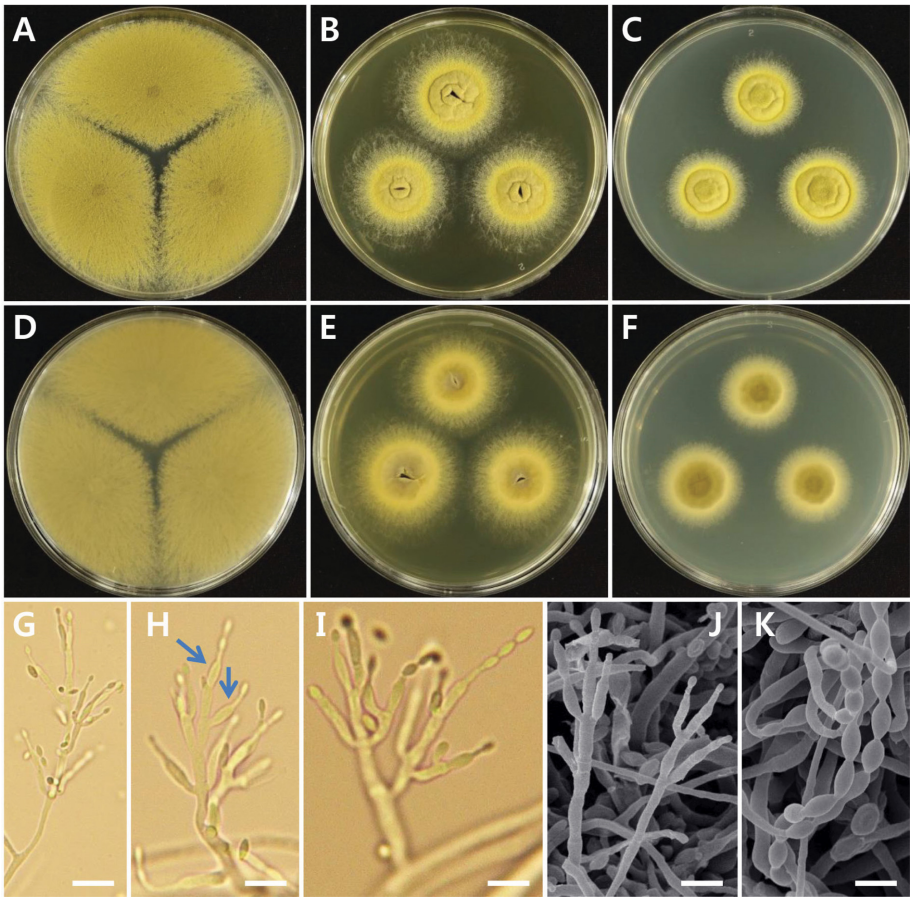

Fig. 4. Morphological and conidial characteristics of the *Paecilomyces variotii* isolate, EML-DG33-1. A, D, Colonies on malt extract agar; B, E, Colonies on yeast extract sucrose agar; C, F, Colonies on Czapeck yeast autolysate agar. A~C, Obverse view; D~F, Reverse view; G~J, Branched conidiophores and club-shaped phialides (arrows); K, Conidial chain (scale bars: G = 50 µm, H~J = 20 µm, K = 10 µm).

to seven in a whorl, and measured 8.5~20.5 µm in length (Fig. 3H). Conidia were ellipsoidal to cylindrical in chains, yellow in color, and measured 4.0~6.0 × 2.5~4.5 µm (Fig. 4K).

Colonies on YES grew faster than those on CYA, and attained a diameter of 53~56 mm after 7 days at 25°C. The color of the colonies was deep yellow, and the color of the colony reverse was dull brown. Conidia grown on YES were often slightly larger than those grown on CYA (Fig. 4B and 4E). Colonies on CYA grew very slowly, and attained a diameter of 40~42 mm after 7 days at 25°C. The color of the colonies was light yellow, and the color of the colony reverse was brownish yellow in the center and white at the margins (Fig. 4C and 4F).

