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ARTICLE

Are alkalitolerant fungi of the *Emericellopsis* lineage (*Bionectriaceae*) of marine origin?

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Abstract: Surveying the fungi of alkaline soils in Siberia, Trans-Baikal regions (Russia), the Aral lake (Kazakhstan), and Eastern Mongolia, we report an abundance of alkalitolerant species representing the *Emericellopsis*-clade within the *Acremonium* cluster of fungi (order *Hypocreales*). On an alkaline medium (pH *ca*. 10), 34 acremonium-like fungal strains were obtained. One of these was able to develop a sexual morph and was shown to be a new member of the genus *Emericellopsis*, described here as *E. alkalina* sp. nov. Previous studies showed two distinct ecological clades within *Emericellopsis*, one consisting of terrestrial isolates and one predominantly marine. Remarkably, all the isolates from our study sites show high phylogenetic similarity based on six loci (LSU and SSU rDNA, RPB2, TEF1-α, β-tub and ITS region), regardless of their provenance within a broad geographical distribution. They group within the known marine-origin species, a finding that provides a possible link to the evolution of the alkaliphilic trait in the *Emericellopsis* lineage. We tested the capacities of all newly isolated strains, and the few available reference ex-type cultures, to grow over wide pH ranges. The growth performance varied among the tested isolates, which showed differences in growth rate as well as in pH preference. Whereas every newly isolated strain from soda soils was extremely alkalitolerant and displayed the ability to grow over a wide range of ambient pH (range 4–11.2), reference marine-borne and terrestrial strains showed moderate and no alkalitolerance, respectively. The growth pattern of the alkalitolerant *Emericellopsis* isolates was unlike that of the recently described and taxonomically unrelated alkaliphilic *Sodiomyces alkalinus,* obtained from the same type of soils but which showed a narrower preference towards high pH.

Key words:
Acremonium
Emericellopsis
alkaline soils
molecular phylogeny
pH tolerance
soda soils

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INTRODUCTION

Alkaline soils (or soda soils) and soda lakes represent a unique environmental niche. There are few studies available on the fungal biodiversity therein. The eye-catching characteristic of these soils is a high pH maintained mainly by the buffering capacities of soluble carbonates present. Soda accumulation is thought to be a common process associated with savannas, steppes and desert regions across the world (Jones *et al*. 1998). Some examples of such extreme occurrences include the Magadi Lake in Kenya and the Natron Lake in Tanzania where the pH values of water are as high as 11–12. Seventy fungi have been isolated from The Dead Sea in Israel, almost half *Eurotiales*, where the salt levels are 340–350 g salt/L (Buchalo *et al.* 2009). In Russia, alkaline soils are mostly restricted to areas adjacent to saline lake basins in south-western Siberia (Sorokin *et al*. 2008).

Naturally, high salts concentration and high environmental pH impose a substantial amount of stress to any living organism. Some have adapted and therefore evolved metabolic pathways in order to thrive in such harsh conditions, such as high osmotic pressures, low water potentials, and, clearly, elevated ambient pHs (>9). The vast majority of so-called alkaliphiles, with a growth optimum at pH above 9, include prokaryotes (Duckworth *et al*. 1996). However, some filamentous fungi have been shown to be able to grow optimally at pH values exceeding 9 (Nagai *et al*. 1995, 1998, Grum-Grzhimaylo *et al*. 2013). Alkaliphily in filamentous fungi is uncommon, while alkalitolerance, on the other hand, is far more widespread. Alkalitolerant fungi, i.e. fungi that can grow to some extent at an alkaline pH but with their optimum still being at neutral pH values, are not only of basic scientific interest for the molecular mechanisms of adaptation, but also in the search for potentially biotechnologically valuable enzymes. It has become more obvious that alkalitolerant fungi may be encountered in many neutral soils (Kladwang *et al.* 2003, Elíades *et al.* 2006). The relative abundance of alkalitolerant fungi has facilitated studies on both their biodiversity and their enzymatic properties. And yet, truly alkaliphilic filamentous fungi have been isolated infrequently. The few existing descriptive studies on alkalitolerant and alkaliphilic fungi show a bias towards fungi with simple

Table 1. Strains used in the current study and characteristics of the sites of isolation. Newly isolated strains are given in bold.

Strain	VKM number	CBS no.	Isolation area	Isolation place	pH of the soil	Total salts (g/kg)	Saltification type
***Acremonium sclerotigenum* A101**	-	-	Trans-Baikal, Russia	near Alla River	8	-	sulfate
***Acremonium sclerotigenum* A130**	-	-	Trans-Baikal, Russia	near Alla River	8	-	sulfate
***Acremonium* sp. A104**	-	-	Kulunda steppe, Altai, Russia	-	taken from *Atriplex verrucifera* MB.		soda
***Acremonium* sp. A105**	-	-	Trans-Baikal, Russia	Orongoyskoe Lake	7.8	26	soda-sulfate
***Acremonium* sp. A106**	-	-	Trans-Baikal, Russia	Sulfatnoe Lake	8.5	3.7	sulfate-soda
***Acremonium* sp. A107**	-	-	Trans-Baikal, Russia	Chedder Lake	9.1	-	soda
***Acremonium* sp. A108**	-	-	Aral lake, Kazakhstan	Cape Aktumsyk	taken from *Sueda salsa*		chloride-sulfate
***Acremonium* sp. A109**	-	-	Trans-Baikal, Russia	Kuchiger area	9	-	sulphate
***Acremonium* sp. A110**	-	-	Trans-Baikal, Russia	Sulfatnoe Lake	10.3	139.4	sulfate-soda
***Acremonium* sp. A111**	-	-	Aral lake, Kazakhstan	Cape Aktumsyk	8	-	chloride-sulfate
***Acremonium* sp. E102**	-	-	Kulunda steppe, Altai, Russia	Bezimyannoe Lake	9.1	47	chloride
***Emericellopsis alkalina* A103**	-	-	Kulunda steppe, Altai, Russia	Mirabilit Lake	9.6	100	soda-chloride-sulfate
***Emericellopsis alkalina* A112**	-	-	North-East Mongolia	Burd Lake	10.1	33	soda
***Emericellopsis alkalina* A113**	FW-1476	-	Choibalsan area, North-East Mongolia	-	11	57	soda
***Emericellopsis alkalina* A114**	FW-1473	-	Kulunda steppe, Altai, Russia	Solyonoe Lake	10	187	chloride
***Emericellopsis alkalina* A115**	FW-1474	-	Kulunda steppe, Altai, Russia	-	9.6	225	chloride-sulfate
***Emericellopsis alkalina* A116**	-	-	Kulunda steppe, Altai, Russia	Mirabilit Lake	9.6	100	soda-chloride-sulfate
***Emericellopsis alkalina* A117**	FW-1471	-	Kulunda steppe, Altai, Russia	Shukurtuz Lake	9.9	53	chloride-sulfate
***Emericellopsis alkalina* A118**	-	-	Kulunda steppe, Altai, Russia	Zheltir' Lake	9.6	137	soda-chloride
***Emericellopsis alkalina* A119**	-	-	Kulunda steppe, Altai, Russia	Bezimyannoe Lake	10.1	38	chloride-sulfate
***Emericellopsis alkalina* A120**	-	-	Kulunda steppe, Altai, Russia	Bezimyannoe Lake	9.9	310	soda
***Emericellopsis alkalina* A121**	-	-	Kulunda steppe, Altai, Russia	Tanatar Lake	10.2	73	soda
***Emericellopsis alkalina* A122**	-	-	Kulunda steppe, Altai, Russia	-	9.5	65	chloride
***Emericellopsis alkalina* A123**	-	-	Kulunda steppe, Altai, Russia	-	taken from *Salicornia europaea* L.		soda
***Emericellopsis alkalina* A124**	-	-	Kulunda steppe, Altai, Russia	south, Berdabay	10.1	60	soda
***Emericellopsis alkalina* A125**	-	-	Trans-Baikal, Russia	Nuhe-Nur Lake	10.1	7.1	soda
***Emericellopsis alkalina* A126**	-	-	Trans-Baikal, Russia	Nuhe-Nur Lake	10.1	1.9	soda
***Emericellopsis alkalina* A127**	-	-	Trans-Baikal, Russia	Nuhe-Nur Lake	10.1	1.9	soda
***Emericellopsis alkalina* A128**	-	-	Trans-Baikal, Russia	Sulfatnoe Lake	10.3	139.4	sulfate-soda
***Emericellopsis alkalina* E101 T**	F-4108	CBS 127350	Kulunda steppe, Altai, Russia	Tanatar Lake	10.1	73	soda
***Emericellopsis alkalina* M14**	F-3905	CBS 120043	Kulunda steppe, Altai, Russia	Bezimyannoe Lake	9.9	310	soda
***Emericellopsis alkalina* M20**	FW-3040	CBS 120044	Kulunda steppe, Altai, Russia	Zheltir' Lake	9.6	137	soda-chloride
***Emericellopsis alkalina* M71**	F-3907	CBS 120049	Trans-Baikal, Russia	Sulfatnoe Lake	10.3	139	sulfate-soda

Table 1. (Continued).

