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Paecilomyces echinosporus sp. nov., a species isolated from soil in China

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Abstract — During a survey of entomopathogenic fungi in China, a new species of *Paecilomyces* was isolated from a soil sample collected from Anhui province in China. It is differentiated from previously described species based on the morphology of its minutely echinulate conidia and conidiophores that possess penicillate phialides. Phylogenetic analyses with ITS region indicate that it is distantly related to *Isaria* and a close relative of *P. carneus*. The new species, *Paecilomyces echinosporus,* is presented with its Latin diagnosis, English description, and illustration. The type isolate and holotype are deposited in the Research Center for Entomogenous Fungi of Anhui Agricultural University (RCEF).

Key words — taxonomy, morphological characteristics, molecular identification

Introduction

The genus *Paecilomyces* was established by Bainier in 1907 and differentiated from the genus *Penicillium* Link by its colony that lacks green color, cylindrical conidiogenous cells, and the slime mass of spores (Samson 1974). The generic concept of *Paecilomyces* was later expanded to include species of genera *Isaria* and *Spicaria* that possess a conidiogenous structure similar to that of *P. variotii*, the type species of *Paecilomyces* (Brown & Smith 1957). The most comprehensive monographic work (Samson 1974) divides *Paecilomyces* species into two sections (i.e., *P.* sect. *Paecilomyces* and *P.* sect. *Isarioidea*) based on their teleomorphic affinities, colony color, odor, and growth temperature.

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Conducting phylogenetic analyses based on the small rDNA subunit to arrive at a natural classification of *Paecilomyces*, Luangsa-ard et al. (2004) showed that *Paecilomyces* is polyphyletic and represents two distantly related classes (i.e., *Sordariomycetes* and *Eurotiomycetes*). As a result, *P.* sect. *Isarioidea* was revised taxonomically with the lectotypification and formal conservation of the generic name, *Isaria* (Gams et al. 2005, Hodge et al. 2005). Following phylogenetic analyses of *P.* sect. *Isarioidea* using the β-tubulin gene and ITS region, ten species of *P.* sect. *Isarioidea* were transferred to *Isaria*.

Liang et al. (2005) reviewed 32 known species of *Paecilomyces* in China, where 12 novel *Paecilomyces* species were reported based on the survey of soil-borne filamentous fungi from 2003–06 (Liang et al. 2009). Of these, six monophialidic species were transferred to a new genus, *Taifanglania,* based on their morphological characteristics and molecular analyses. In this study, we report a new species of *Paecilomyces* that was found during a survey of entomopathogenic fungi in soil in Anhui province, China. The morphological examination and phylogenetic analysis revealed a species with features that differed from previously described *Paecilomyces* species and was distantly related to some *Isaria* taxa. This new species is described below as *Paecilomyces echinosporus*.

Materials and methods

Sample collection and strain isolation

Strain RCEF4111 was isolated from soil samples collected from Qimen, Anhui province, China. A 5 g sample of soil was mixed with 100 ml of sterile distilled water containing 0.05% (v/v) Tween 80. The soil suspension was diluted to a concentration of 10^{-2} after shaking for approximately four hours. A 200 µl of the soil suspension was plated on one plate with the D0C2 selective medium (Shimazu & Sato 1996), and incubated at 25°C for approximately 5 days until the colonies were formed. Colonies that formed conidiogenous structures were transferred to SDAY (Sabouraud's dextrose agar with yeast) slants.

Strain identification

Strain RCEF4111 was transplanted onto Czapek agar, potato dextrose agar (PDA), and Sabouraud's agar according to Brown & Smith (1957) and Samson (1974), and then was incubated at 25°C for 14 days. The isolated fungus was examined using classical mycological techniques based on growth rate, as well as macroscopic and microscopic characteristics. The strain was also tested to investigate its ability to grow on PDA at 35°C. The type strain, RCEF4111 (dried RCEF4111-DPC1, holotype), has been deposited in the Research Center for Entomogenous Fungi (RCEF), Anhui Agriculture University, China.