Culture characteristics: The isolate was observed to grow over a wide range of temperatures with varying growth rates on MEA, YES, and CYA. The average growth rates on MEA, YES, and CYA were 13.5, 8.0, and 6.0 mm/day, respectively. The optimum growth temperature range was 30~35°C, slow growth was observed at below 20°C, and could grow well at temperatures as high as 40°C.

Specimen examined: Republic of Korea, Jeonnam Province, garden of the Chonnam national University located in

Gwangju (35°10′ N, 126°55′ E), from a rat dung, 10 Sep 2014 (EML-DG33-1), deposited at the Culture Collection of NIBR, Incheon, as KOSPFGC000002024.

Taxonomy of EML-NCP50.

Talaromyces amestolkiae Yilmaz, Houbraken, Frisvad & Samson, Persoonia 29: 48 (2012) (Table 3, Fig. 5)

Description: Colonies were characterized after 7 days at 25°C. On the CYA agar, colonies were 21~24 mm in diameter and raised in the center. They had wide, regular margins (2~3 mm), white or yellow mycelium, which was reddish in the center, and moderately dense sporulation (Fig. 5C and 5F). On the YES agar, good sporulation on colonies was observed, whereas pigments and exudates were absent. The colonies were 34~36 mm in diameter. The mycelium on the YES agar was white; it exhibited moderately dense sporulation, narrow margins, and tufts (Fig. 5B and 5E). On the MEA agar, the mycelium was white, sometimes red in the center. Diameter of colonies that had regular wide margins varied within 34~53 mm. Sporulation varied from moderately dense to dense. Soluble pigment was absent (Fig. 5A and 5D).

The texture, conidiophores, conidia, metulae, phialide, and stripes were examined under a compound light

Table 3. Morphological comparison of the isolate EML-NCP50 and closely related species

Characteristic	Present isolate	*Talaromyces amestolkiae*[a]
Colony on CYA	Slow-growing, attaining a diameter of 21~24 mm after 7 days, mycelium white and yellow; reddish in the center.	Slow-growing, attaining a diameter of 29~30 mm after 7 days, mycelium white and yellow; red in the center.
Phialide	3~4 in total, acerose, 9.0~13.5 × 1.5~2.5 μm	3~6 in total, acerose, 9.5~12.0 × 2.5~3.0 μm
Metulae	In verticils of 3~5, 9.5~13.0 × 2.0~3.5 μm	In verticils of 3~5, 9.5~14.0 × 3.0~4.0 μm
Conidia	Smooth-walled, single-celled, ellipsoidal, 2.5~4.0 × 1.0~3.5 μm	Smooth or some rough-walled, ellipsoidal, 2.0~3.0 × 1.5~2.5 μm

[a]From the description by Yilmaz *et al.* [19].