Strain	VKM number	CBS no.	Isolation area	Isolation place	pH of the soil	Total salts (g/kg)	Saltification type
Emericellopsis maritima T	F-1082	CBS 491.71	Black sea Sevastopol area, Crimea, Ukraine	sea water	-	-	-
Emericellopsis minima	F-1057	CBS 871.68	Germany	wheat field soil	-	-	-
Emericellopsis minima T	F-1484	CBS 190.55	Inhaca, Mozambique	mangrove soil	-	-	-
Emericellopsis pallida T	F-925	CBS 490.71	Black sea Sevastopol area, Crimea, Ukraine	sea water	-	-	-
***Sarocladium* sp. A131**	-	-	Aral lake, Kazakhstan	Cape Aktumsyk	8.3	-	chloride-sulfate

conidial morphology, commonly asexual *Acremonium* or *Verticillium* species, and typically, without the development of the any sexual morph (Okada *et al*. 1993, Kladwang *et al.* 2003). Substantial difficulties in identifying *Acremonium* species imposed by their simple morphology have stimulated the use of molecular phylogeny in their identification. The array of fungi with acremonium-like conidiation has been shown to be highly polyphyletic, occupying several lineages throughout *Ascomycota* (Summerbell *et al.* 2011). However, most *Acremonium* species belong to *Hypocreales* (subphylum *Hypocreomycetidae*). One of the well-defined subclades within the hypocrealean acremonia is the *Emericellopsis*-clade (family *Bionectriaceae*), which includes isolates derived from various ecological niches. Notably, previous studies have shown a phylogenetic separation of marine-derived and terrestrial isolates within the *Emericellopsis*-clade (Zuccaro *et al.* 2004). The marine clade also contains fungi derived from soda soils. The current study confirms the evolutionary relationships between marine-borne and soda soil fungi of the genus *Emericellopsis*. Here, we analyse acremonium-like strains isolated from soda soils in western Siberia, the Trans-Baikal area (Russia), the Aral Sea (Kazakhstan) and the Gobi Desert (Mongolia) and elucidate their phylogenetic relationships, with an emphasis on the *Emericellopsis*-clade. A new *Emericellopsis* species, *E. alkalina* sp. nov., is described. We also analysed the newly isolated strains for growth at various pH values, in comparison with reference ex-type strains, and show that the alkalitolerant strains group within the known *Emericellopsis* isolates originated from the marine habitats. We discuss a possible origin of alkalitolerance in this particular lineage of mostly sea-borne fungi.

MATERIALS AND METHODS

Soil samples, strains and media

Soil samples were collected from several locations on the edge of the soda lakes (Table 1). We used alkaline agar (AA) with the antibiotic rifampicin (2 g/L) as a selective medium for alkalitolerant species isolation. For routine subculturing on AA of the newly isolated strains, the antibiotic was not used. The AA medium was prepared as described previously (Grum-Grzhimaylo *et al*. 2013). Several reference ex-type *Emericellopsis* strains were obtained from the KNAW-CBS Fungal Biodiversity Centre (CBS) as well as from the All-Russian Collection of Microorganisms (VKM). For the colony morphology characterization we used several types of media: WA, CZ, MYA, PDA, OA and AA (Mueller *et al.* 2004). The elucidation of the pH optimum was performed in duplicate using race tubes with the media ranging in pH as described previously (Grum-Grzhimaylo *et al*. 2013), with the following modification. Instead of using acetic buffer to generate pH 4 and 5.2, we used a citric acid buffer system. Race tubes and plates were incubated in the dark at 28 °C, and the growth rates were recorded once a week over 2 mo.

Morphology

We used light microscopy (LM) and scanning electron microscopy (SEM) for morphological characterization of the

Table 2. Loci and substitution models used for the phylogenetic analyses.

Phylogenetic analysis	Locus	Model for each partition	Characters	Informative characters	Uninformative variable characters	Invariable characters
1	LSU	TIM1+I+G (GTR+I+G)*	962	162	62	738
2	ITS	TIM1+G (GTR+I+G)*	503	101	62	340
	β-tub	TrN+G (HKY+G)*	333	80	29	224
	RPB2	TIM3+G (GTR+G)*	1070	73	134	863
	TEF1-α	TIM3+G (GTR+G)*	904	54	58	792

* - for MrBayes.

strains, as described previously (Grum-Grzhimaylo *et al.* 2013).

DNA extraction, PCR, and sequencing

Total genomic DNA (gDNA) was extracted from mycelium using DNeasy Plant Mini kit (Qiagen, Chatsworth, CA). We amplified and sequenced six nuclear loci (large and small subunit rDNA, internal transcribed spacers 1 and 2, including 5.8S rDNA, RPB2, TEF1-α and β-tub) from gDNA using the standard primers set. Primer sets, thermo cycling programs and sequencing procedures were performed as described previously (Grum-Grzhimaylo *et al*. 2013). The amplification of beta-tubulin intron 3 (hereafter named as "β-tub") was as in Zuccaro *et al*. (2004).

Phylogenetic analyses

We used five nuclear loci for phylogenetic analysis: large subunit rDNA (LSU), ITS region, RPB2, TEF1-α, and β-tub. The gene for small subunit rRNA (SSU), although sequenced, was not included in our phylogenetic reconstructions since it carried too little phylogenetic signal to contribute to clade differentiation. We constructed separate alignments for each of the analysed genes using the online MAFFT v. 7 service (Katoh & Standley 2013). Ambiguous regions were removed manually from the alignments with BioEdit v. 7.1.3.0 (Hall 1999). Two data sets for different phylogenetic analyses were constructed in order to achieve different degrees of resolution within the studied groups. Appropriate reference sequences were obtained from GenBank. The first analysis included a single LSU gene in order to build a large-scale taxonomy for hypocrealean acremonia. The second, a four-gene (ITS, β-tub, RPB2, and TEF1-α) concatenated super-matrix, was implemented to resolve the recent evolutionary relationships in the *Emericellopsis*-clade and our newly isolated alkalitolerant strains. The four-gene concatenated data set was constructed using Mesquite v. 2.75 (Maddison & Maddison 2011) and divided into four partitions corresponding to each individual gene. The best-fit model for nucleotide substitution for each partition was chosen according to the corrected Akaike Information Criterion (AICc) as implemented in jModelTest v. 2.1.1 (Guindon & Gascuel 2003, Darriba *et al*. 2012) (Table 2). GARLI v. 2.0 (Zwickl 2006) was used for Maximum Likelihood (ML) bootstrap analyses; for both phylogenetic analyses the number of searches was set to five for each of the 200 bootstrap replicates. A 50 % majority rule consensus trees were constructed using SumTrees v. 3.3.1 application within DendroPy v. 3.11.0 package (Sukumaran & Holder 2010) running under Python v. 2.6 platform. Bayesian analysis (BI) was performed using MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001). Two independent searches and four chains were set to run for 10 M generations for both phylogenetic analyses sampling every 100th generation. The convergence of the runs was checked in TRACER v. 1.5 (Rambaut & Drummond 2007). The first 30 % (50 % for four-gene analysis) of the resulting trees was eliminated from the further analysis. The rest were used to generate a 50 % majority rule consensus tree and calculate posterior probabilities (PP). The consensus tree was visualized and edited with TreeGraph v. 2.0.47-206 beta (Stöver & Müller 2010) and Adobe Illustrator CS6 (Adobe Systems, San Jose, CA). The node supports were considered to be strong if they received joint scores of ML>90 and PP>0.94. Newly generated sequences from the studied strains were deposited in GenBank with accessions listed in Table 3. Phylogenetic analyses were deposited in TreeBase (submission ID 14196).

