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Morphological traits and molecular analysis for *Geomyces fujianensis* sp. nov. from China

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Abstract

During a survey of the keratinolytic fungi from China, a new species, described here as *Geomyces fujianensis*, was isolated from the soil of a tree hole in the trunk of a tree in Drum Hill, Fuzhou, Fujian Province, China. It differs from other *Geomyces* species by its obovoid and subglobose conidia (2.5–7.5 × 2.5–5.0 μm), and the absence of intercalary conidia and pigments. Phylogenetic analysis using the ITS region of ribosomal DNA confirmed that *G. fujianensis* is distinct from other species. A synopsis of the morphological characters of the new species is provided.

Key words – acute angle branch – keratin – morphology – phylogeny

Introduction

The genus *Geomyces* was introduced by Traaen (1914) including the type species *G. auratus* Traaen as well as *G. cretaceus*, *G. sulphureus*, and *G. vulgaris*. Several more species have been described subsequently (Dal 1957, Sigler & Carmichael 1976, Oorschot 1980, Hocking & Pitt 1988, Li & Gui 1989, Gargas et al. 2009). Minnis (2013) transferred three species to *Pseudogymnoascus* in a taxonomic revision of *Geomyces* and allies, leaving seven species currently in *Geomyces* (Luo et al. 2016). Despite the small number of described species, members of *Geomyces* have a global distribution. They are especially common in the soils of temperate and high-latitude ecosystems (Kirk et al. 2008), but are also widespread in decaying wood, air, marine environments, as endosymbionts of animals, and as endomycorrhiza in many plants (Dalpé 1989). Some species are keratinolytic, breaking down hair, nails and feathers (Hayes 2012). They are also used as a source of cellulose for food and natural pigments (Duncan et al. 2008, Valmaseda et al. 1989).

A recent survey of keratinolytic fungi in China included the isolation of a member of *Geomyces*, from the soil of a tree hole, in Drum Hill, Fuzhou, Fujian Province, China. Based on its morphological characteristics and phylogenetic analysis, we concluded that it represents a new species and describe it here as *Geomyces fujianensis*.

Materials & Methods

Fungal isolation

Geomyces specimen (G242) was isolated from the soil of a tree hole in Drum Hill, Fuzhou, Fujian Province, China. Soil samples were added to sterilize powdered feathers and kept under

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moist conditions at 5 °C approximately for one month. When fungal growth was observed, the feather powder was mixed with 9mL sterilized water in an Erlenmeyer flask, and 1mL suspensions were evenly spread on plates containing Martin's medium. Plates were incubated at 25 °C for 14d. Pure cultures picked from these plates were then transferred to potato dextrose agar (PDA) slants at 4 °C and the cultures with sterilized 30% glycerol were stored at −70 °C until further use, at the Institute of Fungus Resources, Guizhou University (GZAC).

Morphological examination

Isolates were transferred to PDA and Czapek agar, and incubated at 25 °C for 14 days prior to examination. Fungal micro characteristics were examined with a Motic microscope (Motic Co., Guangzhou, China) and photographed with a Motic microscope (Guangzhou, Motic Co., China). Diagnostic features were then illustrated on the basis of these observations. Fungi were identified based on colony characteristics and conidiogenous structures (Oorschot 1980).

DNA extraction, PCR amplification and sequencing

The strain G242 was incubated on PDA, and fresh sporulating cultures were used for DNA extraction and PCR amplification of the Internal transcribed spacer (ITS) region of ribosomal DNA using primers ITS5 (5'- GGTGAGAGATTTCTGTGC -3') and ITS4 (5'-TCCTCCGCTTATTGA TATGC-3') (White et al. 1990) were carried out as previously described by Liang et al. (2011). Taq polymerase and dNTPs were manufactured by Sangon (Sangon Biotech Co. Ltd, Shanghai, China). PCR products were purified using the UNIQ-10 column PCR Products Purification kit (no. SK1141; Sangon) according to the manufacturer's protocol, and sequenced with the same primers used for PCR, by Sangon. The full ITS sequence (G242) was deposited in GenBank (KX845696).

DNA sequences generated in this study were assembled and edited using Lasergene software (version 6.0, DNASTAR). Sequences of the ITS region from 39 taxa (with *Saccharomyces cerevisiae* as an outgroup) were downloaded from GenBank. Multiple sequence alignment was carried out using MAFFT (Katoh et al. 2013) with the default settings. Manual editing of sequences was performed in MEGA6 (Tamura et al. 2013).

