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FULL PAPER

The correlation among molecular phylogenetics, morphological data, and growth temperature of the genus *Emericella*, and a new species

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Abstract The species of the genus *Emericella* have been classified and identified on the basis of morphological features. However, the phylogenetic relationships in this genus have not been investigated. To clarify the relationships according to molecular phylogenetics, morphological characteristics, and growth temperature regimens in *Emericella*, multilocus sequencing analysis based on recent *Aspergillus* taxonomy was carried out. Various characteristic species formed individual clades, and maximum growth temperature reflected the phylogenetics. *Emericella* species exhibit various ascospore characteristics, although some species do not have distinct ascospore ornamentation. Species that have smooth-walled ascospores with two equatorial crests are polyphyletic. Here, *Emericella pachycristata* is described and illustrated as a new species. Its ascospores are similar to those of *E. nidulans*. These species produce smooth-walled ascospores, but the equatorial crests of *E. pachycristata* are thicker than those of *E. nidulans*. On the phylogenetic trees, *E. pachycristata* is closely related to *E. rugulosa*, which produces ascospores with ribbed convex surfaces. Thus, *E. pachycristata* is considered to be a new species both morphologically and phylogenetically.

Keywords *Emericella pachycristata* · Morphological features · New taxon · Physiological characteristics

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Introduction

Aspergillus nidulans (Eidam) G. Winter is related to the teleomorphic genus *Emericella* Berk. It has been used as a model filamentous fungus to investigate secondary metabolism and signal transduction pathways (Keller et al. 1994; Brown et al. 1996; Kato et al. 2003; Keller 2006). The accumulated data, methods, and techniques of *A. nidulans* can be directly applied to *Emericella*. *Emericella* species have the ability to produce structurally unique metabolites or induce their production (Malmstrom 1999; Malmstrom et al. 2002; Oh et al. 2007). The genus includes a few species that produce aflatoxins and are not part of the species of *Aspergillus* section *Flavi* (Frisvad and Samson 2004; Frisvad et al. 2004; Cary et al. 2005; Zalar et al. 2008). In addition, several species of this genus are reported to be etiological agents in various infections (de Hoog et al. 2000; Horre et al. 2002; Dotis et al. 2003; Gugnani et al. 2004; Balajee et al. 2007). This genus has been classified and identified on the basis of morphological characteristics. Horie (1980) reevaluated the classification of *Emericella* species on the basis of ascospore ornamentation by scanning electron microscopy (SEM). Since then, the species of this genus have been mainly classified according to this criterion.

Horie has investigated *Emericella* and related species in Chinese soils since 1996 and has documented *E. miyajii*, *E. appendiculata*, and *E. qinqixianii* as new species (Horie 1996, 1998, 2000). In 2004, *E. venezuelensis* was reported as a new species on the basis of ascospore ornamentation and aflatoxin B_1 production (Frisvad and Samson 2004). Moreover, four new species of *Emericella*, one of which produces aflatoxin B_1, have been recently reported (Zalar et al. 2008).

Although morphological characteristics are the most important factors in classifying fungi, they depend on

subtle and subjective criteria. In modern descriptions of novel species, traditional morphological characteristics and more objective criteria are combined. These criteria include multilocus sequencing analysis, growth temperature regimens, and extrolite patterns; examination of the combination of these is termed "polyphasic analysis" (Hong et al. 2005, 2008; Yaguchi et al. 2007).

The species of the genus *Emericella* have previously been classified according to the characteristics of their ascospores, but detailed phylogenetic analysis of the genus has never been performed. In this study, we examined *Emericella* species isolated from Chinese soils and identified new species. These strains were similar to *Aspergillus nidulans* var. *roseus* Boeck and Kastner and a sterigmatocystin-producing variant of *Emericella* reported by Klich et al. (2001). In addition, we performed multilocus sequencing analysis on the basis of recent *Aspergillus* taxonomy (Samson et al. 2007) and attempted to clarify the relationships among the multilocus sequencing analysis, morphological characteristics, and growth temperature regimens in the genus *Emericella*.

Materials and methods

Strains in this study

The strains used were preserved at Medical Mycology Research Center, Chiba University (IFM) and the Natural History Museum and Institute, Chiba, Japan (CBM), or were purchased from the Centraalbureau voor Schimmelcultures (CBS), American Type Culture Collection (ATCC), or International Mycological Institute (IMI). Some strains were supplied from the Southern Regional Research Center, Agricultural Research Service, USDA (SRRC; by Dr. Maren Klich). The strains are listed in Table 1.

Incubation and observation

Each strain was grown in incubators at 25 °C or 37 °C for 14 days on Czapek (CZA) or malt extract (MEA) agar. After incubation, colonies were examined using a light microscope (LM) or scanning electron microscope (SEM) (Hitachi S-800, Tokyo, Japan). Colony colors were designated according to the *Methuen Handbook of Colour* (Kornerup and Wanscher 1978).

Growth studies

The maximum growth temperatures of all *Emericella* species were determined according to the method of Balajee et al. (2005): 10 µl of conidial suspension (10^5 conidia or ascospores/ml sterile distilled water) was placed onto the center of an MEA plate, which was then incubated at 40 °, 42 °, 45 °, or 48 °C for 7 days. The presence or absence of fungal growth at the end of the incubation period was recorded.

DNA extraction and sequencing analysis

DNA was extracted from all examined strains with a DNA extraction kit (Dr. GenTLE; Takara Bio, Shiga, Japan) according to the manufacturer's instructions. The parts of the β-tubulin (benA), calmodulin, and actin genes were amplified using primer pairs Bt2a and Bt2b (Glass and Donaldson 1995), cmd5 and cmd6 (Hong et al. 2005), and act-512F and ACT-783R (Carbone and Kohn 1999), respectively. Polymerase chain reaction (PCR) products were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM 3130ABI Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions.

Molecular phylogenetic analysis

DNA sequences were edited using ATGC version 4 sequence assembly software (Genetyx, Tokyo, Japan), and sequence alignment was analyzed using Clustal X software (Thompson et al. 1997). Maximum parsimony (MP) analysis (Fitch 1977) was determined by heuristic search with random addition sequences, branch swapping by tree bisection–reconnection (TBR), and MAXTREES set at 20,000, using PAUP* 4b10 (Swofford 2002). The relative robustness of the individual branches was estimated by bootstrapping (Felsenstein 1985), with 1,000 replicates using heuristic search and branch swapping by TBR and MAXTREES set at 100. For neighbor-joining (NJ) analysis (Saitou and Nei 1987), the distances between base sequences were calculated using Kimura's two-parameter model (Kimura 1980).