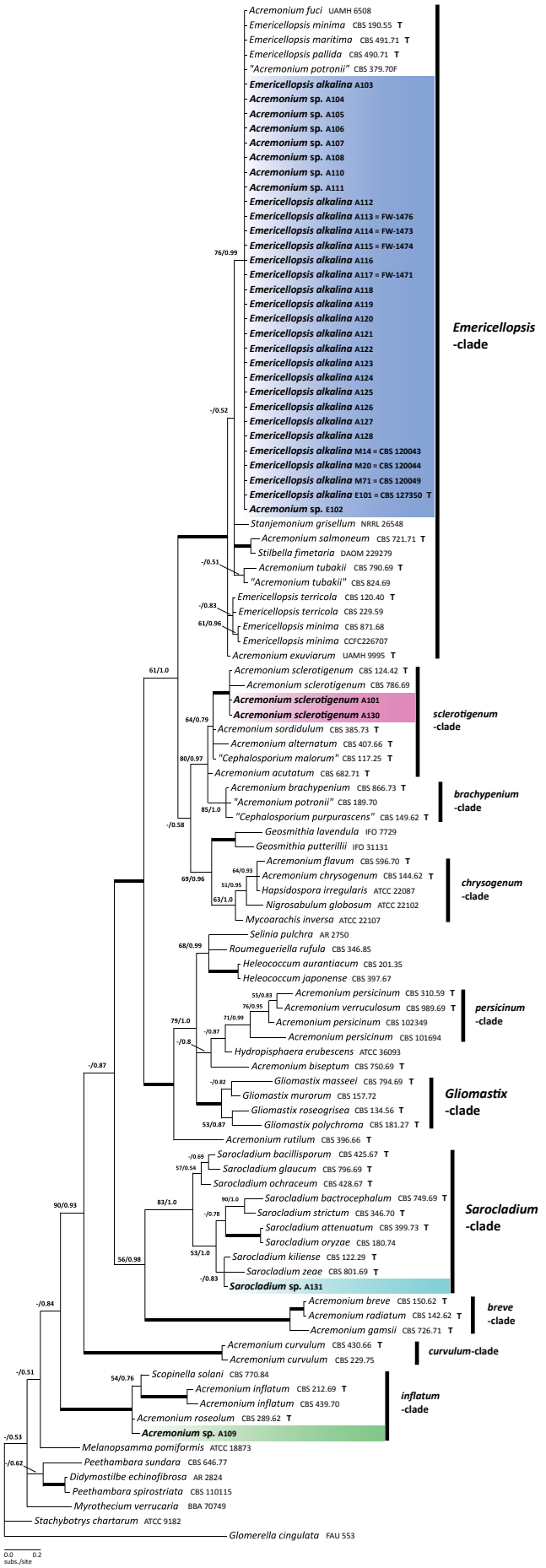
RESULTS

Isolated strains

On the selective AA medium buffered at pH 10 and containing antibiotic, we isolated 34 strains of filamentous fungi from soda soils adjacent to the soda lake basins. Several of the isolated strains were deposited in CBS and VKM. All strains showed asexual acremonium-like sporulation and one displayed comprehensive sexual morphological features and was found to be a new species of the *Emericellopsis* lineage based on molecular, morphological and growth data (*see below*).

Molecular phylogenetic analyses

The alignment for the first phylogenetic analysis using the LSU gene contained 962 characters, with 162 (17 %) being phylogenetically informative (Table 2). The negative log likelihoods (-Ln) of the ML and BI consensus trees were 4696.03 and 5111.82, respectively. The phylogenetic reconstruction based on LSU sequences of our isolates from soda lakes along with the pertinent reference sequences from hypocrealean acremonia is consistent with the topology described by Summerbell *et al*. (2011), hence we follow the clade delineation outlined in that study. As seen in Fig. 1, the new isolates from the soda soils (in coloured boxes) almost exclusively fall into a strongly supported (97/1.0) *Emericellopsis*-clade (*Bionectriaceae*). This clade is known to include marine-borne fungi such as *Acremonium fuci*, *A. tubakii*, *E. maritima,* as well as terrestrial isolates like *E. terricola*, some *Stilbella* species, and the *Stanjemonium*



species. The lizard-associated ex-type-strain of *A. exuviarum* (UAMH 9995), producing chains of conidia, has been shown before to have affinity to the *Emericellopsis*-clade (Sigler *et al.* 2004). Thirty of our new isolates in the *Emericellopsis*-clade stand together within a weakly supported clade (76/0.99) that also includes the ex-type strains of *E. minima* (CBS 190.55), *E. maritima* (CBS 491.71), and *E. pallida* (CBS 490.71), as well as "*A. potronii*" (isolate CBS 379.70F); the latter is a single isolate of an undescribed species that has so far only been isolated from a dolphin skin lesion, apparently not as an agent of infection (Zuccaro *et al.* 2004). The marine species from *Fucus,* a brown seaweed, *A. fuci* (UAMH 6508), also grouped with our isolates from soda soils. There is not enough phylogenetic signal from our LSU-based phylogenetic reconstruction to resolve the *Emericellopsis*-clade further. Four new isolates from soda soils appeared to be in the sister clades, namely, two in the *sclerotigenum*-clade, one in the *Sarocladium*-clade and one in the *inflatum*-clade. They are hence identified accordingly.

The second phylogenetic analysis included partial sequences of four genes (ITS, β-tub, RPB2, TEF1-α) known to have a higher mutation rate than LSU. We sampled a different set of taxa for this low-level taxonomic analysis. The sequences for the *Emericellopsis*-clade had a high degree of similarity, and were easily aligned and edited. The most variable locus in this set was the β-tub region containing introns, and this region thus contributed significantly to the reliability of the resulting tree. The alignment for this analysis had 2 810 characters of which 308 (11 %) were phylogenetically informative (Table 2). The MCMC runs in Bayesian analysis reached stationary status with a deviation of 0.008 after 5M generations. The negative log likelihoods (-Ln) of the ML and BI consensus trees were 8487.81 and 8645.85, respectively.

The tree that was generated for the *Emericellopsis*-clade is displayed in Fig. 2. Here, unlike in the first analysis, the *Emericellopsis*-clade is deeply resolved, displaying several major clades consistent with the previous study by Zuccaro *et al*. (2004). The basal group consists of a highly supported asexual *Stanjemonium* clade, asexual *Stilbella fimentaria* haplotypes, and the soil-derived ex-type isolates of *E. synnematicola*, CBS 176.60, and *E. salmosynnemata*, CBS 382.62. The ex-type isolate of *Acremonium exuviarum,* mentioned earlier, seems to be more distally basal to the rest of the core tree members. Our phylogenetic analysis confirms the presence of the two ecological groups in the *Emericellopsis* lineage, both of which were supported by the molecular studies. The clades designated as marine (M) and terrestrial (T), outlined previously by Zuccaro *et al*. (2004), also appear in our phylogenetic analysis. The T clade (98/1.0) almost

Fig. 1. Phylogenetic reconstruction of *Acremonium* species in *Bionectriaceae* as inferred from the partial LSU gene sequences. New isolates from the soda soils are marked with colour boxes. Clade delineation is from Summerbell *et al.* (2011). Bayesian topology with the ML/PP support values over each node is displayed. Thickened branches indicate strong combined support (ML>90, PP>0.94). **T** – type/ex-type strains.

Table 3. List of taxa used for phylogenetic reconstructions. Strains used in the growth experiments and newly generated accessions are in bold.