Phylogenetic analyses were performed using MrBayes 3.2 (Ronquist et al. 2012) and MEGA6. For the Bayesian MCMC analysis, the GTR+I+G nucleotide substitution model was used, and two runs were executed simultaneously for 10 000 000 generations, saving trees every 500 generations. After the analysis finished, each run was examined with the program Tracer v1.5 (Drummond & Rambaut 2007) to determine burn-in and confirm that both runs had converged. For the parsimony analysis, the MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei & Kumar 2000) with search level 1, in which initial trees were obtained with ten replicates of random sequence addition. Bootstrapping was carried out for MP analysis using 1000 pseudoreplicate datasets in each case. Default parameters were used where they are not specified here. The final alignment and phylogenic tree is available from TreeBASE under submission ID 20139.

Results

Taxonomy

Geomyces fujianensis W.H. Chen, G.P. Zeng, Y. Luo, Z.Q. Liang & Y.F. Han, **sp. nov.** Fig. 1
MycoBank 818287
FOF02661

Etymology – refers to the region, from Fujian Province

Colonies growing at moderate speed on PDA, attaining 43 mm in diam. after 14d at 25 °C; short and fluffy, initially white, usually becoming light pink at the center and pale grey in a clearly marked outer zone; margin white, irregular; reverse dark brown at the center and yellow brown outward. Hyphae septate, hyaline, smooth-walled, 1.1–3.2 μm wide. Conidiophores abundant, always forming verticillate and opposite branches with an acute angle to the axis near the apex.

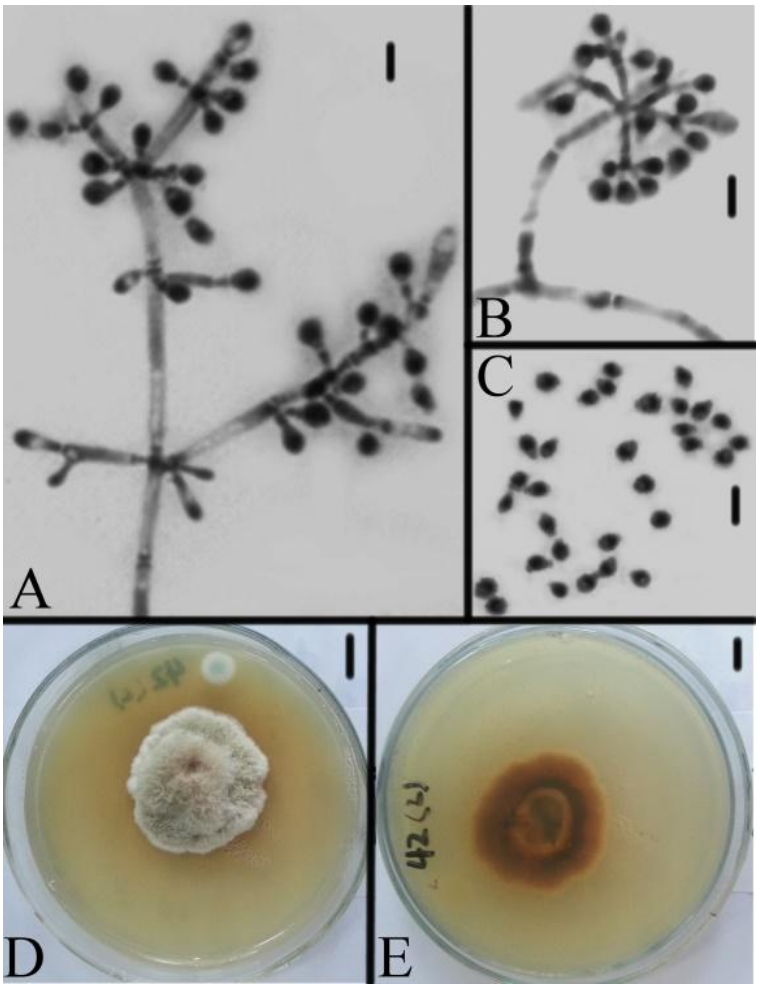

Fig. 1 – Conidiogenous structures and colonies of *Geomyces fujianensis* (Holotype G242.1). A, B: Conidiogenous structures. C: Conidia. D, E: Colonies on PDA. – Bars: A, B, C= 10 µm; D, E=10 mm.

Terminal conidia subhyaline, smooth-walled, obovoid to subglobose, occasionally clavate, 2.5–7.5 × 2.5–5.0 µm, with basal scars 1.1–2.2 µm. Chlamydospores absent.

Type – China, Fujian Province, Fuzhou, N26°03′29.02″, E119°23′25.94″. Holotype G242.1 was isolated from the soil of a tree hole in Fujian Province.

Distribution – Fujian Province, China

Material examined – China, Fujian Province, Fuzhou, from soil in a tree hole, 15 Jul 2015, Y. Luo G242 (G242.1, holotype).

Notes – We compared *G. fujianensis* with similar species of this genus. Three species in *Geomyces* show some similarity to *G. fujianensis*, each having obovoid conidia, *Geomyces guiyangensis* Z.Q. Liang, Y. Luo & Y.F. Han, *Geomyces laevis* Zhong Q. Li & C.Q. Cui, *Geomyces vinaceus* Dal Vesco, but *G. fujianensis* can easily be distinguished from them by its absence of intercalary conidia and pigment.