Results

Multilocus sequencing analysis of the genus *Emericella*

Partial DNA sequences of the β-tubulin, calmodulin, and actin genes were determined in the strains used in this study. All sequences were deposited in the DNA Data Bank of Japan (DDBJ), and the accession numbers are listed in Table 1. The phylogenetic tree of the β-tubulin gene (Fig. 1) yielded 57 equally parsimonious trees based on 97 parsimony informative characters, 361 steps in length, with a consistency index (CI) of 0.447 and a retention index (RI)

Table 1 List of *Emericella* species in this study and its maximal growth temperature

Taxon	Strain number	Origin	GenBank accession number			Maximal growth temperature (°C)
			β-Tubulin	Calmodulin	Actin	
E. acristata (Fennell & Raper) Horie	CBS 119.55^{T}	USA (fabric)	AB248304	AB476805	AB476768	48
E. appendiculata Horie & Li	CBM-FA-865^{T}	China (soil)	AB248345	AB476806	AB476769	<40
E. appendiculata (=*E. filifera* Zalar, Frisvad & Samson)	CBS 113636^{T}	Slovenia (salt water)	EF428372[e]	EU443973[e]	AB524371	
E. astellata (Fennell & Raper) Horie	CBS 134.55^{T}	Ecuador (plant)	AB248330	AB476807	AB476770	<40
E. aurantiobrunnea (Atkins, Hindson & Russell) Malloch & Cain	IMI 74897^{T}	Australia (haversack)	AB248306	AB476808	AB476771	<40
E. bicolor Christensen & States	CBS 425.77^{T}	USA (soil)	AB375872	AB476809	AB524366	<40
E. cleistominuta Mehrotra & Prasad	IMI 131554^{T}	India (soil)	AB248331	AB476810	AB476772	48
E. corrugata Udagawa & Horie	CBM-FA-73^{T}	Thailand (soil)	AB248351	AB476811	AB476773	48
E. dentata (D. K. Sandhu & R. S. Sandhu) Horie	IMI 126693^{T}	India (human)	AB248337	AB476812	AB524367	48
E. desertorum Samson & Mouch	CBS 653.73^{T}	Egypt (soil)	AB248332	AB524034	AB524368	42
E. discophora Samson, Zalar & Frisvad	CBS 469.88^{T}	Spain (soil)	AY339999[e]	EU443970[e]	AB524369	
E. echinulata (Fennell & Raper) Horie	CBM-FA-663	Unknown	AB248354	AB524035	AB524370	48
E. falconensis Horie, Miyaji, Nishimura & Udagawa	CBM-FA-82	Venezuela (soil)	AB248346	AB524036	AB476774	45
E. foeniculicola Udagawa	IFM 54188^{T}	China (soil)	AB524357	AB524037	AB476775	<40
E. foveolata Horie	IFM 42015^{T}	India (herbal drug)	AB248310	AB524038	AB524372	48
E. fruticulosa (Raper & Fennell) Malloch & Cain	CBS 650.73	Egypt (soil)	AB248311	AB524039	AB476776	45
E. heterothallica (Kwon-Chung, Fennell & Raper) Malloch & Cain	ATCC 16847^{T}	Costa Rica (soil)	AB248329	EF652411[b]	AB524373	<40
E. indica Stchigel & Guarro	IMI 378525^{T}	India (soil)	AY339988[a]	–	–	>42[d]
E. miyajii Horie	CBM-FA-833^{NT}	Unknown	AB243110	AB524040	AB476777	48
E. montenegroi Horie, Miyaji & Nishimura	CBM-FA-669^{T}	Brazil (soil)	AB248312	AB524041	AB476778	48
E. navahoensis Christensen & States	CBS 351.81^{T}	USA (soil)	AB248333	AB524042	AB476779	45
E. nidulans (Eidam) Vuillemin	CBS 589.65^{T}	Belgium (unknown)	AB524358	AB524043	AB476780	48
E. nidulans (Eidam) Vuillemin	IFM 51356	Japan (human)	AB375874	AB524044	AB476781	48
E. nidulans (Eidam) Vuillemin	IFM 55368	China (soil)	AB375873	AB524045	AB476782	48
E. nidulans var. *lata* (Thom & Raper) Subramanian	CBS 492.65^{T}	Unknown	AB248334	AB524046	AB476783	48
E. olivicola Frisvad, Zalar & Samson	CBS 119.37^{T}	Italy (decaying fruit)	AY339996[e]	EU443986[e]	AB524374	
E. omanensis Horie & Udagawa	CBM-FA-700^{T}	Oman (soil)	AB248347	AB524047	AB524375	45
E. pachycristata Matsuzawa, Horie & Yaguchi	IFM 55265^{T}	China (soil)	AB375875	AB524062	AB476795	48
E. pachycristata Matsuzawa, Horie & Yaguchi	IFM 55259	China (soil)	AB375876	AB524063	AB476796	48
E. pachycristata Matsuzawa, Horie & Yaguchi	IFM 55260	China (soil)	AB375877	AB524064	AB476797	48
E. pachycristata Matsuzawa, Horie & Yaguchi	IFM 55261	China (soil)	AB375878	AB524065	AB476798	48
E. pachycristata Matsuzawa, Horie & Yaguchi	IFM 55262	China (soil)	AB375879	AB524066	AB476799	48
E. pachycristata Matsuzawa, Horie & Yaguchi	IFM 55263	China (soil)	AB375880	AB524067	AB476800	48
E. pachycristata Matsuzawa, Horie & Yaguchi	IFM 55264	China (soil)	AB375881	AB524068	AB476801	48

