

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

Alexey A. Grum-Grzhimaylo<sup>1</sup>, Marina L. Georgieva<sup>2</sup>, Alfons J.M. Debets<sup>1</sup>, and Elena N. Bilanenko<sup>3</sup>

<sup>1</sup>Laboratory of Genetics, Plant Sciences Group, Wageningen University, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands; corresponding author e-mail: alexey.grumgrzhimaylo@wur.nl

<sup>2</sup>Gause Institute of New Antibiotics, Russian Academy of Medical Sciences, 119021 Moscow, Russia

<sup>3</sup>Faculty of Biology, Lomonosov Moscow State University, 1-12 Leninskie Gory, 119234 Moscow, Russia

**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- $\alpha$ ,  $\beta$ -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

**Article info:** Submitted: 2 July 2013; Accepted: 23 October 2013; Published: 5 November 2013.

## 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, Eliades *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

© 2013 International Mycological Association

You are free to share - to copy, distribute and transmit the work, under the following conditions:

**Attribution:** You must attribute the work in the manner specified by the author or licensor (but not in any way that suggests that they endorse you or your use of the work).

**Non-commercial:** You may not use this work for commercial purposes.

**No derivative works:** You may not alter, transform, or build upon this work.

For any reuse or distribution, you must make clear to others the license terms of this work, which can be found at <http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode>. Any of the above conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author's moral rights.

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)	Satification type
<i>Acremonium sclerotigenum</i> A101	-	-	Trans-Baikal, Russia	near Alla River	8	-	sulfate
<i>Acremonium sclerotigenum</i> A130	-	-	Trans-Baikal, Russia	near Alla River	8	-	sulfate
<i>Acremonium</i> sp. A104	-	-	Kulunda steppe, Altai, Russia	-	taken from <i>Atriplex verrucifera</i> MB.	-	soda
<i>Acremonium</i> sp. A105	-	-	Trans-Baikal, Russia	Orongoskoe Lake	7.8	26	soda-sulfate
<i>Acremonium</i> sp. A106	-	-	Trans-Baikal, Russia	Sulfatnoe Lake	8.5	3.7	sulfate-soda
<i>Acremonium</i> sp. A107	-	-	Trans-Baikal, Russia	Chedder Lake	9.1	-	soda
<i>Acremonium</i> sp. A108	-	-	Aral lake, Kazakhstan	Cape Aktumysyk	taken from <i>Sueda salsa</i>	-	chloride-sulfate
<i>Acremonium</i> sp. A109	-	-	Trans-Baikal, Russia	Kuchiger area	9	-	sulphate
<i>Acremonium</i> sp. A110	-	-	Trans-Baikal, Russia	Sulfatnoe Lake	10.3	139.4	sulfate-soda
<i>Acremonium</i> sp. A111	-	-	Aral lake, Kazakhstan	Cape Aktumysyk	8	-	chloride-sulfate
<i>Acremonium</i> sp. E102	-	-	Kulunda steppe, Altai, Russia	Bezimyanno Lake	9.1	47	chloride
<i>Emericellopsis alkalina</i> A103	-	-	Kulunda steppe, Altai, Russia	Mirabilit Lake	9.6	100	soda-chloride-sulfate
<i>Emericellopsis alkalina</i> A112	-	-	North-East Mongolia	Burd Lake	10.1	33	soda