Phylogenetic analyses

The alignment obtained for the ITS region was 463 bp long. Phylogenies obtained using Maximum Parsimony and Bayesian MCMC analyses were mostly congruent. The majority of branches were strongly supported in both analyses. *Geomyces fujianensis* G242 and *Geomyces pannorum* clustered with moderate bootstrap support (63/0.59).

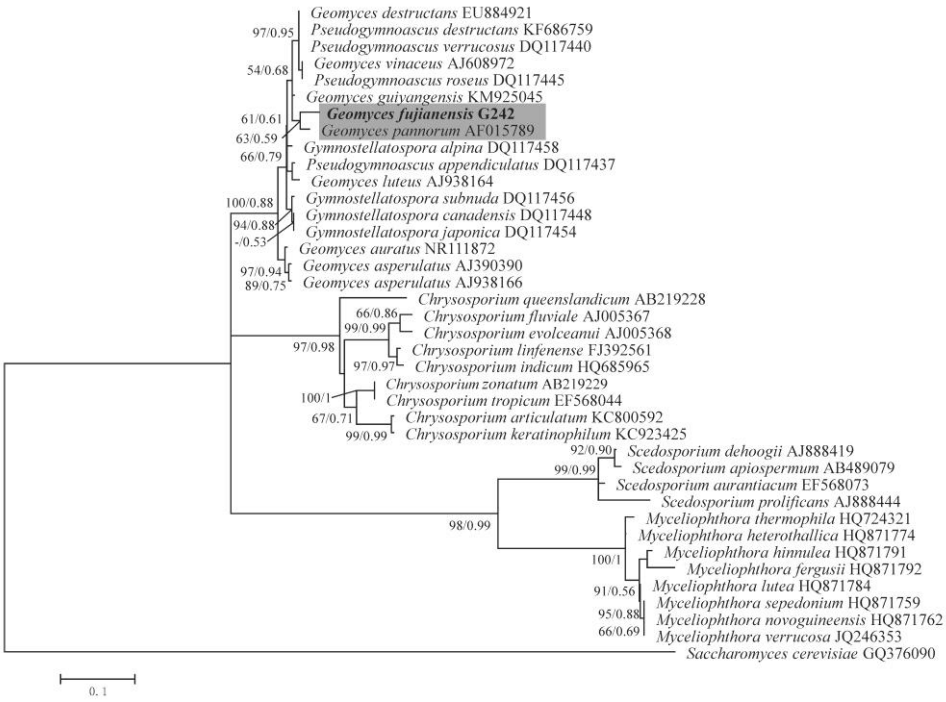

Fig. 2 – Phylogenetic tree generated from maximum parsimony and Bayesian method analysis based on the ITS sequences. Statistical support values of Bayesian posterior probabilities (≥0.5) and Maximum-parsimony bootstrap values (≥50 %) are shown at nodes.

Discussion

As described by Oorschot (1980), the main diagnostic criteria of the genus *Geomyces* are colonies that spread only slightly, often with scattered tufts of aerial hyphae. Here, fertile hyphae were frequent, positioned at acute angles, often once or twice verticillate with 2–4 branches per whorl, showing repeated verticillate branching. *Geomyces fujianensis* G242 thus fulfills the criteria for assignment to *Geomyces*. Three species in *Geomyces* show some similarity to *Geomyces fujianensis*, each having obovoid conidia (Table 1), but *Geomyces fujianensis* can easily be distinguished from them by its absence of intercalary conidia and pigment.

Geomyces taxonomy is primarily based on morphological characters. As molecular phylogenetic has developed, numerous loci have been used in analyses of *Geomyces* and its allies, including ITS, LSU, MCM7, RPB2, TEF1; however the ITS region is typically used to resolve relationships in *Geomyces* (Rice & Currah 2006, Gargas et al. 2009, Minnis 2013). Phylogenetic results obtained here support the morphological identification of *Geomyces fujianensis* as belonging to *Geomyces*; it clustered with *G. pannorum* with moderate bootstrap support (63/0.59).

Table 1 Morphological comparison among *Geomyces fujianensis* and related species.

Species name	***Conidia (μm)***	***intercalary conidia***	***pigment***
G. guiyangensis	obovoid 5.0–7.5 × 2.5–5.0	absent	black
G. laevis	pyriform, cuneate or obovate 1.5–2.5 × 3.5–4.0	present	-
G. vinaceus	obovoid, somewhat cuneiform, or ellipsoid 3–5 × 2–3.5	present	reddish brown
G. fujianensis	obovoid, subglobose 2.5–7.5 × 2.5–5.0	absent	absent

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