Taxon	Voucher	Appearance in phylogenetic analysis (1,2)	LSU	ITS	β-tub	RPB2	TEF1-α	SSU
Acremonium acutatum T	CBS 682.71	1	HQ231965					
Acremonium alternatum T	CBS 407.66	1	HQ231988					
Acremonium biseptum T	CBS 750.69	1	HQ231998					
Acremonium brachypenium T	CBS 866.73	1	HQ232004					
Acremonium breve T	CBS 150.62	1	HQ232005					
Acremonium chrysogenum T	CBS 144.62	1	HQ232017					
Acremonium curvulum T	CBS 430.66	1	HQ232026					
Acremonium curvulum	CBS 229.75	1	HQ232021					
Acremonium exuviarum T	UAMH 9995	1,2	HQ232036	AY882946	AY882947	-	-	
Acremonium flavum T	CBS 596.70	1	HQ232037					
Acremonium fuci T	CBS 112868	2		AY632653	AY632690	-	-	
Acremonium fuci	CBS 113889	2		AY632652	-	-	-	
Acremonium fuci	UAMH 6508	1	HQ232038					
Acremonium gamsii T	CBS 726.71	1	HQ232040					
Acremonium inflatum T	CBS 212.69	1	HQ232050					
Acremonium inflatum	CBS 439.70	1	HQ232051					
Acremonium persicinum T	CBS 310.59	1	HQ232077					
Acremonium persicinum	CBS 101694	1	HQ232085					
Acremonium persicinum	CBS 102349	1	HQ232086					
"Acremonium potronii"	CBS 189.70	1	HQ232094					
"Acremonium potronii"	CBS 379.70F	1,2	HQ232096	AY632655	AY632691	-	-	
Acremonium radiatum T	CBS 142.62	1	HQ232104					
Acremonium roseolum T	CBS 289.62	1	HQ232123					
Acremonium rutilum T	CBS 396.66	1	HQ232124					
Acremonium salmoneum T	CBS 721.71	1	HQ232125					
Acremonium salmoneum	JS-NJ01	2		HM747162	-	-	-	
Acremonium sclerotigenum T	CBS 124.42	1	HQ232126					
***Acremonium sclerotigenum* A101**		1	**KC987215**	**KC987139**	**KC987101**	**KC998999**	**KC998961**	**KC987177**
***Acremonium sclerotigenum* A130**		1	**KC987242**	**KC987166**	**KC987128**	**KC999024**	**KC998988**	**KC987204**
Acremonium sclerotigenum	CBS 786.69	1	HQ232130					
Acremonium sordidulum T	CBS 385.73	1	HQ232136					
***Acremonium* sp. A104**		1,2	**KC987217**	**KC987141**	**KC987103**	**KC999001**	**KC998963**	**KC987179**
***Acremonium* sp. A105**		1,2	**KC987218**	**KC987142**	**KC987104**	**KC999002**	**KC998964**	**KC987180**
***Acremonium* sp. A106**		1,2	**KC987219**	**KC987143**	**KC987105**	**KC999003**	**KC998965**	**KC987181**
***Acremonium* sp. A107**		1,2	**KC987220**	**KC987144**	**KC987106**	**KC999004**	**KC998966**	**KC987182**
***Acremonium* sp. A108**		1,2	**KC987221**	**KC987145**	**KC987107**	**KC999005**	**KC998967**	**KC987183**
***Acremonium* sp. A109**		1	**KC987222**	**KC987146**	**KC987108**	**KC999006**	**KC998968**	**KC987184**
***Acremonium* sp. A110**		1,2	**KC987223**	**KC987147**	**KC987109**	**KC999007**	**KC998969**	**KC987185**
***Acremonium* sp. A111**		1,2	**KC987224**	**KC987148**	**KC987110**	**KC999008**	**KC998970**	**KC987186**
***Acremonium* sp. E102**		1,2	**KC987248**	**KC987172**	**KC987134**	**KC999030**	**KC998994**	**KC987210**
Acremonium tubakii T	CBS 790.69	1	HQ232148					
Acremonium tubakii	CBS 111360	2		AY632654	AY632689	-	-	
"Acremonium tubakii"	CBS 824.69	1	HQ232149					
Acremonium verruculosum T	CBS 989.69	1	HQ232150					
"Cephalosporium malorum" T	CBS 117.25	1	HQ232015					
"Cephalosporium purpurascens" T	CBS 149.62	1	HQ232071					

Table 3. (Continued).

Taxon	Voucher	Appearance in phylogenetic analysis (1,2)	LSU	ITS	β-tub	RPB2	TEF1-α	SSU
Didymostilbe echinofibrosa	AR 2824	1	AY489706					
***Emericellopsis alkalina* A103**		1,2	**KC987216**	**KC987140**	**KC987102**	**KC999000**	**KC998962**	**KC987178**
***Emericellopsis alkalina* A112**		1,2	**KC987225**	**KC987149**	**KC987111**	**KC999009**	**KC998971**	**KC987187**
***Emericellopsis alkalina* A113**	FW-1476	1,2	**KC987226**	**KC987150**	**KC987112**	**KC999010**	**KC998972**	**KC987188**
***Emericellopsis alkalina* A114**	FW-1473	1,2	**KC987227**	**KC987151**	**KC987113**	**KC999011**	**KC998973**	**KC987189**
***Emericellopsis alkalina* A115**	FW-1474	1,2	**KC987228**	**KC987152**	**KC987114**	**KC999012**	**KC998974**	**KC987190**
***Emericellopsis alkalina* A116**		1,2	**KC987229**	**KC987153**	**KC987115**	**-**	**KC998975**	**KC987191**
***Emericellopsis alkalina* A117**	FW-1471	1,2	**KC987230**	**KC987154**	**KC987116**	**KC999013**	**KC998976**	**KC987192**
***Emericellopsis alkalina* A118**		1,2	**KC987231**	**KC987155**	**KC987117**	**KC999014**	**KC998977**	**KC987193**
***Emericellopsis alkalina* A119**		1,2	**KC987232**	**KC987156**	**KC987118**	**KC999015**	**KC998978**	**KC987194**
***Emericellopsis alkalina* A120**		1,2	**KC987233**	**KC987157**	**KC987119**	**KC999016**	**KC998979**	**KC987195**
***Emericellopsis alkalina* A121**		1,2	**KC987234**	**KC987158**	**KC987120**	**KC999017**	**KC998980**	**KC987196**
***Emericellopsis alkalina* A122**		1,2	**KC987235**	**KC987159**	**KC987121**	**KC999018**	**KC998981**	**KC987197**
***Emericellopsis alkalina* A123**		1,2	**KC987236**	**KC987160**	**KC987122**	**KC999019**	**KC998982**	**KC987198**
***Emericellopsis alkalina* A124**		1,2	**KC987237**	**KC987161**	**KC987123**	**KC999020**	**KC998983**	**KC987199**
***Emericellopsis alkalina* A125**		1,2	**KC987238**	**KC987162**	**KC987124**	**KC999021**	**KC998984**	**KC987200**
***Emericellopsis alkalina* A126**		1,2	**KC987239**	**KC987163**	**KC987125**	**KC999022**	**KC998985**	**KC987201**
***Emericellopsis alkalina* A127**		1,2	**KC987240**	**KC987164**	**KC987126**	**-**	**KC998986**	**KC987202**
***Emericellopsis alkalina* A128**		1,2	**KC987241**	**KC987165**	**KC987127**	**KC999023**	**KC998987**	**KC987203**
***Emericellopsis alkalina* E101 T**	CBS 127350 (=VKM F-4108)	1,2	**KC987247**	**KC987171**	**KC987133**	**KC999029**	**KC998993**	**KC987209**
***Emericellopsis alkalina* M14**	CBS 120043 (=VKM F-3905)	1,2	**KC987244**	**KC987168**	**KC987130**	**KC999026**	**KC998990**	**KC987206**
***Emericellopsis alkalina* M20**	CBS 120044 (=VKM F-3040)	1,2	**KC987245**	**KC987169**	**KC987131**	**KC999027**	**KC998991**	**KC987207**
***Emericellopsis alkalina* M71**	CBS 120049 (=VKM F-3907)	1,2	**KC987246**	**KC987170**	**KC987132**	**KC999028**	**KC998992**	**KC987208**
Emericellopsis donezkii T	CBS 489.71	2		AY632658	AY632674	-	-	
Emericellopsis glabra T	CBS 119.40	2		AY632657	AY632673	-	-	
Emericellopsis glabra	A.R. 3614	2		HM484860	HM484879	-	HM484843	
Emericellopsis humicola T	CBS 180.56	2		AY632659	AY632675	-	-	
Emericellopsis maritima T	CBS 491.71 (=VKM F-1082)	1,2	**KC987251**	**KC987175**	**KC987137**	**KC999033**	**KC998997**	**KC987213**
Emericellopsis microspora T	CBS 380.62	2		AY632663	AY632679	-	-	
Emericellopsis minima T	CBS 190.55 (=VKM F-1484)	1,2	**KC987249**	**KC987173**	**KC987135**	**KC999031**	**KC998995**	**KC987211**
Emericellopsis minima	CBS 111361	2		AY632661	AY632677	-	-	
Emericellopsis minima	CBS 871.68 (=VKM F-1057)	1,2	**KC987250**	**KC987174**	**KC987136**	**KC999032**	**KC998996**	**KC987212**
Emericellopsis minima	CCFC226707	1	AY283560					
Emericellopsis mirabilis	CBS 177.53	2		AY632656	-	-	-	
Emericellopsis pallida T	CBS 490.71 (=VKM F-925	1,2	**KC987252**	**KC987176**	**KC987138**	**KC999034**	**KC998998**	**KC987214**
Emericellopsis pallida	CBS 624.73	2		AY632667	AY632683	-	-	
Emericellopsis robusta	CBS 489.73	2		AY632664	AY632680	-	-	
Emericellopsis salmosynnemata	CBS 382.62	2		AY632666	AY632682	-	-	

Table 3. (Continued).

