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A new species of *Emericella* from Tibet, China

Li-chun Zhang[1, 2 A*], Juan Chen[2], Wen-han Lin[1] & Shun-xing Guo[2 B*]

[1] *The State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100193, People's Republic of China.*

[2] *Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, 100193, People's Republic of China*

* Correspondence to: [A] *zlclily@sina.com* & [B] *sxguo1986@163.com**

Abstract — *Emericella miraensis* sp. nov. is described and illustrated. It was isolated from the alpine plant *Polygonum macrophyllum* var. *stenophyllum* from Tibet, China, and is characterized by ascospores with star-shaped equatorial crests. The new species is distinguished from other *Emericella* species with stellate ascospores (e.g., *E. variecolor, E. astellata*) by its violet ascospores and verrucose spore ornamentation. ITS and β-tubulin sequence analyses also support *E. miraensis* as a new species.

Key words —*Aspergillus*, endophytic fungi, phylogeny, taxonomy

Introduction

Berkeley (1857) established *Emericella,* a teleomorph genus associated with *Aspergillus,* for the type species *E. variecolor* Berk. & Broome (Geiser 2009, Peterson 2012). To date, 36 species have been described and recorded worldwide (Kirk et al. 2008). *Emericella* species are usually isolated from soil (Samson & Mouchacca 1974, Horie et al. 1989, 1990, 1996, 1998, 2000; Stchigel & Guarro 1997) but sometimes also from stored foods, herbal drugs, and grains or occasionally from hypersaline water (Zalar et al. 2008) or living plants (Berbee 2001, Thongkantha et al. 2008, Zhang et al. 2011).

During a survey of endophytic fungi associated with *Polygonum macrophyllum* var. *stenophyllum* (Meisn.) A.J. Li in Tibet, China, an unidentified endophytic fungus was isolated from the roots of the alpine medicinal plant. Our morphological and molecular data confirm the fungus as a new *Emericella* species.

Materials & methods

Plant samples of *P. macrophyllum* var. *stenophyllum* were collected at 4850 m altitude from the mountain steppe of Mira Hill at Nyingchi County, in Tibet, China, in June

2010. Plant samples and their rhizosphere soils were collected and kept in plastic bags together for transporting to the laboratory.

For fungal isolation, three pieces of healthy roots of individual plants were randomly selected and removed from each of the five *P. macrophyllum* var. *stenophyllum* plants, rinsed under tap water to remove soil and litter, and washed in sterile de-ionized water. Each root was cut into 20 mm long segments, and the surfaces were sterilized in 75% ethanol for 1 min, 3% NaClO for 3 min, and 75% ethanol for 30 s, and then rinsed in sterilized water three times. These root segments were cut into approximately 1 mm long portions and inoculated on potato dextrose agar (PDA) in 90 mm diam. Petri dishes. The cultures were kept in a dark incubator at 25°C to obtain their anamorphic stages. The fungal mycelium was sub-cultured in Czapek yeast extract agar (CYA), PDA, and malt extract agar (MEA) for morphological observations.

The fungal isolates were identified morphologically from colony characters (e.g., colour, growth rate, hyphae, and margin characters) and micro-characters (conidia and ascospores) on CYA and PDA medium. Strains were incubated at 25°C for 14 d, after which colonies and mycelia were observed using a light microscope (Zeiss Axio ImagerA1) and scanning electronic microscope (SEM; JSM-6510LV, Japan). Cultures have been conserved in the Chinese General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (CGMCC).

DNA was extracted from the examined strain using the E.Z.N.A.™ Fungal DNA Mini Kit (Omegabiotek, Norcross, USA) according to the manufacturer's protocol. The internal transcribed spacer (ITS) region was amplified with primer pair ITS1/ITS4 (White et al. 1990) and the β-tubulin gene with primer pair T1/T22 (O'Donnell & Cigelnik 1997) according to Zhang et al. (2012). PCR products were examined in 1% agarose gels (mixed with goldview) by electrophoresis visualized under UV light. After purification with mini-columns (Sangon, China), purified DNA was directly sequenced in the ABI Prism 377 DNA sequencer (Applied Biosystems, USA).

Similar sequences were retrieved from GenBank using the NCBI Blast program (Altschul et al. 1997), most previously published in Peterson (2008) and Zalar et al. (2008). *Aspergillus karnatakaensis* was chosen as outgroup based on Peterson's (2008) analyses. Sequences were aligned with Clustal X 1.81. Neighbor joining (NJ) trees were constructed with the Kimura two-parameter model (Kimura 1980) and maximum parsimony (MP) trees with the close-neighbor-interchange (CNI) search method on random trees on Mega version 5.0 (Tamura et al. 2011).