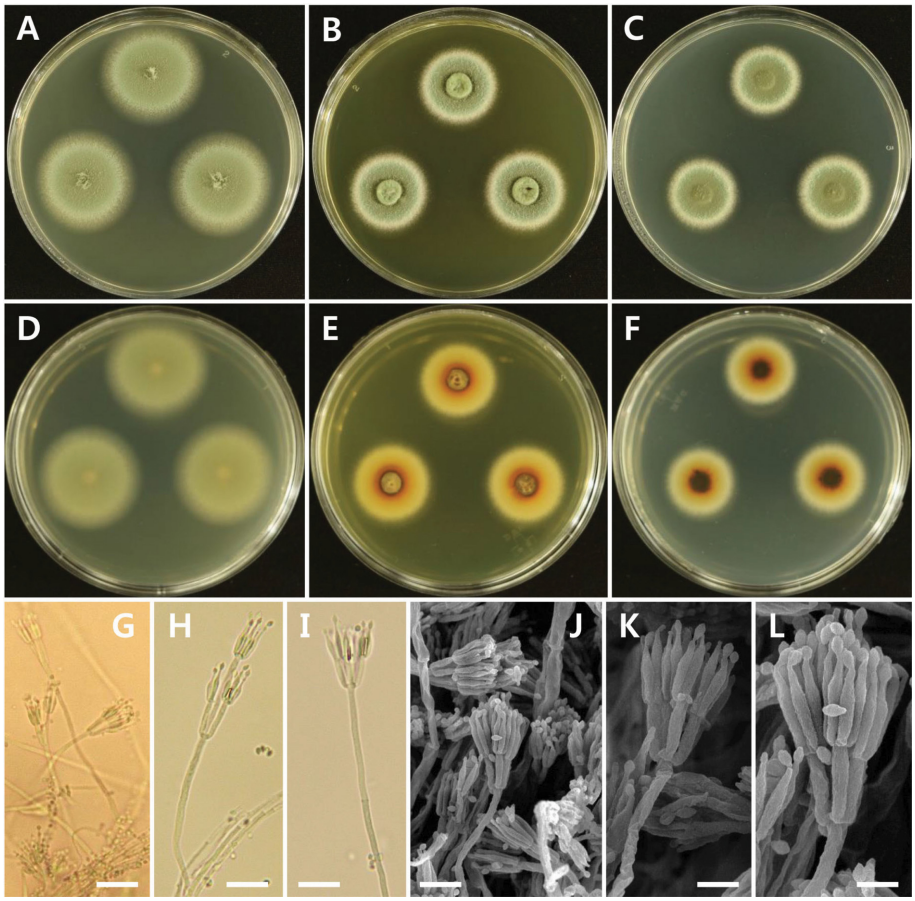

Fig. 5. Morphological characteristics of the *Talaromyces amestolkiae* isolate, EML-NCP50. Colonies on malt extract agar; B, E, Colonies on yeast extract sucrose agar; C, F, Colonies on Czapeck yeast autolysate agar. A~C, Obverse view; D~F, Reverse view; G~K, Conidiophores; L, Conidiophore and conidia (scale bars: G = 50 μm, H~J = 20 μm, K, L = 10 μm).

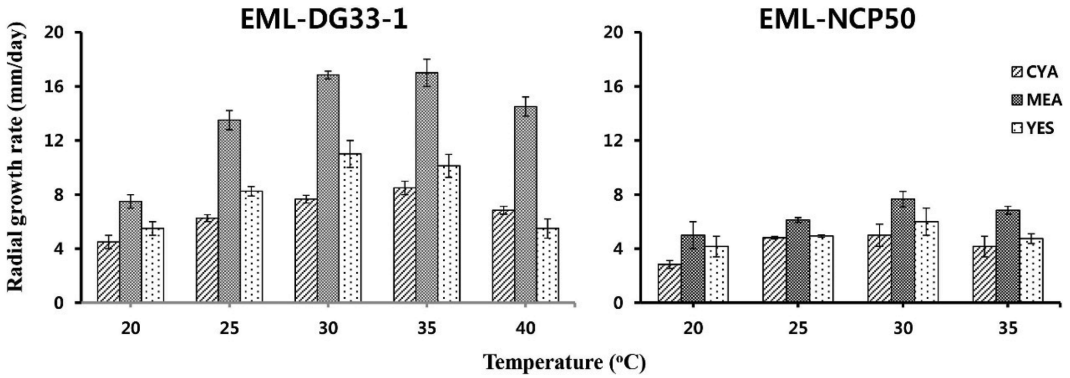

Fig. 6. Effect of temperature and culture medium on mycelial growth of *Paecilomyces variotii* EML-DG33-1 and *Talaromyces amestolkiae* EML-NCP50. Mycelia were grown on malt extract agar (MEA), Czapeck yeast autolysate agar (CYA), and yeast extract sucrose agar (YES) at different temperatures, as indicated.

microscope and SEM. Densely textured conidiophores were borne solitary or in small fascicles, simple or branched, smooth-walled, and pale to light brown in color (Fig. 5G and 5J). Conidia were smooth-walled, single-celled, ellipsoidal, and their size varied in the range of 2.5~4.0 × 1.0~3.5 µm (Fig. 5L). Metulae were arranged in verticils of 3~5; their length ranged from 9.5~13.0 µm and the width varied from 2.0~3.5 µm. There were 3~4 acerose phialides in total and their size varied in the range of 9.0~13.5 × 1.5~2.5 µm. Branches of stripes (2~3) were observed; they were smooth-walled, 86~201 µm long, and 2.0~3.0 µm wide.