DNA extraction

For DNA extraction, spores were inoculated to Petri dish containing SDAY medium overlaid with a disc of sterilized cellophane. After incubating at 25°C for approximately

Table 1. Accession numbers, strain numbers, and origins of *Paecilomyces* spp. and other taxa used for phylogenetic analysis.

GenBank #	Name	Strain #	References
AJ786573	*Cordyceps militaris* (L.) Link	3856.H.	Stensrud et al. (2005)
AY624168	*Isaria amoenerosea* Henn.	CBS 107.73 T	Luangsa-ard et al. (2005)
AY624172	*I. cateniannulata* (Z.Q. Liang) Samson & Hywel-Jones	CBS 152.83	Luangsa-ard et al. (2005)
AY624175	*I. cicadae* Miq.	BCC 2574	Luangsa-ard et al. (2005)
AY624176	*I. coleopterorum* (Samson & H.C. Evans) Samson & Hywel-Jones	CBS 102.73	Luangsa-ard et al. (2005)
AY624181	*I. farinosa* (Holmsk.) Fr.	CBS 111113	Luangsa-ard et al. (2005)
AY624184	*I. fumosorosea* Wize	CBS 107.10	Luangsa-ard et al. (2005)
AY624186	*I. javanica* (Frieder. & Bally) Samson & Hywel-Jones	CBS 134.22	Luangsa-ard et al. (2005)
AY624196	*I. tenuipes* Peck	ARSEF 5135	Luangsa-ard et al. (2005)
AY624202	*Mariannaea camptospora* Samson	CBS 209.73	Luangsa-ard et al. (2005)
AF135210	*Metarhizium anisopliae* (Metschn.) Sorokīn var. *anisopliae*	FI1029	Driver et al. (2000)
AF368270	*M. cylindrosporum* Q.T. Chen & H.L. Guo	ACCC 30114 T	Huang et al. (2004)
AF138270	*M. flavoviride* W. Gams & Rozsypal var. *flavoviride*	FI 38	Driver et al. (2000)
AF368501	*Nomuraea rileyi* (Farl.) Samson	RCEF 0292	Huang et al. (2004)
AY624170	*Paecilomyces carneus* (Duché & R. Heim) A.H.S. Br. & G. Sm.	CBS 399.59	Luangsa-ard et al. (2005)
AY624174	*P. cinnamomeus* (Petch) Samson & W. Gams	CBS 398.86	Luangsa-ard et al. (2005)
GU108582	*P. echinosporus* Ming J. Chen, G.H. Sung & B. Huang	RCEF 4111	In this study
AJ536552	*P. gunnii* Z.Q. Liang	ZSU 20872	Unpublished
AY624189	*P. lilacinus* (Thom) Samson	CBS 284.36 T	Luangsa-ard et al. (2005)
AY624193	*P. marquandii* (Massee) S. Hughes	CBS 182.27 T	Luangsa-ard et al. (2005)
AY624192	*P. niphetodes* Samson	CBS 364.76	Luangsa-ard et al. (2005)
AY624194	*P. penicillatus* (Höhn.) Samson	CBS 448.69	Luangsa-ard et al. (2005)
AY624197	*P. viridis* Segretain et al. ex Samson	CBS 348.65	Luangsa-ard et al. (2005)
EU004811	*Taifanglania curticatenata* (Z.Q. Liang & Y.F. Han) Z.Q. Liang et al.	HC 125-2 T	Liang et al. (2009)

7 days, genomic DNA was extracted from the mycelia scraped from the cellophane using benzyl chloride (Zhu et al. 1994). The extracted DNA was stored in 100 μL TE buffer (10mM Tris-HCl, PH8.0; 1mM EDTA) at 4°C, and was diluted 10-fold with TE buffer for the following PCR reactions.