Table 1 continued

Taxon	Strain number	Origin	GenBank accession number			Maximal growth temperature (°C)
			β-Tubulin	Calmodulin	Actin	
E. parvathecia (Raper & Fennell) Malloch & Cain	IMI 139280^{T}	USA (human)	AB243111	AB524048	AB476784	48
E. pluriseminata Stchigel & Guarro	NBRC 33028^{T}	India (soil)	AB524359	AB524049	AB476785	>37[c]
E. purpurea Samson & Mouchacca	CBS 754.74^{T}	Egypt (soil)	AB248315	AB524050	AB476786	<40
E. qinqixianii Horie, Abliz & Li	CBM-FA-866^{T}	China (soil)	AB524360	AB524051	AB476787	<40
E. quadrilineata (Thom & Raper) Benjamin	IMI 89351^{NT}	USA (soil)	AB248335	AB524052	AB476788	48
E. rugulosa (Thom & Raper) Benjamin	CBS 133.60^{T}	Brazil (soil)	AB524361	AB524053	AB476789	48
E. rugulosa var. *lazulina* Horie, Miyaji & Nishimura	CBM-FA-710^{T}	Brazil (soil)	AB248319	AB524054	AB476790	48
E. similis Horie, Udagawa, Abdullah & Al-Bader	IFM 54235^{T}	Iraq (soil)	AB248321	AB524055	AB524376	48
E. spectabilis Christensen & Raper	CBS 429.77^{T}	USA (soil)	AB248320	AB524056	AB476791	<40
E. stella-maris Zalar, Frisvad & Samson	CBS113638^{T}	Slovenia (salt water)	EF428367[e]	EU443978[e]	AB524377	
E. striata (Rai, Tewari & Mukerji) Malloch & Cain	IMI 163899	India (plant seed)	AB248322	AB524057	AB524378	48
E. sublata Horie	IFM 42029^{T}	Japan (herbal drug)	AB248323	AB524058	AB476792	48
E. undulata Kong & Qi	CBM-FA-715^{T}	China (soil)	AB248324	EU443989[d]	AB476793	<40
E. unguis Malloch & Cain	ATCC 16812^{T}	USA (shoe)	AB248325	AB524059	AB524379	<40
E. variecolor Berkeley & Broome	NBRC 32302^{T}	India (plant seed)	AB524362	AB524060	AB476794	<40
E. venezuelensis Frisvad & Samson	CBS 868.97^{T}	Venezuela (sponge)	AY339998[a]	–	–	>37[a]
E. violacea (Fennell & Raper) Malloch & Cain	CBS 138.55^{T}	Ghana (soil)	AB248336	AB524061	AB524380	48
Emericella sp.	SRRC1398	USA (soil)	AB524363	AB524069	AB476802	48
Emericella sp.	SRRC1402	USA (soil)	AB524364	AB524070	AB476803	48
Aspergillus nidulans var. *roseus* & Kastner (*E. nidulans* var. *roseus*)	ATCC 58397	USA (soil)	AB524365	AB524071	AB476804	48
Aspergillus clavatus Desmazières	CBS 514.65		AB489851	AB489852	AB489853	

[a] Data of Frisvad et al. (2004)

[b] Data of Peterson (2008)

[c] Data of Stchigel and Guarro (1997)

[d] Stchigel et al. (1999)

[e] Data of Zalar et al. (2008)

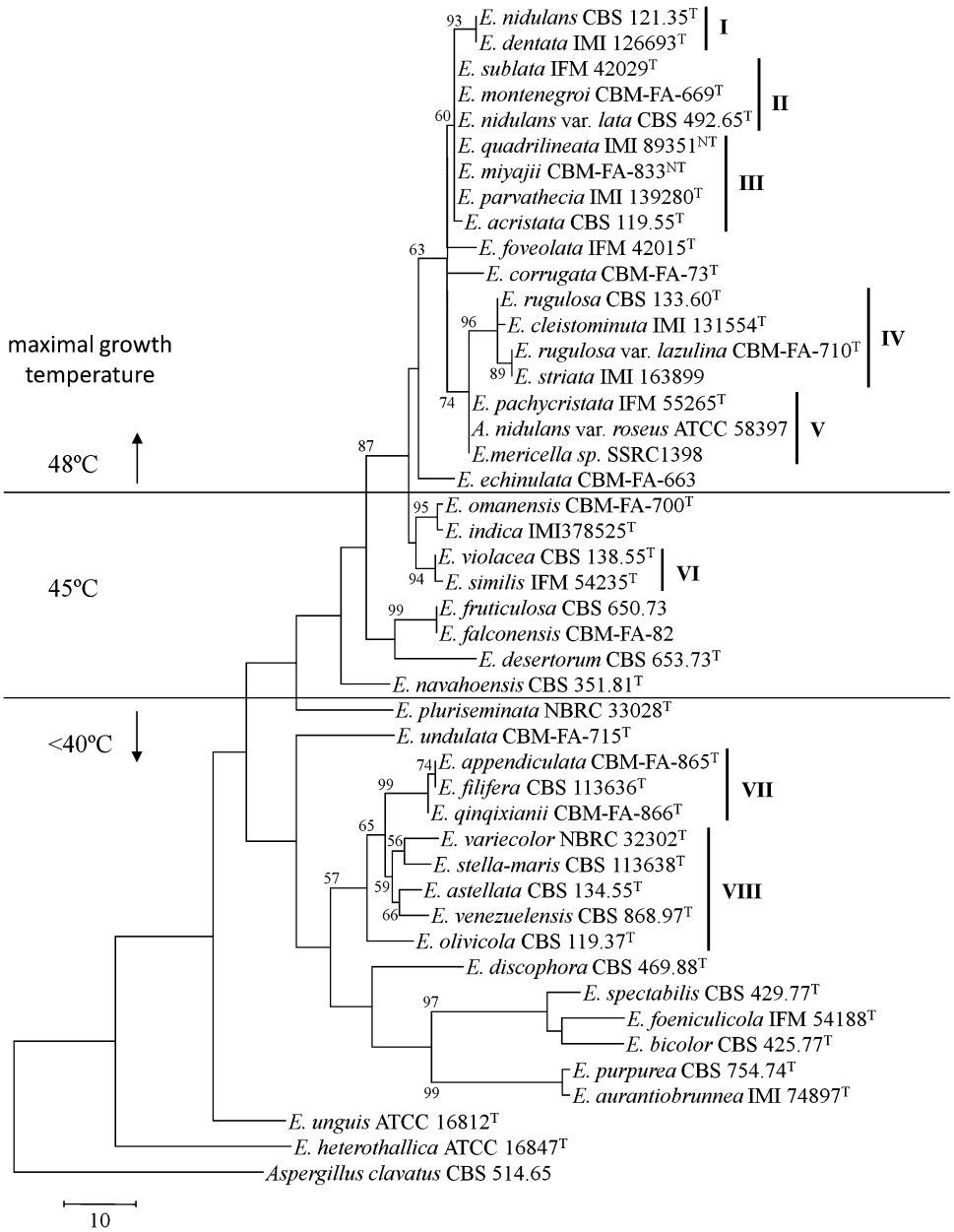

Fig. 1 One of 57 equally parsimonious trees obtained from analysis of the β-tubulin gene using PAUP. Trees were 361 steps in length with a consistency index (CI) of 0.447 and a retention (RI) of 0.761. *Numbers above* or *below nodes* represent bootstrap values >50 % (of 1,000 bootstrap replications)