Culture characteristics: The isolate was observed to grow over a wide range of temperatures with varying growth rates on MEA, YES, and CYA. The average growth rates on MEA, YES, and CYA were 6.5, 5.0, and 4.8 mm/day, respectively. Optimal growth was observed around 25~30°C, slow growth was observed at below 20°C, and no growth at 40°C (Fig. 6).

Specimen examined: Republic of Korea, Jeonnam Province, garden of the Chonnam national University located in Gwangju (35°10′ N, 126°55′ E), from a figs leaf, June 2014 (EML-NCP50), and deposited at the Culture Collection of NIBR, Incheon, as KOSPFGC000002029.

DISCUSSION

In this study, we combined a morphological description with a multigene analysis to assess the phylogenetic placement of a poorly reported species, *Paecilomyces variotii* in Korea. Although the species of *Paecilomyces variotii* has been only mentioned in several studies in Korea [15, 21, 28, 29], there were no phylogenetic placement analyses or morphological descriptions or official record. Especially, the species of *P. variotii* has been used as a test microorganism in Korea, but it has not been described as undescribed species [30]. Because of lack of clear information about the species of *P. variotii*, confirmation of this species as undescribed species in Korea based on the detailed descriptions of the morphology and multigene phylogenetic analyses are required.

Dung is not only a rich source of nutrients, including carbohydrates, nitrogen, vitamins, minerals, and growth factors, but also contains a high amount of water with a neutral pH of around 6.5 [31, 32]. Thus, it is considered a good substratum for fungal growth in a niche. A number of studies on the diversity of fungi have been reported with regard to different animal dung substrates [33-35]. Nyberg and Persson [36] observed 24 species in 14 genera in mouse dung, whereas Richardson [37] found 32 species in 17 genera from the dung of sheep, deer, cattle, rabbit, hare, and grouse. Although species of rat are among the most common animals worldwide, there is lack of information regarding the *Paecilomyces* species occurring in rat dung. Recently, the species *P. variotii* was isolated from the hair of golden hamster by Bagy *et al.* [38]. Notably, their study indicated that *P. variotii* and *Aspergillus niger* were the dominant groups of fungi recovered from the hair of golden hamsters. Furthermore, many species of fungi that have been isolated from dung produce secondary metabolites [32, 39, 40], such as appenolides A~C and coniochaetone-A, produced by *Podospora appendiculata* isolated from deer dung and *Coniochaeta saccardoi* isolated from lemming dung, respectively.

The ability of different species of the genus *Paecilomyces* to produce mycotoxin and other biological metabolites has been reported in previous studies [9]. According to Bokhari *et al.* [41], *P. variotii* produces the genotoxic mycotoxin, patulin. Peptide mycotoxins known as leucinostatins, with activity against fungi, are extracted from *P. lilacinus* and possess high toxicity to experimental animals [42]. In addition, *Paecilomyces* was used as a biological control. Perveen *et al.* [43] reported that the *Paecilomyces* species exhibit antifungal activity against *Sclerotium rolfsii* and *Pythium aphanidermatum*; whereas, *P. lilacinus* affects nematodes that attack plant roots [44].