<i>Emericellopsis alkalina</i> A113	FW-1476	-	Choibalsan area, North-East Mongolia	-	11	57	soda
<i>Emericellopsis alkalina</i> A114	FW-1473	-	Kulunda steppe, Altai, Russia	Solyono Lake	10	187	chloride
<i>Emericellopsis alkalina</i> A115	FW-1474	-	Kulunda steppe, Altai, Russia	-	9.6	225	chloride-sulfate
<i>Emericellopsis alkalina</i> A116	-	-	Kulunda steppe, Altai, Russia	Mirabilit Lake	9.6	100	soda-chloride-sulfate
<i>Emericellopsis alkalina</i> A117	FW-1471	-	Kulunda steppe, Altai, Russia	Shukurtuz Lake	9.9	53	chloride-sulfate
<i>Emericellopsis alkalina</i> A118	-	-	Kulunda steppe, Altai, Russia	Zheltir' Lake	9.6	137	soda-chloride
<i>Emericellopsis alkalina</i> A119	-	-	Kulunda steppe, Altai, Russia	Bezimyanno Lake	10.1	38	chloride-sulfate
<i>Emericellopsis alkalina</i> A120	-	-	Kulunda steppe, Altai, Russia	Bezimyanno Lake	9.9	310	soda
<i>Emericellopsis alkalina</i> A121	-	-	Kulunda steppe, Altai, Russia	Tanatar Lake	10.2	73	soda
<i>Emericellopsis alkalina</i> A122	-	-	Kulunda steppe, Altai, Russia	-	9.5	65	chloride
<i>Emericellopsis alkalina</i> A123	-	-	Kulunda steppe, Altai, Russia	-	taken from <i>Salicornia europaea</i> L.	-	soda
<i>Emericellopsis alkalina</i> A124	-	-	Kulunda steppe, Altai, Russia	south, Berdabay	10.1	60	soda
<i>Emericellopsis alkalina</i> A125	-	-	Trans-Baikal, Russia	Nuhe-Nur Lake	10.1	7.1	soda
<i>Emericellopsis alkalina</i> A126	-	-	Trans-Baikal, Russia	Nuhe-Nur Lake	10.1	1.9	soda
<i>Emericellopsis alkalina</i> A127	-	-	Trans-Baikal, Russia	Nuhe-Nur Lake	10.1	1.9	soda
<i>Emericellopsis alkalina</i> A128	-	-	Trans-Baikal, Russia	Sulfatnoe Lake	10.3	139.4	sulfate-soda
<i>Emericellopsis alkalina</i> E101 T	F-4108	CBS 127350	Kulunda steppe, Altai, Russia	Tanatar Lake	10.1	73	soda
<i>Emericellopsis alkalina</i> M14	F-3905	CBS 120043	Kulunda steppe, Altai, Russia	Bezimyanno Lake	9.9	310	soda
<i>Emericellopsis alkalina</i> M20	FW-3040	CBS 120044	Kulunda steppe, Altai, Russia	Zheltir' Lake	9.6	137	soda-chloride
<i>Emericellopsis alkalina</i> 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
<i>Emericellopsis maritima</i> T	F-1082	CBS 491.71	Black sea Sevastopol area, Crimea, Ukraine	sea water	-	-	-
<i>Emericellopsis minima</i>	F-1057	CBS 871.68	Germany	wheat field soil	-	-	-
<i>Emericellopsis minima</i> T	F-1484	CBS 190.55	Inhaca, Mozambique	mangrove soil	-	-	-
<i>Emericellopsis pallida</i> T	F-925	CBS 490.71	Black sea Sevastopol area, Crimea, Ukraine	sea water	-	-	-
<b><i>Sarocladium</i> sp. A131</b>	-	-	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
	$\beta$ -tub	TrN+G (HKY+G)*	333	80	29	224
	RPB2	TIM3+G (GTR+G)*	1070	73	134	863
	TEF1- $\alpha$	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- $\alpha$  and  $\beta$ -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 " $\beta$ -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- $\alpha$ , and  $\beta$ -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,  $\beta$ -tub, RPB2, and TEF1- $\alpha$ ) concatenated supermatrix, 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 100<sup>th</sup> 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