Taxon	Voucher	Appearance in phylogenetic analysis (1,2)	LSU	ITS	β-tub	RPB2	TEF1-α	SSU
Emericellopsis stolkiae T	CBS 159.71	2		AY632668	AY632684	-	-	
Emericellopsis synnematicola T	CBS 176.60	2		AY632665	AY632681	-	-	
Emericellopsis terricola T	CBS 120.40	1,2	U57082	U57676	-	-	-	
Emericellopsis terricola	CBS 229.59	1,2	AY305034	AY632662	AY632678	-	-	
Emericellopsis terricola	CCF3815	2		FJ430737	-	-	-	
Emericellopsis terricola	NRRL 54109	2		HQ698592	-	-	-	
Geosmithia lavendula	IFO 7729	1	D88325					
Geosmithia putterillii	IFO 31131	1	AB047215					
Gliomastix masseei T	CBS 794.69	1	HQ232060					
Gliomastix murorum	CBS 157.72	1	HQ232067					
Gliomastix polychroma T	CBS 181.27	1	HQ232091					
Gliomastix roseogrisea T	CBS 134.56	1	HQ232121					
Glomerella cingulata	FAU 553	1	AF543786					
Hapsidospora irregularis	ATCC 22087	1	AF096192					
Heleococcum aurantiacum	CBS 201.35	1	JX158442					
Heleococcum japonense	CBS 397.67	1	JX158441					
Hydropisphaera erubescens	ATCC 36093	1	AY545726					
Melanopsamma pomiformis	ATCC 18873	1	AY489709					
Mycoarachis inversa	ATCC 22107	1	GQ505991					
Mycopepon smithii	SMH 1609	1	AF279400					
Myrothecium verrucaria	BBA 70749	1	AJ301999					
Nigrosabulum globosum	ATCC 22102	1	AF096195					
Peethambara spirostriata	CBS 110115	1	AY489724					
Peethambara sundara	CBS 646.77	1	AF193245					
Roumegueriella rufula	CBS 346.85	1	DQ518776					
Sarocladium attenuatum T	CBS 399.73	1	HQ232165					
Sarocladium bacillisporum T	CBS 425.67	1	HQ231992					
Sarocladium bactrocephalum T	CBS 749.69	1	HQ231994					
Sarocladium glaucum T	CBS 796.69	1	HQ232041					
Sarocladium kiliense T	CBS 122.29	1	HQ232052					
Sarocladium ochraceum T	CBS 428.67	1	HQ232070					
Sarocladium oryzae	CBS 180.74	1	HQ232166					
***Sarocladium* sp. A131**		1	**KC987243**	**KC987167**	**KC987129**	**KC999025**	**KC998989**	**KC987205**
Sarocladium strictum T	CBS 346.70	1	HQ232141					
Sarocladium zeae T	CBS 801.69	1	HQ232152					
Scopinella solani	CBS 770.84	1	AY015632					
Selinia pulchra	AR 2750	1	AF193246					
Selinia pulchra	AR 2812	2		HM484859	HM484884	-	HM484841	
Stachybotrys chartarum	ATCC 9182	1	AY489714					
Stanjemonium grisellum	NRRL 26548	1	AF049171					
Stanjemonium grisellum T	CBS 655.79	2		AY632671	AY632687	-	-	
Stanjemonium ochroroseum T	CBS 656.79	2		AY632672	AY632688	-	-	
Stilbella fimetaria	D99026	2		AY952467	-	-	-	
Stilbella fimetaria	DAOM 229279	1	HQ232176					
Stilbella fimetaria	MH178	2		FJ430712	-	-	-	
Stilbella fimetaria	SES201	2		FJ939394	-	-	-	
Verrucostoma freycinetiae T	MAFF 240100	2		HM484866	HM484885	-	HM484853	

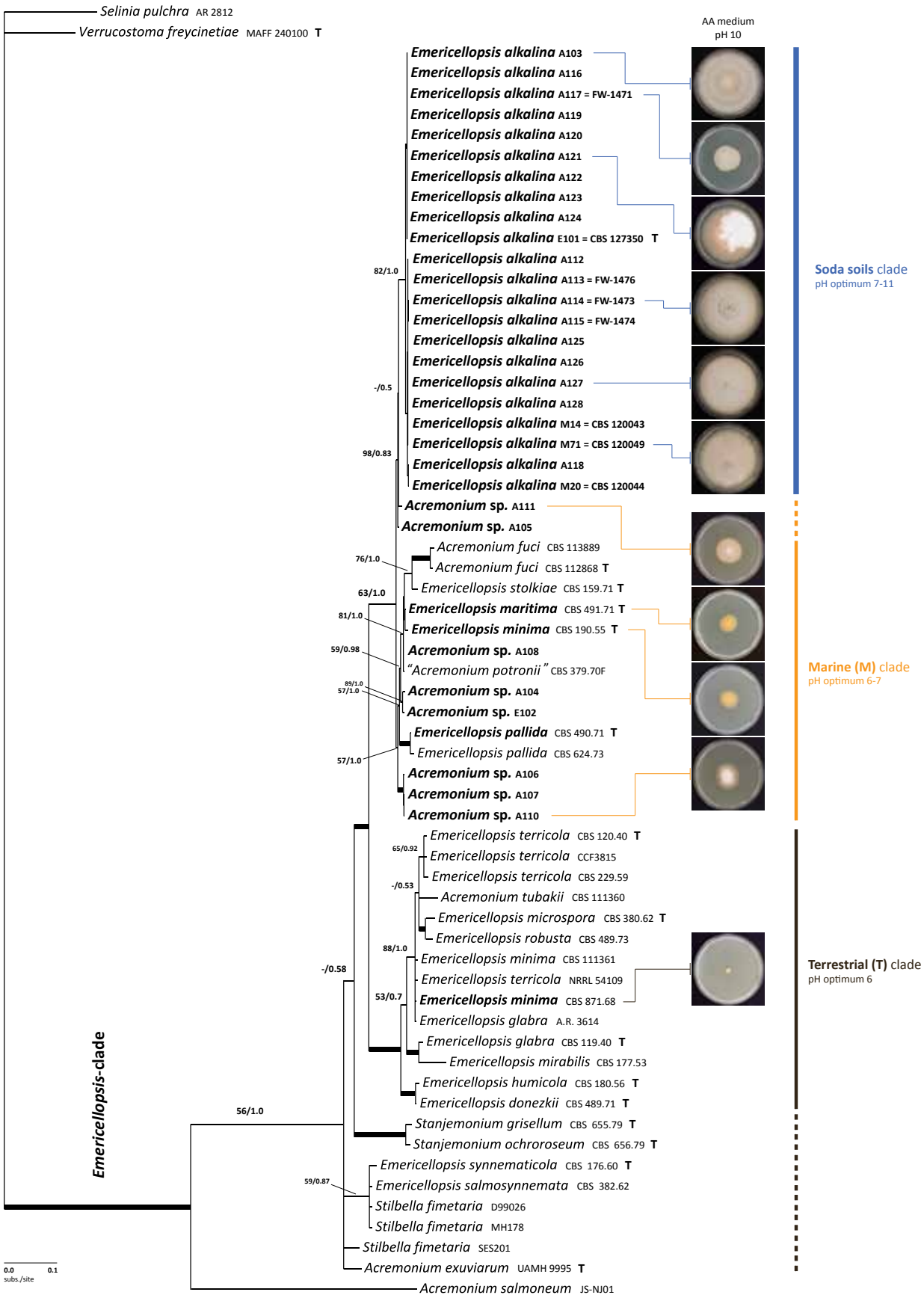


Fig. 2. Four-gene phylogeny of the new alkalitolerant isolates within the *Emericellopsis*-clade based on partial sequences for ITS (including 5.8S rDNA), β-tub, RPB2 and TEF1-α genes. All strains studied are in bold. Bayesian topology is displayed with the ML/PP support values over each node. Thickened branches indicate strong combined support (ML>90, PP>0.94). **T** – type/ex-type strains. Representative strains from each delineated clade are shown on AA medium plates (11-d-old).