In recent years, endophytes have received considerable scientific attention because they have been recognized as biological control agents. Vaz *et al.* [45] screened endophytic fungi from the leaves of *Myrciaria floribunda*, *Alchornea*

castaneifolia, and *Eugenia* aff. *bimarginata* to examine their antimicrobial activity against pathogenic microorganisms. Thirty-eight fungal extracts exhibited antimicrobial activity against at least one of the target microorganisms tested. Similar results were obtained by Paul *et al.* [46, 47], who showed that non-pathogenic endophytic fungi might reduce the growth of pathogenic *C. acutatum*, *F. oxysporum,* and *Phytophthora capsici*. In this study, the isolate EML-DG33-1 showed antifungal activity against *A. alternata* and *F. oxysporum*. The endophytic isolate EML-NCP50, weakly to moderately inhibited the growth of eight fungal pathogens (data not shown). This result is consistent with previous reports demonstrating that many endophytes exhibit antifungal activity against different pathogenic fungi [45]. Our study suggests that the strains, EML-DG33-1 and EML-NCP50, might be a source of biologically active secondary metabolites and biological control agents.

In addition, in the present study, we demonstrated the thermal tolerance of this EML-DG33-1 strain. In comparison with previous results [7], the colony morphology and cultural characteristics of the present isolate on MEA medium was similar to that of *P. variotii*.

Based on the sequences of the 18S and ITS rDNA regions, Luangsa-ard *et al.* [48] and Inglis and Tigano [49] demonstrated that *Paecilomyces* is polyphyletic across two Ascomycetes orders, the Eurotiales and the Hypocreales. Luangsa-ard *et al.* [48] analyzed the 18S rDNA and showed that the species *P. variotii* and its thermophilic relatives belong to the order Eurotiales (Trichocomaceae), and the mesophilic species related to *P. farinosus* are in the order Hypocreales (Clavicipitaceae and Hypocreaceae). In another aspect, Samson *et al.* [9] studied the genus *Byssochlamys* and its *Paecilomyces* anamorphs using a polyphasic approach to differentiate the species by analyzing each of ITS region, parts of the Tub2 and calmodulin gene. Our results showed that the strains EML-DG33-1 and EML-DG33-2 belonged to a *variotii* group containing *P. variotii* species. There was only two RPB2 sequences data in the *Paecilomyces* & *Byssochlamys* clade available in GenBank (Fig. 1). The results of phylogenetic analysis showed that this species belongs to the same clade presented by Houbraken *et al.* [27].

To our knowledge, the genus *Talaromyces* has been frequently isolated from soil and root aquatic in Korea [50, 51]. Figs trees are rarely found in Korea. There have been no studies of fungal endophytes on the host in Korea. Thus, this finding suggest that figs tree may be useful source of fungal endophytics. Endophytic microorganisms have been discovered in all plant families, representing various species in different climatic regions of the world [52]. Species of fig tree (*Ficus* sp.) are native throughout the tropical region with several species extending into the semi-warm temperate zones. In 2001, Suryanarayanan and Vijaykrishna [53] isolated the endophytes from the aerial root of fig tree (*Ficus benghalensis*) in India. Wang *et al.* [54] investigated the fungal diversity on fallen leaves of *Ficus* in northern Thailand in 2008.

So far, several studies to evaluate and compare the phylogenetic relationships between some species belonging to the genus *Talaromyces* have been carried out, using the ITS region, β-tubulin, and RPB2 [18, 19]. Phylogenetic analyses of three genes showed that EML-NCP50 belonged to the genus *Talaromyces*, including *T. amestolkiae*, *T. stollii*, and *T. ruber*. Morphological characteristics of the isolate EML-NCP50 were similar to those of *T. amestolkiae* described by Yilmaz *et al.* [19]. Therefore, these results confirmed that the isolate EML–NCP50 belongs to the species *T. amestolkiae* within sect. *Talaromyces*.

Some genera including *Paecilomyces* and *Talaromyces* belonging to Eurotiales within class Ascomycetes are polyphyletic (Fig. 1). Thus, to include unrecorded species in a national species list, it is very important to confirm the first records of poorly known species based on the detailed morphological descriptions and the current molecular phylogenetic placement analyses.

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