PCR amplification and determination of ITS sequencing

The PCR amplification of ITS region was performed using the primers of ITS5 and ITS4 (White et al. 1990). The PCR conditions are as follows: 94°C for 5 mins, 35 cycles of 94°C for 1 min, 52°C for 1 min, 72°C for 2 mins and 72°C for 10 mins. The PCR reaction

was conducted in 25 μL volume with the following components: 2.5 μL of 10 × reaction buffer, 0.5 μL of each dNTP, 1 μL of each primer, and 2 units of Taq DNA polymerase, 2 μL of the diluted DNA and 16.8 μL of ddH_2O. The resulting PCR product was examined on 1.2% TBE agarose gel stained with ethidium bromide. After purifying PCR product using EasyPure quick gel extraction kit (TransGen Biotech), DNA sequencing was carried out at Sangon Company (Shanghai, China) and the resulting ITS sequence of RCEF 4111 was submitted to GenBank with accession number GU108582.

Sequence alignment and phylogenetic analysis

DNA sequences that are generated in this study and downloaded form GenBank were aligned using Clustal X 1.81 (Thompson et al. 1997). The alignment was manually adjusted to maximize homology. Maximum parsimony analyses were conducted using PAUP* 4.0b10 (Swofford 2002) with 1,000 replicates of heuristic search of random sequence additions, branch swapping by tree bisection-reconnection (TBR) and MulTrees in effect. In the parsimony analyses, unambiguously aligned gaps were treated as a new state and all characters were equally weighted. Branch support was estimated by bootstrapping using 1,000 replicates of 10 replicates of heuristic search with the same option (Felsenstein 1985). We also performed a BLAST search with the obtained sequence of the new taxon as a query to find the close relatives in GenBank database.

Results

Taxonomy

Paecilomyces echinosporus Ming J. Chen, G.H. Sung & B. Huang, **sp. nov.** Fig. 1

MycoBank 518113; GenBank GU108582

Coloniae in agaro Czapekii ad 30–37 mm diam post 14 dies 25°C, in medio modice sulcatae, albae, pulverulentae, margine regulari; reversum luteolum; 35°C haud crescit. Hyphae vegetativae hyalinae, septatae, ramosae, leves, 2.0–3.5 μm latae. Apparatus conidialis elongatus vel compactus, seu phialides singulae seu capitula verticillos ramorum et phialidum ferentia; stipites ex hyphis aeriis orientes, vulgo 45–95 × 2.5 μm. Phialides ad quinae verticillatae, 9.5–15.5 × 2.0–3.0 μm, e basi cylindrica et collulo angusto minus quam 0.5 μm lato composita. Conidia unicellularia, minute echinulata, subglobosa vel elllipsoidea, 2.7–5.0 × 2.0–3.0 μm. Chlamydosporae absentes.

Holotype — RCEF4111 was isolated by B. Huang & N. Zhou from soil of Qimen, Anhui province, China, in March, 2008, deposited in the Research Center for Entomogenous Fungi (RCEF).

Colony on Czapek agar attaining a diameter of 30 to 37 mm within 14 days at 25°C, slightly ridged at the center, white, powdery, regular in the margin; reverse yellowish. Colony growth not observed at 35°C. Vegetative hyphae hyaline, septate, branched, smooth-walled, 2.0–3.5 μm wide. Conidial structures elongated to compact, varying in complexity from single detached phialides to heads with a terminal whorl of phialides and whorl of branches, conidiophores arising from aerial hyphae, normally 45–95 × 2.5 μm. Phialides up to 5 in a whorl, 9.5–15.5 × 2.0–3.0 μm, consisting of a cylindrical basal portion, tapering into a thin neck, less than 0.5 μm wide. Conidia one-celled, minutely echinulate, subglobose to ellipsoidal, 2.7–5.0 × 2.0–3.0 μm. Chlamydospores absent.