of 0.761. The phylogenetic tree of the calmodulin gene sequences (Fig. 2) yielded 44 equally parsimonious trees based on 147 parsimony informative characters, 651 steps in length, with a CI of 0.464 and an RI of 0.762. Last, the phylogenetic tree of the actin gene sequences (Fig. 3) yielded 21 parsimonious trees based on 129 parsimony informative characters, 532 steps in length, with a CI of 0.513 and an RI of 0.773. No differences were found between tree topologies from MP and NJ analyses (NJ trees not shown) of the β-tubulin, calmodulin, and actin genes. The three trees based on the three loci were similar. There was a correlation between molecular phylogenetics and morphological data on the phylogenetic tree of the three genes. The ascospores of *E. nidulans* have smooth convex walls with two equatorial crests, whereas those of *E. dentata* (D.K. Sandhu & R.S. Sandhu) Y. Horie have smooth convex walls with two dentate equatorial crests; these two species formed a clade (Figs. 1, 2, 3; clade I). Moreover, various characteristic species formed individual clades: *E. sublata* and *E. montenegroi*, which have ascospores with broad equatorial crests (Figs. 1, 2, 3; clade II); *E. quadrilineata*, *E. parvathecia*, *E. miyajii*, and *E. acristata*, which have ascospores with four equatorial crests (Figs. 1, 2, 3; clade III); *E. rugulosa*, *E. rugulosa* var. *lazulina* and *E. cleistominuta*, which have ascospores with ribbed convex surfaces (Figs. 1, 2, 3; clade IV); *E. violacea* and *E. similis*, which have ascospores with cancellous convex surfaces (Figs. 1, 2, 3; clade VI); *E. appendiculata*,

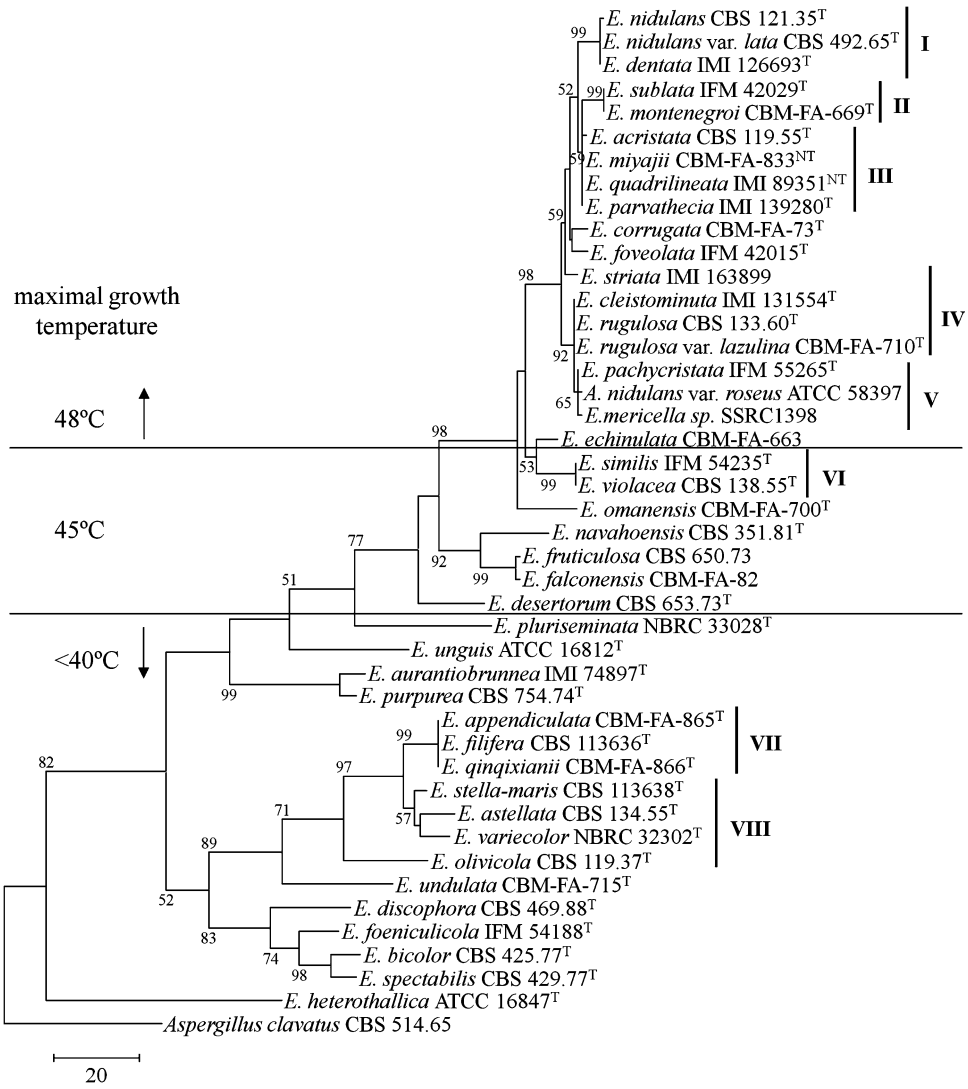

Fig. 2 One of 44 equally parsimonious trees obtained from analysis of the calmodulin gene using PAUP. Trees were 651 steps in length with a CI of 0.464 and an RI of 0.762. *Numbers above* or *below nodes* represent bootstrap values >50 % (of 1,000 bootstrap replications)

E. qinqixianii, and *E. filifera*, which produce ascospores with appendaged threads (Figs. 1, 2, 3; clade VII); and *E. variecolor*, *E. astellata*, *E. venezuelensis*, *E. stella-maris*, and *E. olivicola*, which produce stellate ascospores or ascospores with widely waved equatorial crests (Figs. 1, 2, 3; clade VIII).

The maximum growth temperatures of all strains are listed in Table 1. Approximately half the species in this genus were able to grow up to 48 °C. *Emericella nidulans*, *E. rugulosa*, and *E. echinulata* were typical species capable of growing at 48 °C. Six species of this genus (except *E. indica*) grew at 45 °C. *Emericella undulata*, *E. appendiculata*, *E. qinqixianii*, and the other nine species of this genus grew at temperatures less than 40 °C. On the dendrograms of all genes, the species of *Emericella* were clearly separated into three groups with respect to maximum growth temperature. As a result, correlations among molecular phylogenetics, morphological data, and growth temperature were apparent.

Taxonomic position of *Emericella pachycristata*

We found seven strains isolated from Chinese soils (IFM 55259–55265) in clade V (Figs. 1, 2, 3). On the phylogenetic trees of the three genes, these strains were closely related to *E. rugulosa*. *Emericella pachycristata* formed individual clades supported by high bootstrap values. Although *E. rugulosa* produces ascospores with ribbed convex surfaces, *E. pachycristata* does not: it shows ascospore morphology similar to that of *E. nidulans* (Fig. 4e, f). However, *E. pachycristata* differed from *E. nidulans* with respect to the thickness of the equatorial crests. The equatorial crests of ascospores of *E. pachycristata* were thicker than those of *E. nidulans*. Therefore, *E. pachycristata* is considered to be phylogenetically distinct from *E. nidulans*. Here, we propose this species as a novel species, *Emericella pachycristata* sp. nov.