Results

Taxonomy

Emericella miraensis L.C. Zhang, Juan Chen & S.X. Guo, **sp. nov.** Fig. 1

MycoBank MB 800444

Differs from *Emericella stella-maris* and *E. variecolor* by its violet ascospores with verrucose convex walls.

Type: China, Tibet, Nyingchi County, Mira Hill, 29°53′N 92°50′E, 4850 m, from roots of *Polygonum macrophyllum* var. *stenophyllum*, June 2010, Li-Chun Zhang (**Holotype**, CGMCC3.14984; GenBank, JQ268604, KC342577).

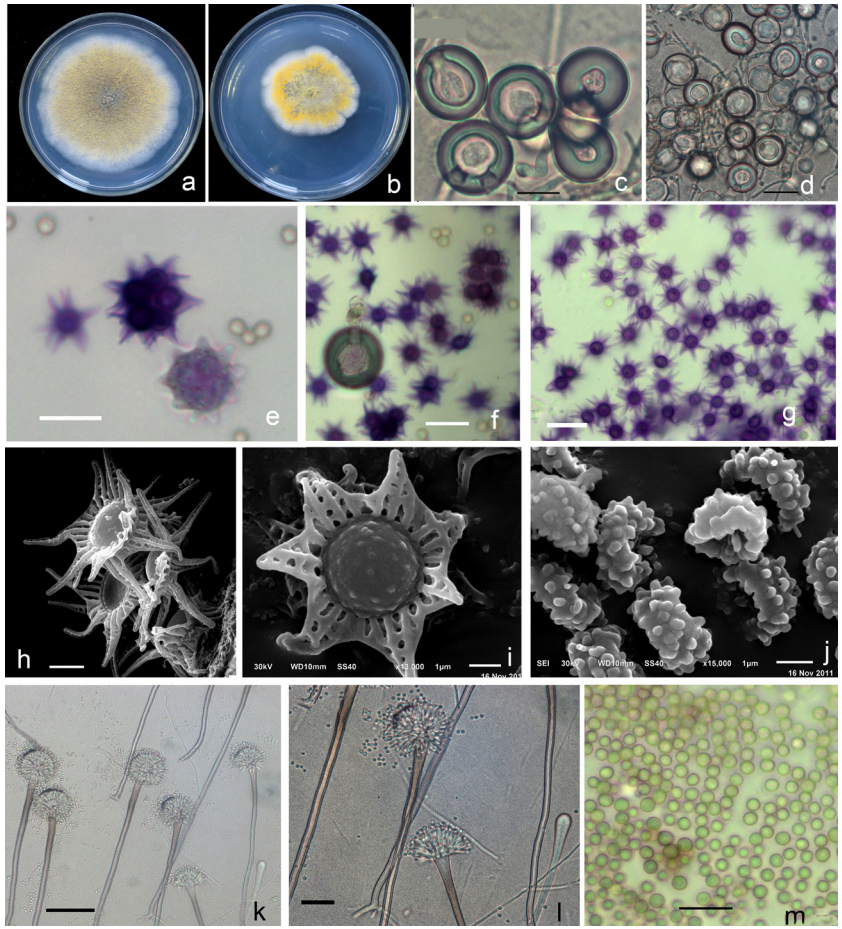

Figure 1. *Emericella miraensis* (holotype). Morphological characters. **a**. Colonies on PDA after 2 week at 25°C; **b.** Colonies on CYA after 2 week at 25°C; **c, d**. Hülle cells; **e**. Asci in different developmental stages views; **f.** Hülle cells, asci and ascospores; **g**. Stellate ascospores; **h, i**. Scanning electron micrograph of ascospores showing the side views and verrucose ornamentation on the convex surface; **j**, Scanning electron micrograph of conidia; **k, l.** Conidiophores; **m**. Conidia. Bars: c, e–g, m = 10 µm; d, l = 20 µm; h = 2 µm; i, j = 1 µm; k = 100 µm.

Etymology — *miraensis* refers to the type locality, Mira Hill.

Colonies on PDA spreading broadly, attaining 7.2–7.8 cm diam. in 14 days at 25°C. Aerial mycelium khaki, marginal mycelium white, irregular, and small amounts exudates droplets and granular produced in center after two weeks, reverse pale yellowish white, and then becoming gray purple-brown. Ascomata

abundantly produced, gray green, subglobose, with numerous, globose to ovoid shaped Hülle cells, 15–20 mm diam.

Asci 8-spored, globose, 10 × 20 μm, firstly pink-purple, becoming violet in mature. Ascospores violet, with 6–7 μm denotative crests stellate in surface view, 6.0–10.0 μm (including stellate tentacles); spore bodies subglobose, verrucose on the surface, 4.0–5.0 μm diam., convex-lens form, with two stellate, broad equatorial crests in side view; undissected part of crests ≤ 1 μm, every angle of stellate crests regularly ornamented with three longitudinal striations, transverse striation arranged irregularly between two longitudinal striations.