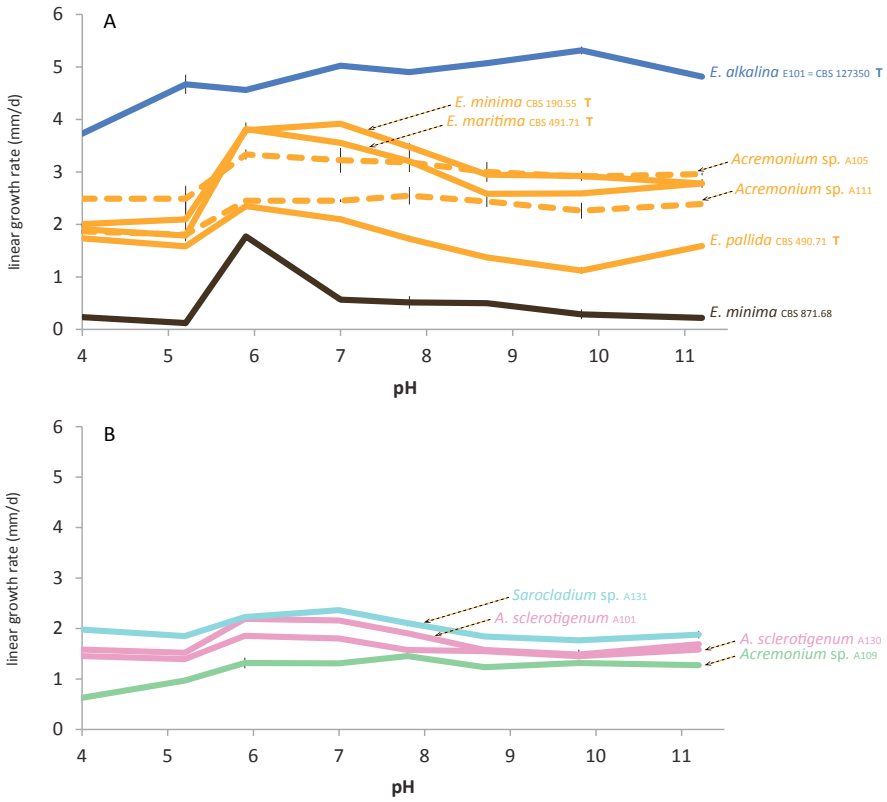


Fig. 3. Growth patterns of the representative strains at pH 4 through 11.2 based on MYA medium. **A.** strains from the T, M and soda soils clades within the *Emericellopsis* lineage including intermediate *Acremonium* sp. isolates A105 and A111; **B.** isolated alkalitolerant strains from the sister clade of the *Emericellopsis* lineage.

exclusively contains terrestrial species of *Emericellopsis*, such as *E. robusta*, *E. terricola*, and *E. microspora*. There are a few exceptions, namely, *E. donezkii* CBS 489.71, *E. minima* CBS 111361, and *A. tubakii* CBS 111360, which were found in aquatic environments. The very weakly supported M clade (57/1.0) predominantly contains isolates from marine and soda lake habitats, with the exception of *E. pallida* CBS 624.73 and the ex-type isolate of *E. minima*, CBS 190.55. Interestingly, eight of our new isolates from the soda soils fall into the M clade while the majority (22 strains) form a well-supported sister clade (82/1.0). We name that clade the "soda soils" clade. It comprises 22 of our isolates that collectively represent a new species named *E. alkalina* sp. nov. here. Of those 22 strains, one formed ascomata, while the others only displayed asexual structures. These structures were identical to those seen in CBS 127350, the sexual strain from which we derived the type of *E. alkalina*.

SSU sequences showed almost no variation among our newly isolated strains in the *Emericellopsis*-clade. We found only two variable sites among 1 637 base pairs.

Growth patterns

In order to link our phylogenetic data to ecological preferences, we conducted a growth experiment testing the growth ability of all studied strains at different ambient pH values. As seen in Fig. 3A, the pH preferences vary among the members of the different clades within the *Emericellopsis* lineage. A reference member of the T clade, *E. minima* (CBS 871.68), displayed a very narrow growth optimum at pH 6 with no ability to cope with both lower and higher pH values. Three reference members of the M clade, the ex-type strains of *E. maritima* (CBS 491.71), *E. minima* (CBS 190.55), and *E. pallida* (CBS 490.71), had an optimum growth at pH 6–7, but were able to tolerate higher pH values. Identical growth patterns were seen in our strains *Acremonium* sp. A104, A105, A106, A107, A108, A110, A111, and E102 (data not shown) which also fall into the M clade. Two strains (A105 and A111) seem to be paraphyletic to the M clade, but based on their growth patterns they belong to the M clade (dashed line). Members of the M clade grew faster than *E. minima* (CBS 871.68) from the T clade. All new isolates of *E. alkalina* (except A117, which had very low growth rate and no pH preference) showed a higher growth rate than that seen in the members of the M and T clades. They had a broad pH optimum in the 7–11 range, and displayed a wide tolerance across the pH scale.

Isolates *Acremonium* sp. A109, *A. sclerotigenum* A101, A130 and *Sarocladium* sp. A131, which fall into a sister-clade to the *Emericellopsis*-clade, had an overall slow growth rate with a slight preference for neutral pH combined with the ability to tolerate higher pH values. This pattern somewhat resembled that seen in the M clade (Fig. 3B).

TAXONOMY

Emericellopsis alkalina Bilanenko & Georgieva, **sp. nov.**
MycoBank MB804572
(Figs 4–5)

Etymology: Epithet taken from the ability to grow at high ambient pH.

Diagnosis: *Asci* saccate, 12–15 µm long, unitunicate. *Ascospores* ellipsoid, pale brown, with uneven surfaces,

4.5–5.5 × 2.5–3.0 µm, surrounded by 3, but frequently 5 longitudinal, subhyaline, smooth-edged alar appendages, width up to 1.0 µm. *Asexual morph* acremonium-like.

Type: **Russia**: Altai, Kulunda steppe, soda soil (total salts 73 g kg^{-1}, pH 10.1) on the edge of the basin of Tanatar Lake, August 2002, *D. Sorokin* (CBS H-21412 – holotype; culture ex-type E101 = CBS 127350 = VKM F-4108).

Description: *Ascomata* dark brown, superficial on the substratum, globose, 50–120(–180) µm diam, non-ostiolate, wall 6–10 µm thick. *Peridium* multi-layered, pseudoparenchymatous, composed of 3–5 layers of compressed cells. *Asci* saccate, 12–15 µm long, with thin deliquescent wall, soon dissolving, unitunicate, scattered irregularly in the ascocarp. *Ascospores* ellipsoid, pale brown, with uneven surfaces, 4.5–5.5 × 2.5–3.0 µm, surrounded by 3, but frequently 5 longitudinal, subhyaline, smooth-edged alar appendages, width up to 1.0 µm. *Asexual morph* acremonium-like. *Conidiation* abundant, mostly plectonematogenous, partially nematogenous. *Conidiophores* mostly simple orthotropic. *Conidiogenous cells* 20–35 µm long, tapering from 1.5–1.8 µm at the base to 0.7–0.8 µm at the apex, sometimes lateral branches form. *Conidia* narrowly ellipsoid, smooth-surfaced, 3.5–6.0 × 1.8–2.2 µm, about the same length as ascospores but narrower, hyaline, adhering in slimy heads. *Chlamydospores* absent.

Culture characteristics: Colonies on alkaline agar (AA, pH 10.0–10.2) fast-growing, reaching 70–80 mm diam in 10 d at 25°C. On MEA (pH 6.5) growing slower, reaching 32–38 mm diam in 10 d. Colonies orange-salmon-pink, later darkening in centre due to the formation of ascomata with tufted aerial mycelium sometimes forming concentric zones upon exposure to light. Reverse colourless. Exudate absent. Decumbent vegetative hyphae thin-walled, hyaline, 0.5–2.0 µm wide. Mycelium consisting of hyaline, smooth-walled, septate hyphae, 1–3 µm wide, often fasciculate.

Additional specimens examined: A103, A112, A113 (= VKM FW-1476), A114 (= VKM FW-1473), A115 (= VKM FW-1474), A116, A117 (= VKM FW-1471), A118, A119, A120, A121, A122, A123, A124, A125, A126, A127, A128, M14 (= VKM F-3905 = CBS 120043), M20 (= VKM FW-3040 = CBS 120044), M71 (= VKM F-3907 = CBS 120049).

Notes: The current study shows a well-supported clade (82/1.0) as inferred from four phylogenetic loci (ITS, β-tub, RPB2, TEF1-α) containing 22 isolates including the type E101. Although only the type E101 strain formed a sexual morph, we assign the remaining 21 isolates to *E. alkalina* as well, based on sequence similarity and the identity of asexual morphology. All 22 isolates of *E. alkalina* showed essentially the same growth patterns with a wide pH tolerance culminating in an optimum at pH 7–11. Isolate A117 is the only exception, showing a highly reduced growth rate in general, and no obvious pH optimum.

Morphological differences from sister species: The ascomata of the type of *Emericellopsis alkalina* (CBS 127350), have a multilayered peridium, composed mostly of five layers of flattened cells. The peridium of *E. pallida* ex-type isolate CBS 490.71 is thinner, 1–2 layered. The ascospore morphology of the type of *E. alkalina* (CBS 127350) looks similar to that of *E. pallida* and *E. minima*. However, *E. alkalina* ascospores have an uneven surface with (3–)5 alar appendages, while *E. pallida*, as represented by ex-type CBS 490.71, has smooth ascospores often with three alar appendages. The ex-type of *E. minima* (CBS 190.55), unfortunately did not produce ascomata during our investigation. A non-type isolate of *E. minima,* CBS 871.68, has wider (2 µm) alar appendages with flexuose rims, while *E. alkalina* (CBS 127350) has narrow (1 µm) appendages with smooth rims.