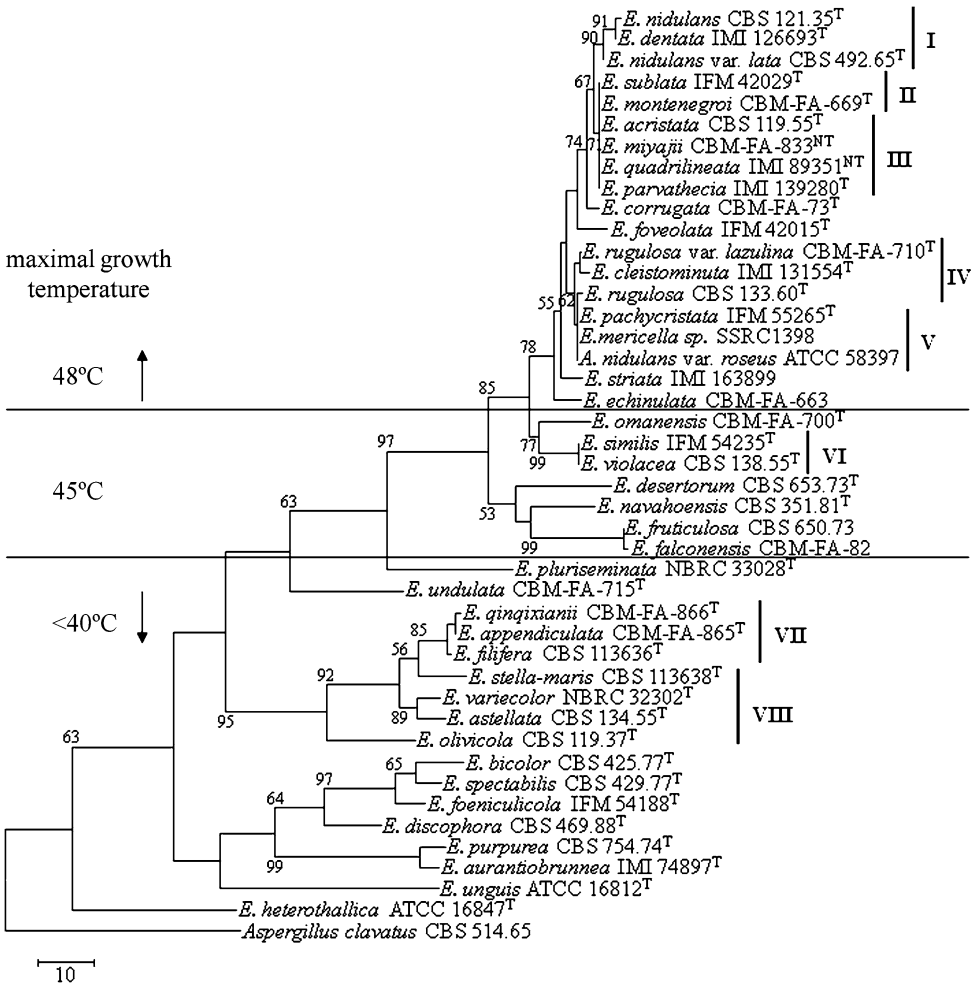

Fig. 3 One of 21 equally parsimonious trees obtained from analysis of the actin gene using PAUP. Trees were 532 steps in length with a CI of 0.513 and an RI of 0.773. *Numbers above* or *below nodes* represent bootstrap values >50 % (of 1,000 bootstrap replications)

Taxonomy

Emericella pachycristata Matsuzawa, Horie & Yaguchi sp. nov. Figs. 4 and 5

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Coloniae in agaro maltoso expansae restrictae, ascomata abundanter producentia, conidiogenesis abundanter dilute aurantiacae vel griseo-viridia vel hebes virides; reversum brunneo-aurantiacum.

Ascomata, rabro-purpurea vel atro rubro-brunnea, globosa vel subglobosa, 210–320 μm diametro, cum cellulis dictis "hülle" numerosis, crassitunicatis, globosis vel ovoideis, 15–25 × 12.5–25 μm circumcinctis; peridium griseo-flavum, membranaceum. Asci 8-spori, globosi vel subglobosi vel ovoidei, 9–11 × 8.5–10 μm, evanescentes. Ascosporae dilute rubidae vel rubro-brunneae, lenticulares, 4–5 × 3.5–4 μm, cristis aequatorialibus duabus praeditae, parte convexa laevi. Status anamorphus: *Aspergillus pachycristatus.*

Holotypus. IFM 55265. colonia exsiccata in culturea ex solo, in Pichan, Xinjiang, Sina, VIII-2006, a T. Yaguchi isolata et ea collectione fungorum Medical Mycology Reserch Center, Chiba University (IFM) conservata.

Anamorphosis. *Aspergillus pachycristatus* sp. nov. Capitula conidica griseo-olivacea, radiantia vel brevi-columnaria 35–75 × 30–40 μm, conidiophora ex mycelio basali oriunda, usque 175 μm longa, ad medium 3.5–6 μm crassa, laevia; vesiculae hemisphaericae vel ampulliformes, 7.5–12.5 μm diametro. Aspergilla in summa 2/3 vel 1/3 vesicula insidentia, biseriata; metulae griseo-brunneae 5–7 × 3.5–5 μm; phialides griseo-brunneae 5–9 × 2.5–3.5 μm. Conidia hyalina vel dilute griseo-viridia, globosa vel subglobosa, 3–4 μm, echinulata. Status teleomorphus: *Emericella pachycristata*.

Colonies on Czapek's solution agar growing restrictedly, attaining a diameter of 20–21 mm in 14 days, floccose, consisting of a thin mycelial felt and loose aerial hyphae, ascomata and conidial heads few in number, orange white (5A2–6A2, after Kornerup and Wanscher 1978); reverse greyish orange (6B3) to brownish orange (6C5).