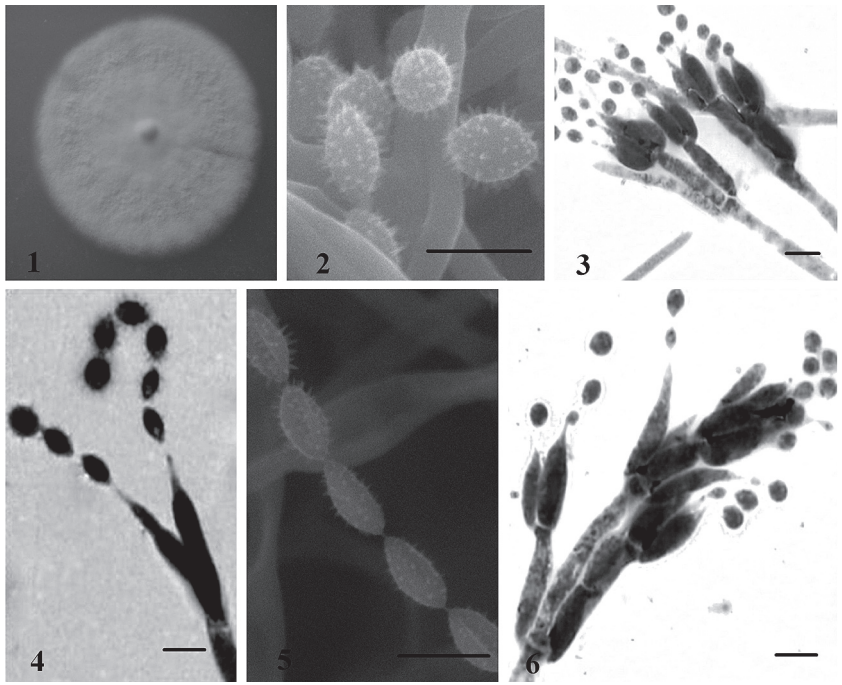

Fig. 1. Colony and conidiogenous structure of *Paecilomyces echinosporus* (Bars = 5μm). 1—Colony on Czapek agar; 2, 5—echinulate conidia; 3,4, 6—phialides and echinulate conidia.

Molecular Characteristics of *Paecilomyces echinosporus*

The ITS (ITS1, 5.8S rDNA, and ITS2) region is 538 bp long. ITS dataset with 23 strains contains 732 characters including 259 parsimony-informative characters. The single tree generated from maximum parsimony (TL= 1078, CI= 0.5965, HI = 0.4035, RI = 0.6432, RC = 0.3836) is shown in Fig. 2. The phylogenetic tree inferred from the ITS sequence data clusters isolate RCEF4111 with *P. carneus* with 95% bootstrap support. In addition to the phylogenetic analysis, we performed a BLAST search with ITS sequence of *P. echinosporus* as a query. Search results imply that *P. echinosporus* is most comparable to *P. marquandii* (ARSEF 3047, EU553322, 97%), *P. lilacinus* (CG 348, EU553317, 97%), and *P. carneus* (CBS 399.59, AY624170, 90%). A NCBI BLAST search yielded a sequence max identity of the *P. echinosporus* ITS sequence of 100% with Malian strain ARSEF 3047 and Brazilian strain CG 348 and showed the closest relative of these two isolates as *P. carneus* (GC 525, EU553292, 91%). Therefore, ARSEF 3047 and CG 348 appear either closely related to or conspecific with *P. echinosporus,* indicating the presence of the species in Brazil and Mali.