DISCUSSION

Here we provide phylogenetic evidence that our newly isolated alkalitolerant fungi from geographically diverse soda soils, are derived from marine-borne species within the genus *Emericellopsis*. Based on pH growth preference, the highly alkalitolerant strains form a “soda soils” clade distinct from the moderately alkalitolerant “marine” clade and the neutrophilic “terrestrial” clade. The genus *Emericellopsis*, previously considered to belong to *Eurotiales*, was erected in 1940, based on the isolation of *E. terricola* and its variant *E. terricola* var. *glabra* (eventually renamed *E. glabra*; Backus & Orpurt 1961). Van Beyma (1939–40) described *E. terricola* based on an isolate from soil collected near the town of Baarn in The Netherlands. The generic name came from the close morphological resemblance of the ascospore ornamentation to that of *Emericella nidulans*, which was originally thought to be taxonomically related. Subsequent studies described additional soil-borne *Emericellopsis* species from various parts of the world (Stolk 1955, Gilman 1957, Mathur & Thirumalachar 1960, 1962, Backus & Orpurt 1961). At the beginning of the 1960s, the genus contained five species and one variety. Ascospore size and shape constituted the major criteria used to distinguish species (Durrell 1959).

The beginning of the 1970s marked a new period in the study of *Emericellopsis* with the establishment of marine mycology. New *Emericellopsis* species were discovered in the sediments of soda lakes and along the seacoasts. *Emericellopsis stolkiae*, for instance, was isolated from the soil on the edge of the soda lake in south-western Wyoming, USA (Davidson & Christensen 1971). That species had larger ascospores than previously known *Emericellopsis* species, and also had distinct alar appendages. Tubaki (1973) suggested the conidial genus *Cephalosporium* was characteristic of aquatic sediments, and he linked *Emericellopsis* as the corresponding sexual state.

Emericellopsis was revised by the Russian mycologist Belyakova (1974) who analysed the morphological features of the then known *Emericellopsis* species and compiled an identification key for 12 species. She also described three new aquatic species: *Emericellopsis donezkii* isolated from the basin of the North Donetz River (Ukraine), and *E. maritima* and *E. pallida* from the intertidal zone of the Black Sea in the Crimean peninsula (Ukraine) (Belyakova 1970, 1974).

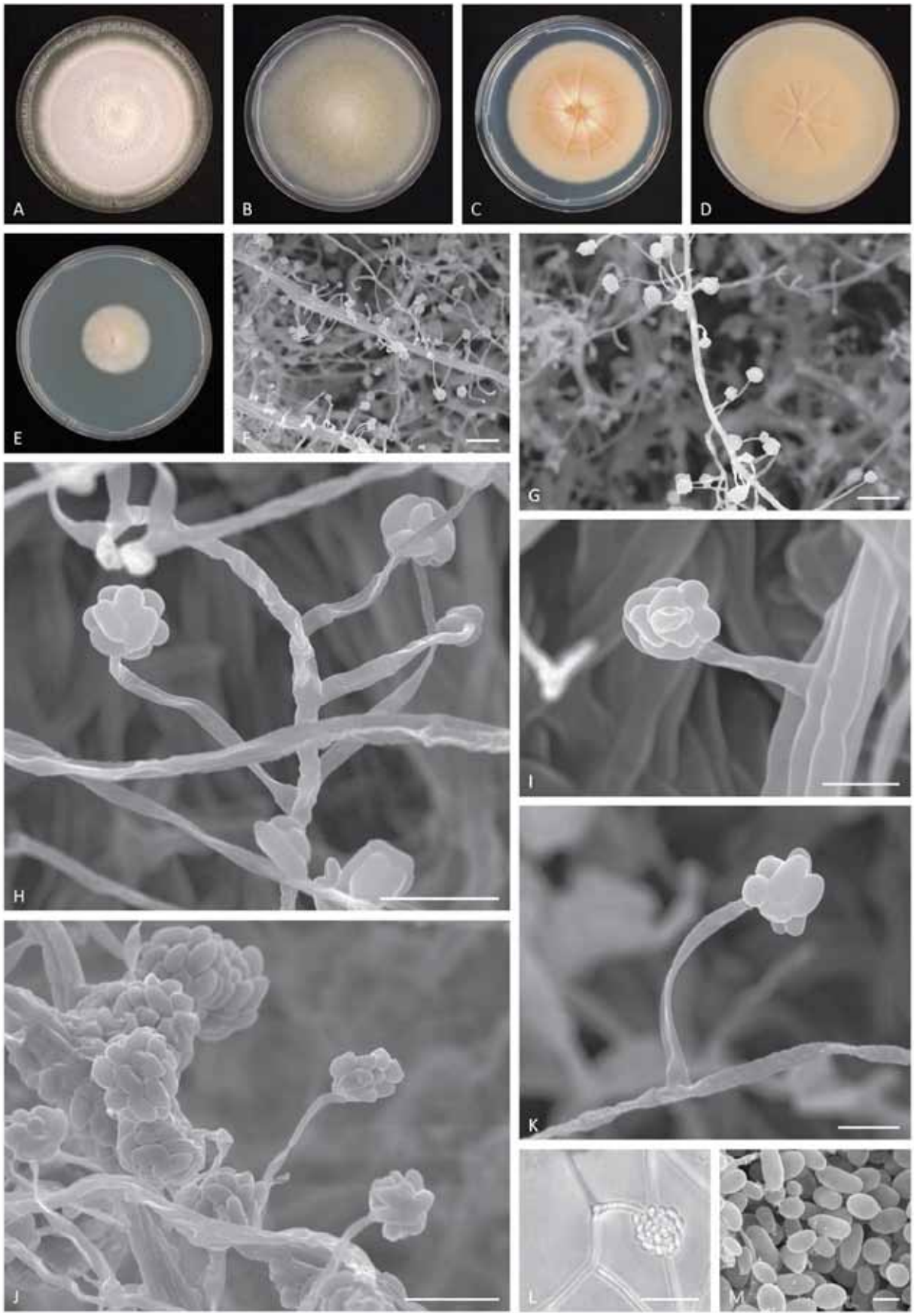


Fig. 4. *Emericellopsis alkalina* (CBS 127350). **A–E.** 11-d-old (28 °C, dark regime, 9 cm Petri dish) colony on alkaline agar (AA), Czapek agar (CZ), potato dextrose agar (PDA), oatmeal agar (OA), malt yeast extract agar (MYA). **F–G.** Hyphal bundles with acremonium-like conidiation (SEM). **H.** Conidiogeous cells emerging from single hypha (SEM). **I.** Conidial head on a single conidiogenous cell emerging from the hyphal bundle (SEM). **J.** Matured conidial heads (SEM). **K.** Single conidiogenous cell with young conidial head (SEM). **L.** Conidial head (LM). **M.** Conidia (SEM). Bars F–G = 20 µm; H, J and L = 10 µm; I and K = 5 µm; and M = 2 µm.