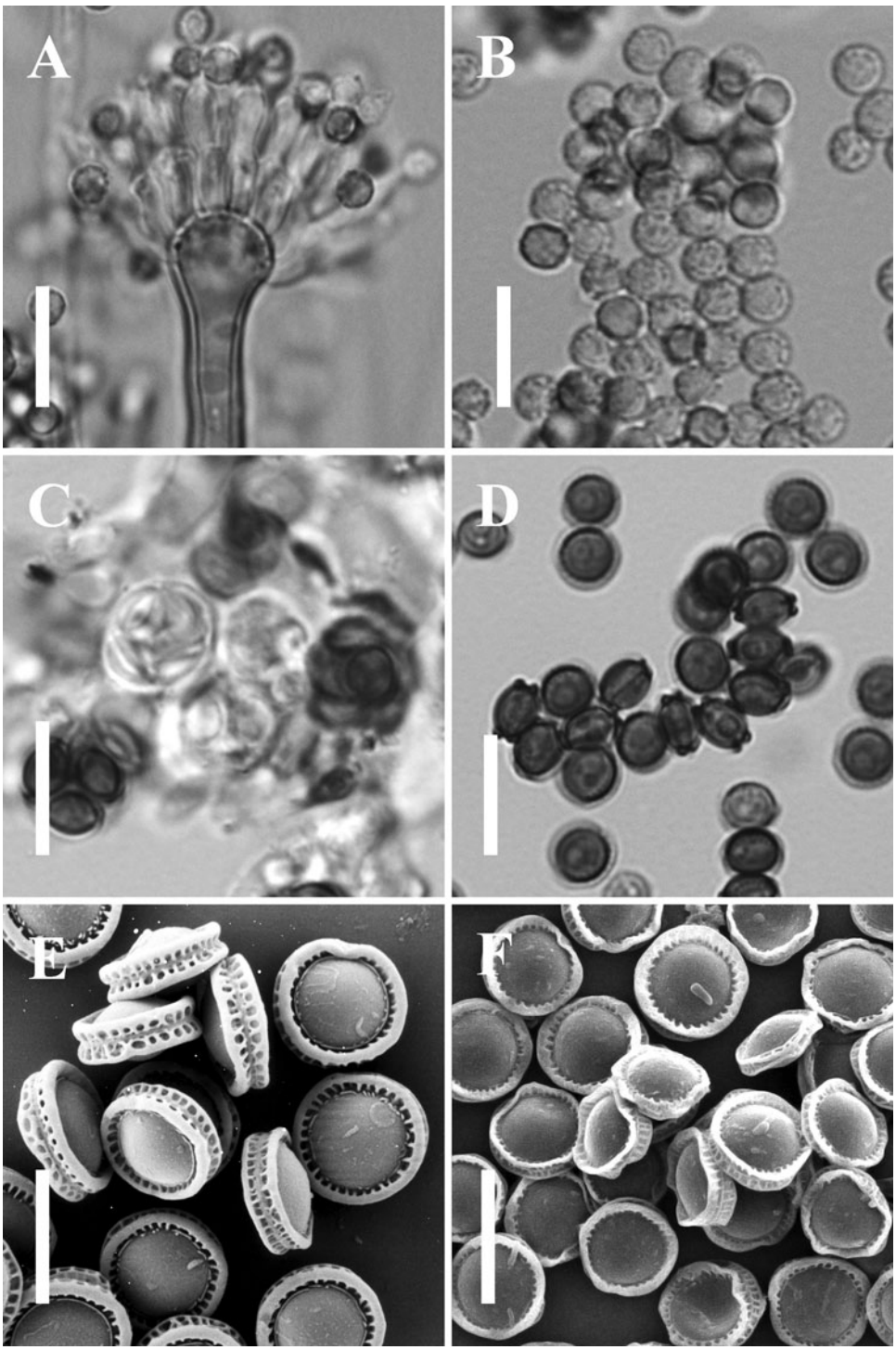

Fig. 4 *Emericella pachycristata* sp. nov. **a** Aspergillum (light microscopy: LM). **b** Conidia (LM). **c** Asci (LM). **d** Ascospores (LM). **e** Ascospores (scanning electron microscopy: SEM). **f** Ascospores of *E. nidulans* IFM 55368 (SEM). *Bars* **a–d** 10 μm; **e–f** 5 μm

Colonies on malt agar growing restrictedly, attaining a diameter of 22–23 mm in 14 days, consisting of a dense basal mycelium and loose aerial hyphae, ascomata abundantly produced, conidial heads abundantly produced, pale orange (6A3) to greyish green (29D5) or deep green (30D8); reverse brownish orange (6C6) to brown (6E7).

At 37 °C, growth rate better than at 25 °C, and with increased production of ascomata. Ascomata, reddish purple to dark reddish brown, globose to subglobose, 210–320 μm in diameter, surrounded by hyaline to pale yellowish brown, globose to ovate, thick-walled hülle cells measuring 15–25 × 12.5–25 μm; peridium grayish yellow, membranaceous, consisting of a angular cells. Asci 8-spored, globose to subglobose or ovate, 9–11 × 8.5–10 μm, evanescent. Ascospores at first hyaline to pale yellowish brown, then becoming dull red to reddish brown