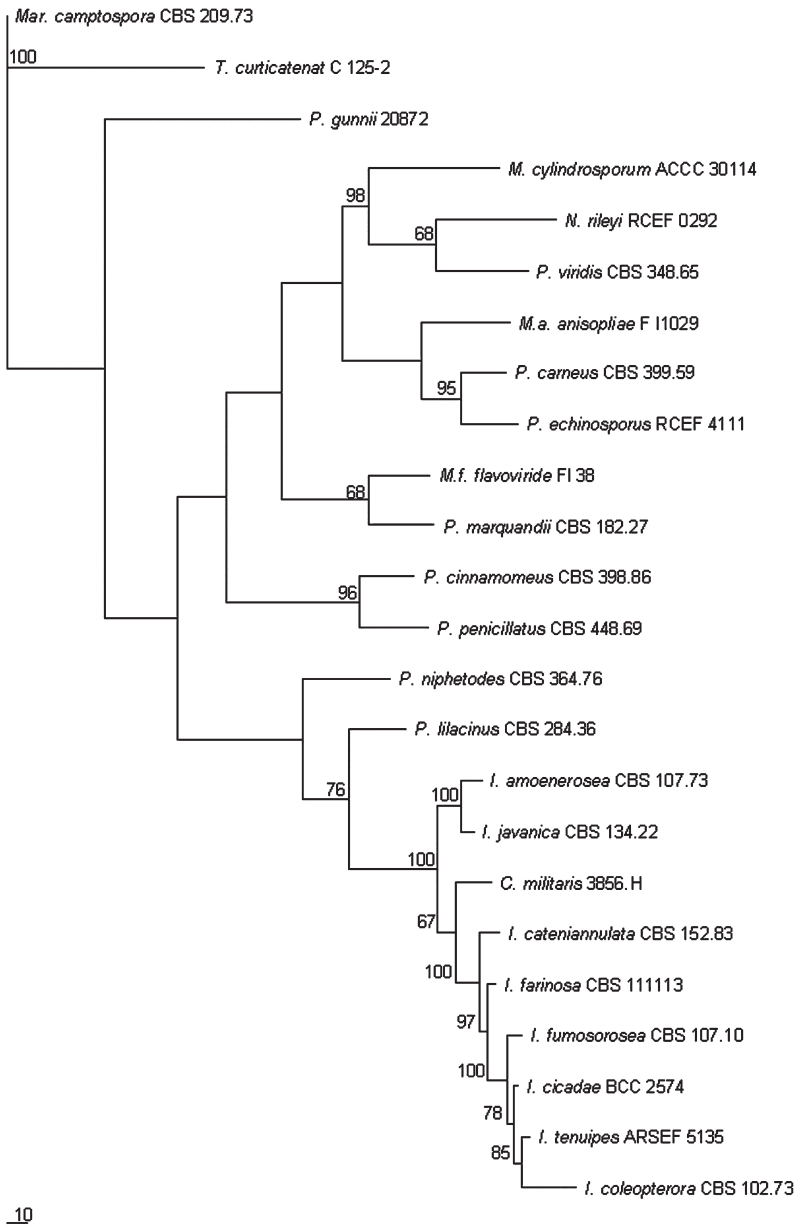

Fig. 2. Phylogenetic tree generated from parsimony analysis based on ITS rDNA sequences. Numbers at the nodes give bootstrap support derived from 1000 replicates. *Mariannaea camptospora* was used as outgroup.

Discussion

In *Paecilomyces*, based on the morphological characters, species that produce echinulate or rough conidia include *P. carneus*, *P. gunnii*, *P. marquandii,* and *P. lilacinus* (Samson 1974, Liang 1985, Han et al. 2005). Although the conidia are echinulate in both *P. carneus* and *P. gunnii*, they can be differentiated by the color of the reverse side of the colony in culture; *P. carneus* is dark green, while *P. gunnii* produces a dark brown colony and chlamydospores. Meanwhile, conidia are rough in *P. marquandii* and *P. lilacinus* but possess purple or vinaceous conidial heads. In addition, Chlamydospore-like cells are usually present in *P. marquandii* and *P. lilacinus* conidiophores are pigmented and rough-walled, while *P. echinosporus* does not produce chlamydospores and possesses white and smooth conidiophores.

Our phylogenetic analysis of *Paecilomyces* species clusters *P. echinosporus* and *P. carneus* together in a clade and distinctly related to the other four species that produce echinulate or rough conidia (Fig. 2). Although the new species resembles *P. carneus* in the echinulate conidia, *P. echinosporus* and *P. carneus* share only 91% sequence similarity. In morphological comparison, *P. echinosporus* produces conidiophores with penicillate branches and short-necked phialides and a white colony with a yellow reverse. In contrast, *P. carneus* produces conidiophores with verticillate branches and phialides that taper into a thin long neck and a pink (after sporulation) colony with a mostly green to dark green reverse. Our combined traditional morphological study and molecular analyses identify strain RCEF4111 isolated from soil sample as a new species of *Paecilomyces, P. echinosporus.*

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