Anamorphic stage: Conidial heads grayish olive, radiate to short columnar. Conidiophores abundant, columnar, straightforward, stipes smooth, brownish and nonseptate, generally 275–345 μm long × 8.5–13.0 μm wide, dark colour near the conidia; vesicles abundant, radiate, subclavate to flask-shaped, hyaline to pale brownish green, 2.0–3.5 μm wide × 9.0–17.0 μm long, covered in the upper half by metulae, which are hyaline to pale brown, 15.5–18.5 × 9.0–10.0 μm; phialides flask-shaped, hyaline, 10–16.5 × 3.5–5.5 μm.

Conidia globose to subglobose, verrucose, appearing grayish green in mass, diameter 1.5–4.0 μm, strawberry appearance in SEM. Colonies on MEA spreading broadly, attaining a diameter of 5.3–5.7 cm in 14 days at 25°C; appearance same as on PDA, but ascomata arranged in concentric circles, particularly on colony margins, grayish green, edge mycelium white. Colonies on CYA spreading slowly, attaining 4.0–4.5 cm diam. after 14 d at 25°C, bright yellow, white edge, consisting of a thin mycelial felt, slightly granular in colony center due to formation of Hülle cells (thick-walled refractive cells like chlamydospores) and conidia abundant, reverse yellowish brown, edge mycelium white.

Phylogenetic analysis

The sequences for our new taxon have been deposited in GenBank. The ITS data set consisted of 28 sequences and 550 characters, of which 98 are parsimony informative sites. MP analysis produced 97 trees (TL = 139, CI = 0.813, RI = 0.834), of which one is shown in Fig. 2. NJ analysis (not shown) created similar topology to MP analysis. All *Emericella* sequences analyzed were divided into two large clades and four subclades. *Emericella miraensis* formed a well-supported clade (Clade II; BP = 100%) with other related species, such as *E. stella-maris, E. astellata, E. variecolor, E. qinqixianii, E. appendiculata,* and *E. filifera*. These taxa all have star-shaped ascospores, but are distinguished from one another by color or ascospore ornamentation (Table 1).

The β-tubulin data set contained 19 sequences and 420 characters, of which 84 were parsimony informative sites. MP analysis produced 9 trees

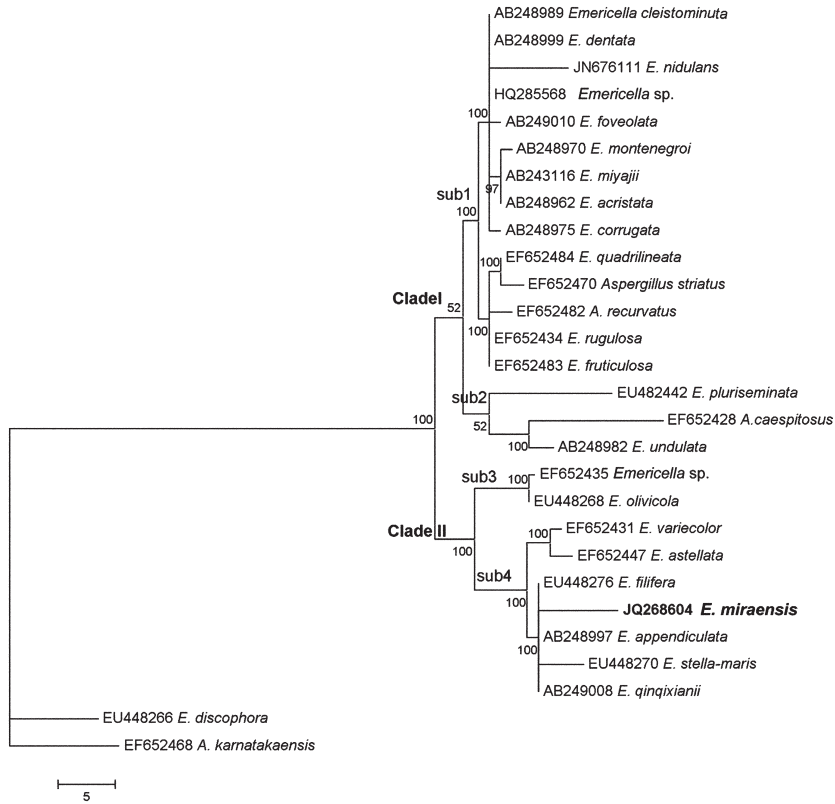

Figure 2. One of the MP trees obtained based on phylogenetic analysis of ITS sequence data of *Emericella/Aspergillus*. Numbers above branches are bootstrap values ≥ 50%.