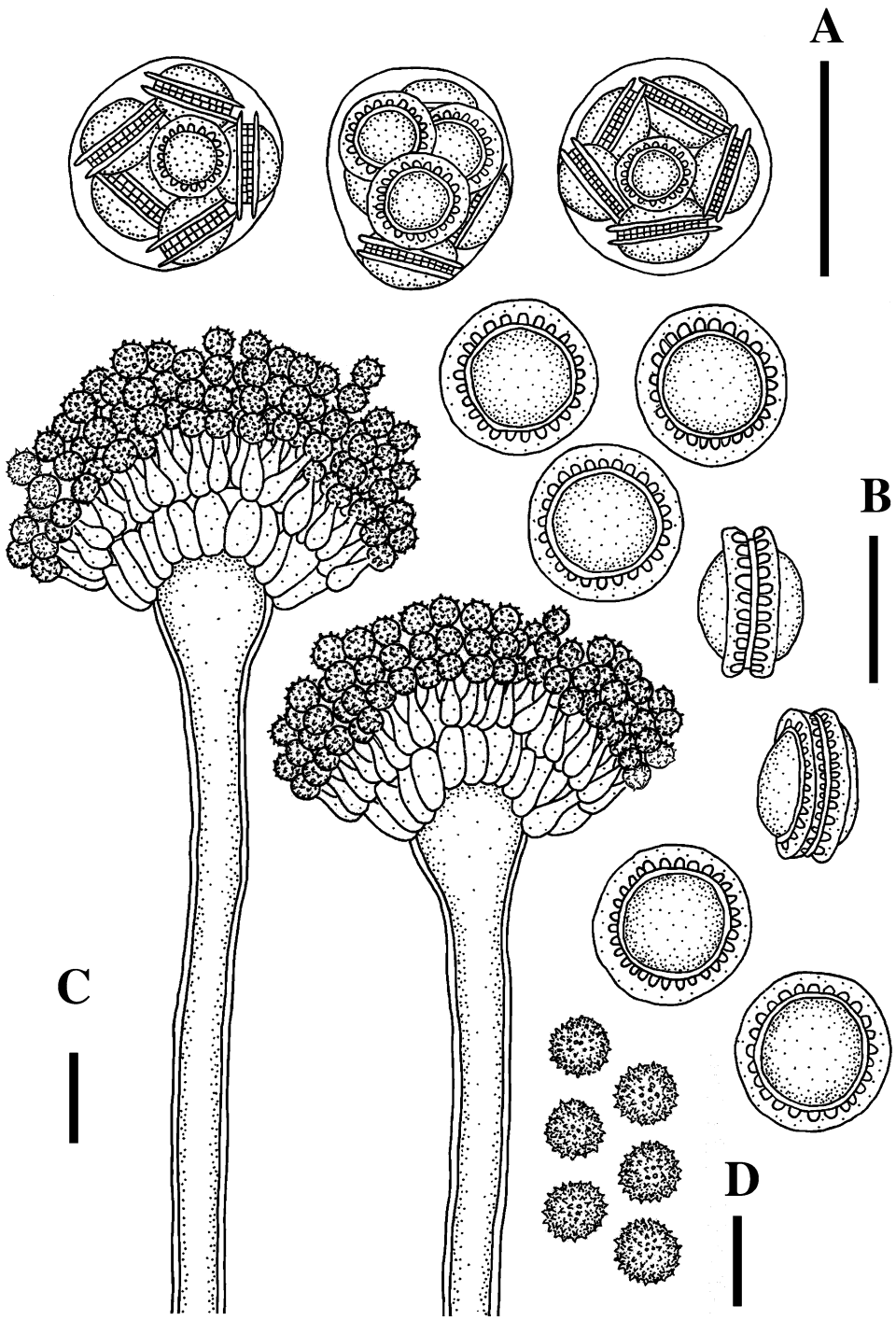

Fig. 5 *Emericella pachycristata* sp. nov. **a** Asci. **b** Ascospores. **c** Aspergillum. **d** Conidia. *Bars* **a**, **c** 10 μm; **b**, **d** 5 μm

at maturity, lenticular, spore bodies 4–5 × 3.5–4 μm, provided with two equatorial crests measuring 1.0 μm wide, with convex surfaces smooth.

Conidial heads grayish olive, radiate to short columnar, 35–75 × 30–40 μm. Conidiophores grayish brown to reddish brown, smooth, arising from the basal mycelium or aerial hyphae, up to 175 μm long, 3.5–6 μm diameter at the middle, vesicles grayish brown, hemispherical to flask shaped, 7.5–12.5 μm diameter with metulae covering the upper 2/3–1/3 of surfaces. Aspergilla biseriate; metulae grayish brown 5–7 × 3.5–5 μm; phialides grayish brown, 5–9 × 2.5–3.5 μm. Conidia hyaline to pale greenish gray, globose to subglobose, 3–4 μm diameter, echinulate.

Etymology. From Latin, *pachy-* = thick- and *cristata* = crest, referring to the ascospore ornamentation.

Specimen examined. IFM 55265 (holotype), a dried culture derived from an isolate of vineyard soil, Pichan (Shanshan), Pichan Prefecture, Xinjiang Uygur autonomous

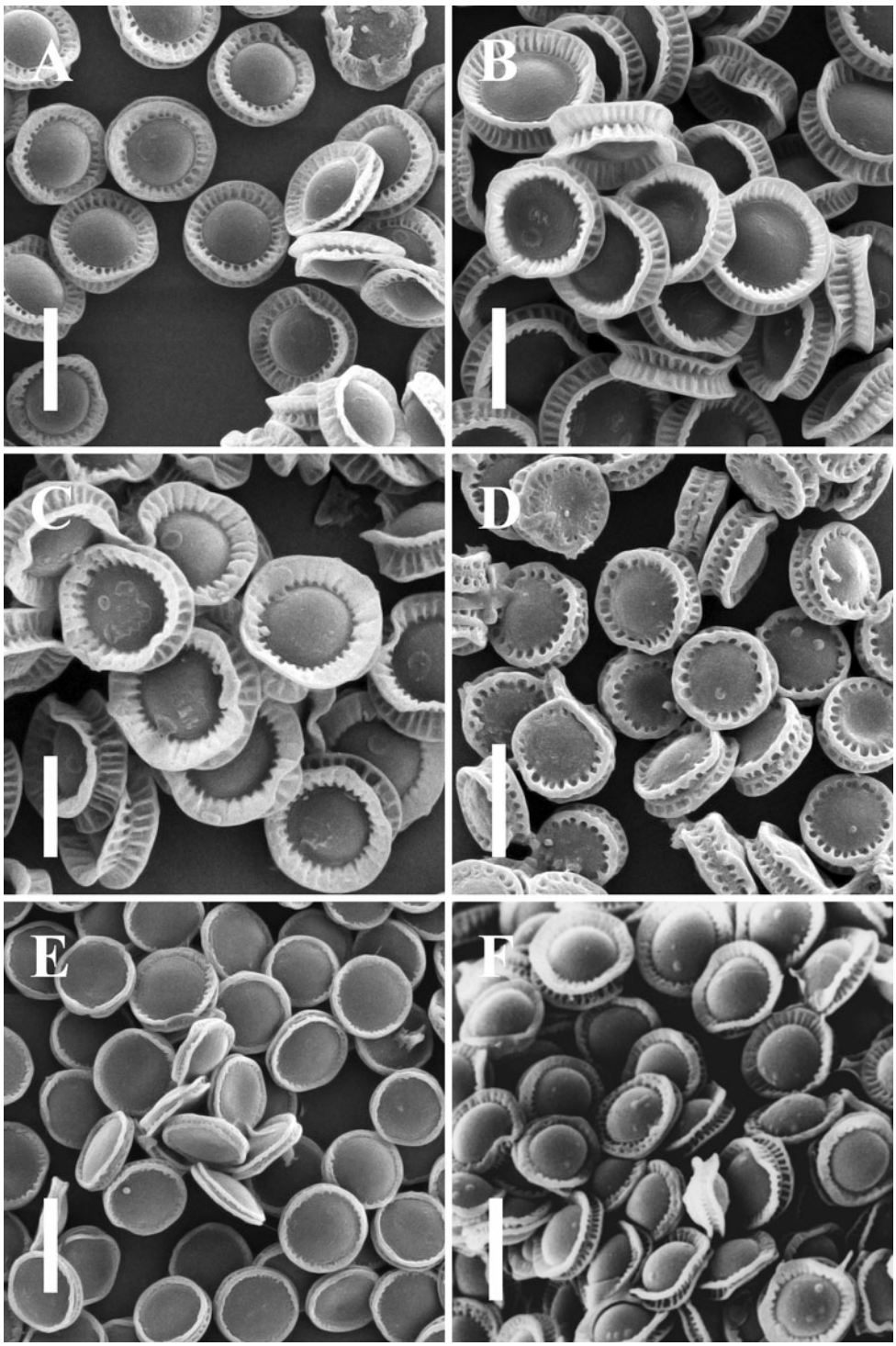

Fig. 6 Comparison of the species of *Emericella* that have smooth-walled ascospores with two equatorial crests. **a** *E. sublata* IFM42029. **b** *E. fruticulosa* CBS 650.73. **c** *E. falconensis* CBM-FA-82. **d** *E. aurantiobrunnea* IMI 74897. **e** *E. foeniculicola* IFM 42201. **f** *E. spectabilis* CBS 429.77. *Bars* **a**–**f** 5 μm

region, China, collected by Paride Abliz, isolated and developed by T. Yaguchi in the laboratory of the Department of Dermatology, Xinjiang Medical University, Urumuqi, as isolate strain No. E-100, August 2006. The living culture was deposited in NITE Biological Resource Center (NBRC) as NBRC 104790.