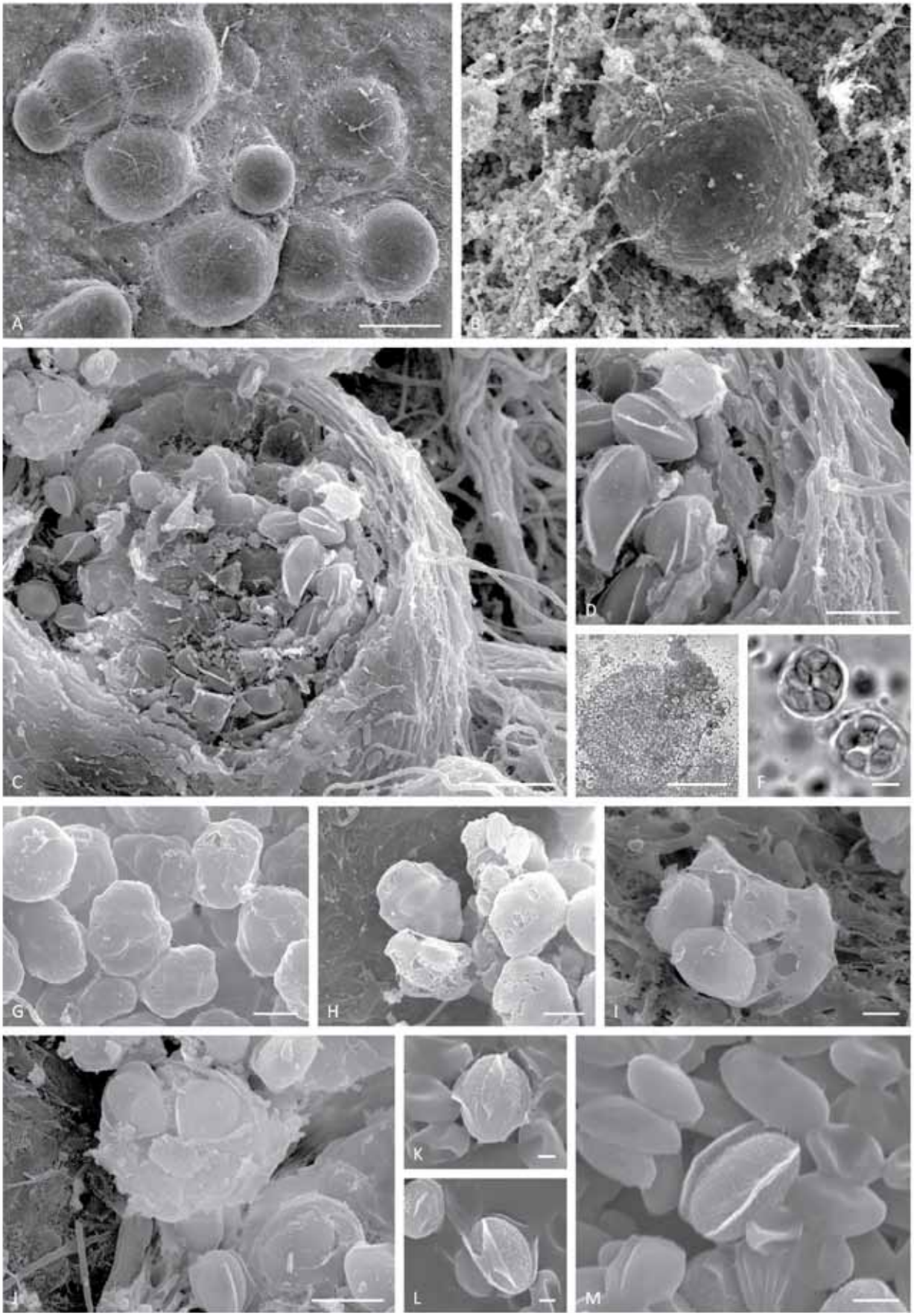


Fig. 5. *Emericellopsis alkalina* (CBS 127350). **A.** Cleistothecia (SEM). **B.** Cleistothecium surrounded by the asexual sporulation (SEM). **C.** Open cleistothecium (SEM). **D.** Magnified view on the multilayered peridium (SEM). **E.** Open cleistothecium (LM). **F.** Young asci (LM). **G.** Young asci (SEM). **H–J.** Lysing asci (SEM). **K–M.** Ascospores with alar appendages (SEM). Bars: A and E = 100 µm; B = 20 µm; C = 10 µm; D, F–H, and J = 5 µm; I and M = 2 µm; and K–L = 1 µm.

At the moment, *Emericellopsis* comprises homothallic saprobic cleistothecial species with acremonium-like conidiation; one species, *E. synnematicola*, also forms stilbella-like synnemata. However, different authors accept different numbers of species in the genus. Currently, 16 species with four varieties are listed in the MycoBank database (Crous *et al*. 2004). All authors have so far supported the opinion that the main distinguishing features among species are the morphology of the ascospores and their alar appendages. Molecular studies conducted in the late 1990s placed *Emericellopsis* in *Hypocreales* (Glenn *et al*. 1996). Analysis of SSU and LSU revealed it as a member of the family *Hypocreaceae* (Ogawa *et al*. 1997), as it was then defined, although it was subsequently assigned to *Bionectriaceae* (Rossman *et al.* 1999, 2001). The genus appears to be monophyletic, with strong support values obtained in the analysis of the ITS and beta-tubulin sequences (Zuccaro *et al*. 2004). The *Emericellopsis* lineage *s. lat.* also harbours the asexual genera *Stilbella* and *Stanjemonium,* along with the marine species *Acremonium tubakii* and *A. fuci* (Summerbell *et al.* 2011).

The accumulated knowledge on the genus *Emericellopsis* suggests a wide ecological amplitude and worldwide distribution. This includes typical species of soils undergoing periodic flooding (e.g. rice paddies), as well as species found in bogs, the sediments of freshwater and seawater basins, and even the soils around subterranean wasp nests where humidity and alkalinity are elevated (Batra *et al.* 1973, Tubaki 1973, Domsch *et al*. 2007). Some species have a broad ecological distribution, such as *E. terricola*, which has been isolated from alkaline soils at the Mono Lake in California as well as from both acidic and saline soils in the Czech National Park (Steiman *et al*. 2004, Hujslová *et al*. 2010). A survey of ascomycetous fungi in limestone soils in Argentina formed by mollusc shells yielded *E. minima,* with its ability to grow from pH 5 to 11 (Elíades *et al.* 2006). The pattern of marine and other salt-associated isolations has suggested that marine habitats might harbour a large number of the *Emericellopsis* species. The ability to survive in high salinity and pH does not always coincide with the ability to develop the full life-cycle in those conditions, making the salts-adapted species difficult to discriminate from “transit” species and hampering efforts to estimate their ecological contribution (Kohlmeyer & Volkmann-Kohlmeyer 2003). A study by Zuccaro *et al*. (2004) revealed the presence of distinct marine and terrestrial clades within *Emericellopsis,* as noted above. The M clade contained isolates from saline habitats, including the recently described *A. fuci* from the thalli of the seaweed *Fucus serratus and F. distichus.* Members of the marine clade within *Emericellopsis* showed an ability to utilize sugars present in seaborne brown algae (e.g. fucoidan, fucose). The presence of marine water appeared to be necessary for conidial germination in *A. fuci*.

Involvement of additional loci in our phylogenetic analysis confirms the presence of the M and T clades (Fig. 2). Our new alkalitolerant isolates are exclusively linked to the M clade, with our 22 *E. alkalina* isolates displaying an extreme alkalitolerant phenotype. Both growth patterns and molecular data suggest that the *E. alkalina* group originated from the marine isolates of the M clade, linking evolutionary development in the marine habitat with that of the soda soils. Clearly, these environments share high salinity and elevated ambient pH values. As far as we know, however, such an ecological overlap has not been demonstrated for other marine fungal lineages. To address this issue, we need systematic biodiversity research on the fungi from soda lakes.

That the intron of the β-tub gene contributed extensively to the phylogenetic signal in our study suggests a relatively recent divergence of *E. alkalina* from the M clade. Our *Acremonium* sp. strains A105 and A111 seem to be intermediate isolates situated in a statistically ambiguous position between the alkaline and marine lineages. The growth pattern of these isolates contributed significantly to our decision to include them within the M clade.

Emericellopsis alkalina grew well at pHs from 4 to 11.2, with a slight preference towards 7–11. However, a few isolates of this species, namely A113, A118, A122, A126, A127, and M20, displayed a significant dip in growth rate at neutral pH values (data not shown). This feature could be seen as a physiological trade-off that has evolved in some strains of *E. alkalina* that thrive along with alkalitolerant strains from the M clade. Interestingly, A128 from the soda soils clade, and A110, were isolated from the same soil sample at Sulfatnoe Lake. And yet, this trend does not extend to all *E. alkalina* strains that were jointly isolated with M clade strains. It is unclear what makes the majority of *E. alkalina* strains grow more vigorously than the M clade members essentially at every pH value we tested. That *E. alkalina* performs well along a large section of the pH scale makes it difficult to specify the ecology of this species in conventional terms. It is technically not correct to label it an ‘alkaliphile’, since it is capable of growth at low pH as well as at high pH. Nor is the term ‘alkalitolerant’ entirely true, since the optimal growth pH is above neutral. The term ‘pH-tolerant’ with the preference towards alkaline conditions might be suitable. As opposed to the soda soils clade, members of the M clade can be appropriately called ‘alkalitolerant’, while *E. minima* (CBS 871.68) from clade T can safely be termed a ‘neutrophile’.

A link between marine and soda soil inhabitants has previously been observed in bacteria. In metabolic studies of fungi, specifically *Fusarium oxysporum*, it has been shown that the expression of the gene *ena1* encoding P-type Na^+-ATPase, which is believed to be an important player in the halotolerance adaptation cascade response, is up-regulated as the ambient pH goes up (Caracuel *et al.* 2003). Therefore, halophilic or halotolerant species may hold a clue towards elucidating the mechanisms of the ability to thrive at high pH. The molecular aspects of the ability to cope with high ambient pH have not been studied in filamentous fungi. Future work aimed at revealing these molecular properties could be carried out by contrasting the genomics of neutrophiles and alkaliphiles. Such a project might provide answers to the intriguing questions inherent in the alkaliphily phenomenon.

ACKNOWLEDGEMENTS

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