(TL = 172, CI = 0.784, RI = 0.804; one tree is shown in Fig. 3). MP analysis of β-tubulin produced topologies very similar to those obtained by ITS analysis. All *Emericella* sequences analyzed were divided into three large clades, with *E. miraensis* forming a well-supported clade (BP = 100%) with three other species (*E. stella-maris, E. astellata, E. variecolor*).

Discussion

The *Emericella miraensis* specimens were isolated from the roots of an alpine plant. Microscopic examination showed ascomata embedded in masses of Hülle cells and violet ascospores with two stellate equatorial crests. In addition, their anamorphic *Aspergillus* stage also was observed on CYA,

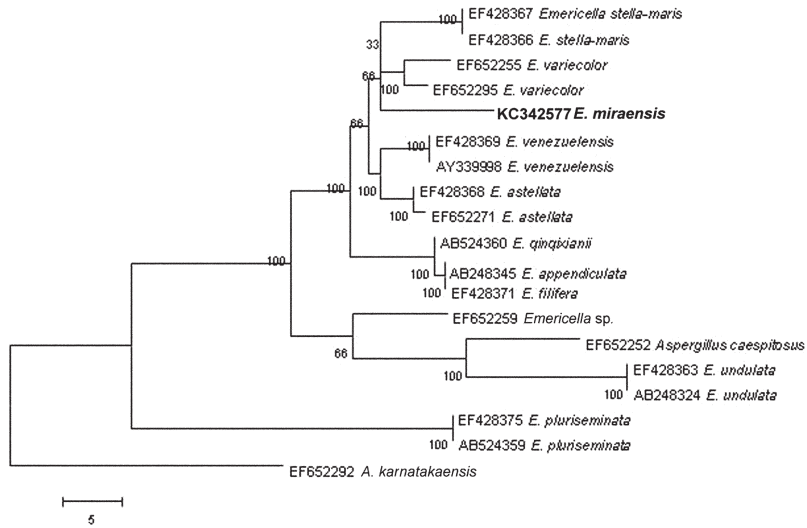

Figure 3. One of the MP trees obtained based on phylogenetic analysis of β-tubulin sequence data from *Emericella/Aspergillus*. Numbers above branches are bootstrap values ≥ 50%.

PDA, and MEA. These are some typical morphological features associated with *Emericella* (Malloch & Cain 1972). *Emericella miraensis* differs from the closely related *E. stella-maris* and *E. variecolor* in having ascospores with tuberculate or verrucose convex walls and of a different color (Table 1). In previous studies, the six *Emericella* species having ascospores ornamented in a star-shape pattern included *E. variecolor* (Berkeley 1857), *E. astellata* (Horie 1980), *E. pluriseminata* (Stchigel & Guarro 1997), *E. venezuelensis* (Frisvad & Samson 2004), *E. stella-maris*, and *E. olivicola* (Zalar et al. 2008). *Emericella pluriseminata* did not produce aspergilla on any media (Frisvad & Samson 2004, Zalar et al. 2008), while *E. miraensis* forms conidiophores and conidia on three conventional media: CYA, PDA, and MEA. Although *E. miraensis* resembles *E. variecolor* in ascospore colour, its ascospores are obviously verrucose as seen in SEM, while *E. variecolor* ascospores have smooth convex walls. The phylogenetic trees (Figs 2–3) indicate a close relationship between *E. miraensis* and *E. stella-maris,* which differs in its orange-red ascospore color. *Emericella miraensis* also can easily be distinguished from *E. venezuelensis*, which has violet brown ascospores with triangular flaps on their convex sides. In addition, the nonseptate conidiophores of *E. miraensis* distinguish it from *E. stella-maris* and *E. astellata*, whose conidiophores are always septate. Ascospore morphology is the most important diagnostic character for *Emericella* species (Horie 1980).

Table 1. Ascospore characters of *Emericella miraensis* and related species.

Species	Ascospores		
	Ascospore shape	Color	Surface ornamentation
E. variecolor	stellate	violet	smooth
E. stella-maris	stellate	orange-red	smooth
E. astellata	stellate	reddish purple–reddish brown	smooth
E. qinqixianii	lenticular	violet-brown	smooth
E. appendiculata	lenticular	violet-brown	capitate appendages
E. filifera	subglobose	brownish red	capitate appendages
E. venezuelensis	stellate	violet brown	triangular flaps
E. miraensis	stellate	violet	verrucose

Most *Emericella* species are more adapted to dry warm climates than to humid cold environments (Samson & Mouchacca 1974, Zalar et al. 2008). However the host of *E. miraensis*, *P. macrophyllum* var. *stenophyllum,* is an alpine plant that occurs in mountain steppes up to 4850 m, with annual precipitation of 443.6 mm (Luo et al. 2003).

Acknowledgments

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