Discussion

Emericella species exhibit various ascospore phenotypes, although several species do not exhibit remarkable ascospore phenotypes (Fig. 6). In this study, we indicated that species having smooth-walled ascospores with two

Table 2 Comparison of properties of the species that are related to *Emericella pachycristata*

Species	Convex wall	Colony color	Conidial head color	Conidiophorous size	Maximal growth temperature (°C)
E. nidulans	Smooth	Deep dull yellow green	Dark green	75–100 × 2.5–3 μm	48
E. sublata	Smooth	Dull brown to grayish olive	Grayish olive green to dull green	Up to 210 × 4–6 μm	48
E. rugulosa	Ribbed	Purple-gray to purple-brown	Green to dark green	50–150 × 4.5–7.5 μm	48
E. pachycristata	Smooth	Pale orange to greyish green or deep green	Grayish olive	Up to 175 × 3.5–6 μm	48
E. fruticulosa	Smooth	Grey green near pale lumiere green	Greyish green to olive yellow	40–60 × 2.2–4.4 μm	45
E. falconensis	Smooth	Orange to bright brown	Dull green	75–240 × 3–6 μm	45
E. spectabilis	Smooth	Vinaceous gray	Dark green	190–364 × 5.7–10.3 μm	<40
E. foeniculicola	Smooth	Pale vinaceous to purplish gray	Grayish green	20–160 × 3–5 μm	<40
E. aurantiobrunnea	Smooth	Cream to buff	Light to dull buff	Up to 250 × up to 8 μm	<40

equatorial crests are polyphyletic groups. The typical species correspond to *E. nidulans*, *E. sublata*, *E. fruticulosa*, *E. falconensis*, *E. spectabilis*, *E. foeniculicola*, *E. aurantiobrunnea*, and *E. astellata*. *Emericella nidulans* has smooth-walled ascospores with two equatorial crests. However, *E. sublata* has ascospores with two broad equatorial crests and is distinguishable from *E. nidulans* based on the width of its equatorial crests (Horie 1979). *Emericella spectabilis* radically differs from *E. nidulans* with respect to conidiophore size, hülle cell prominence, and color in mass (vinaceous in *E. spectabilis*) (Christensen 1978). *Emericella foeniculicola* differs from *E. nidulans* with respect to anamorph morphology (Udagawa and Muroi 1979). *Emericella aurantiobrunnea* does not produce conidial heads until the culture becomes several weeks old; its conidial heads exhibit light to dull buff shades and fail to show any blue-green color (Raper and Fennell 1965). *Emericella astellata* has a characteristic ability to produce aflatoxin B_1 and B_2 (Frisvad et al. 2004). In addition to ascospore ornamentation, the morphological characteristics of anamorph and mycotoxin production are important characteristics to identify *Emericella* species. Moreover, the maximum growth temperatures of *E. spectabilis*, *E. foeniculicola*, and *E. aurantiobrunnea* were less than 40 °C, whereas *E. nidulans* grew in temperatures up to 48 °C. These differences were reflected in the analysis of molecular phylogenetics. Other species that have smooth-walled ascospores with two equatorial crests also differed from *E. nidulans* with respect to morphological and physiological characteristics other than ascospore ornamentation (Table 2).

Emericella pachycristata also has smooth-walled ascospores with two equatorial crests, and the maximum growth temperature is 48 °C. However, the phylogenetic position of *E. pachycristata* was close to that of *E. rugulosa*. Klich et al. (2001) reported a new sterigmatocystin-producing variant of *Emericella* (strain SSRC1398) that exhibits a growth rate on standardized media and Southern blots of genomic DNA similar to *E. rugulosa*; however, it produces smooth-walled ascospores. According to this report, this variant is likely to be *E. pachycristata.* The molecular phylogenetic relationship between *E. rugulosa* and *E. pachycristata* apparently supports these morphological and physiological characteristics (Figs. 1, 2, 3; clades IV and V).

We conducted multilocus sequencing analysis based on recent *Aspergillus* taxonomy and indicated the correlations among molecular phylogenetics, morphological data, and growth temperature. However, several species, particularly species belonging to clades I–III, were undistinguishable by phylogenetic analysis alone. *Emericella dentata* produces smooth-walled ascospores with two dentate equatorial crests (Raper and Fennell 1965). Although it belongs to clade I, with *E. nidulans*, these species have very different ascospore crests. In the phylogenetic trees based on the *β*-tubulin and actin genes, species that have ascospores with two broad equatorial crests (clade II) and four equatorial crests (clade III) formed the same clade. However, the species that belong to clades II and III exhibit substantially different morphological characteristics. Thus, it is very difficult to identify *Emericella* species by phylogenetic analysis alone.

Zalar et al. (2008) reported four new *Emericella* species. Among them is *E. filifera*, which forms ascospores with appendaged threads. They also discuss the similarity between the ascospores of *E. filifera* and *E. appendiculata*. In addition, *E. appendiculata* produces ascospores with appendaged threads. We reevaluated *E. filifera* according to phylogenetic analysis of sequence data and morphological characteristics, and found that *E. filifera* is identical

to *E. appendiculata*. Therefore, we conclude that *E. filifera* is a synonym of *E. appendiculata* based on sequence data and ascospore ornamentation. Moreover, *E. appendiculata* (synonym: *E. filifera*) and *E. qinqixianii* are unique species that produce ascospores with appendaged threads.

In the morphological taxonomy of the genus *Aspergillus* and its teleomorphs, species are distinguished by phenotypic characteristics such as colony and microscopic characteristics of the conidia and ascospores. Colony diameter at 7 days after inoculation on standard media is another important characteristic (Klich et al. 2001). *Emericella* species have been mainly classified on the basis of ascospore ornamentation since the reevaluation performed by Horie. However, the results of the present study indicate that morphological characteristics other than ascospore ornamentation and physiological characteristics are important as well. Thus, to identify species in this genus, it is necessary to evaluate both phylogenetic analysis and morphological and physiological characteristics.

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