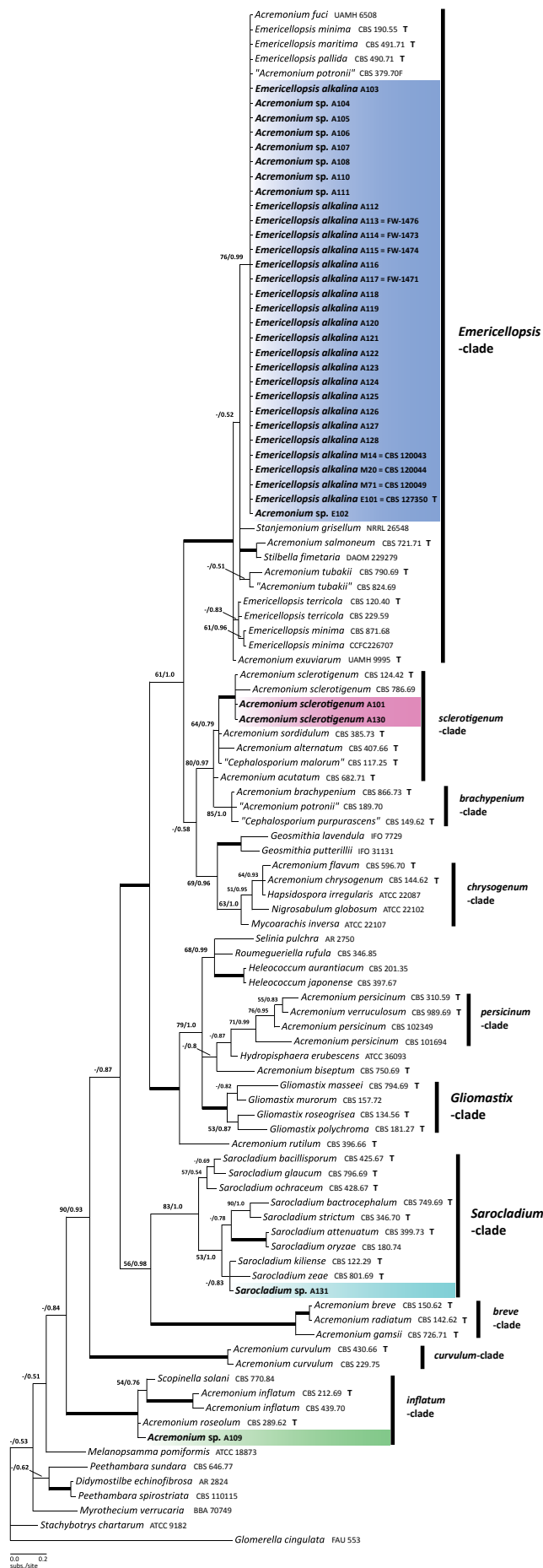
### Isolated strains

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,  $\beta$ -tub, RPB2, TEF1- $\alpha$ ) known to have a higher mutation rate than LSU. We sampled a different set of taxa for this low-level taxonomic analysis. The sequences from the *Emericellopsis*-clade had a high degree of similarity, and were easily aligned and edited. The most variable locus in this set was the  $\beta$ -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. synnemata*, 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 LSU phylogenetic analysis (1,2)	ITS	$\beta$ -tub	RPB2	TEF1- $\alpha$	SSU
<i>Acremonium acutatum</i> T	CBS 682.71	1	HQ231965				
<i>Acremonium alternatum</i> T	CBS 407.66	1	HQ231988				
<i>Acremonium bisseptum</i> T	CBS 750.69	1	HQ231998				
<i>Acremonium brachyphenium</i> T	CBS 866.73	1	HQ232004				
<i>Acremonium breve</i> T	CBS 150.62	1	HQ232005				
<i>Acremonium chrysogenum</i> T	CBS 144.62	1	HQ232017				
<i>Acremonium curvulum</i> T	CBS 430.66	1	HQ232026				
<i>Acremonium curvulum</i>	CBS 229.75	1	HQ232021				
<i>Acremonium exuviarum</i> T	UAMH 9995	1,2	HQ232036	AY882946	AY882947	-	-
<i>Acremonium flavum</i> T	CBS 596.70	1	HQ232037				
<i>Acremonium fuci</i> T	CBS 112868	2		AY632653	AY632690	-	-
<i>Acremonium fuci</i>	CBS 113889	2		AY632652	-	-	-
<i>Acremonium fuci</i>	UAMH 6508	1	HQ232038				
<i>Acremonium gamsii</i> T	CBS 726.71	1	HQ232040				
<i>Acremonium inflatum</i> T	CBS 212.69	1	HQ232050				
<i>Acremonium inflatum</i>	CBS 439.70	1	HQ232051				
<i>Acremonium persicinum</i> T	CBS 310.59	1	HQ232077				
<i>Acremonium persicinum</i>	CBS 101694	1	HQ232085				
<i>Acremonium persicinum</i>	CBS 102349	1	HQ232086				
" <i>Acremonium potronii</i> "	CBS 189.70	1	HQ232094				
" <i>Acremonium potronii</i> "	CBS 379.70F	1,2	HQ232096	AY632655	AY632691	-	-
<i>Acremonium radiatum</i> T	CBS 142.62	1	HQ232104				
<i>Acremonium roseolum</i> T	CBS 289.62	1	HQ232123				
<i>Acremonium rutilum</i> T	CBS 396.66	1	HQ232124				
<i>Acremonium salmoneum</i> T	CBS 721.71	1	HQ232125				
<i>Acremonium salmoneum</i>	JS-NJ01	2		HM747162	-	-	-
<i>Acremonium sclerotigenum</i> T	CBS 124.42	1	HQ232126				
<b><i>Acremonium sclerotigenum</i> A101</b>		1	<b>KC987215</b>	<b>KC987139</b>	<b>KC987101</b>	<b>KC998999</b>	<b>KC998961</b> <b>KC987177</b>
<b><i>Acremonium sclerotigenum</i> A130</b>		1	<b>KC987242</b>	<b>KC987166</b>	<b>KC987128</b>	<b>KC999024</b>	<b>KC998988</b> <b>KC987204</b>
<i>Acremonium sclerotigenum</i>	CBS 786.69	1	HQ232130				
<i>Acremonium sordidulum</i> T	CBS 385.73	1	HQ232136				
<b><i>Acremonium</i> sp. A104</b>		1,2	<b>KC987217</b>	<b>KC987141</b>	<b>KC987103</b>	<b>KC999001</b>	<b>KC998963</b> <b>KC987179</b>
<b><i>Acremonium</i> sp. A105</b>		1,2	<b>KC987218</b>	<b>KC987142</b>	<b>KC987104</b>	<b>KC999002</b>	<b>KC998964</b> <b>KC987180</b>
<b><i>Acremonium</i> sp. A106</b>		1,2	<b>KC987219</b>	<b>KC987143</b>	<b>KC987105</b>	<b>KC999003</b>	<b>KC998965</b> <b>KC987181</b>
<b><i>Acremonium</i> sp. A107</b>		1,2	<b>KC987220</b>	<b>KC987144</b>	<b>KC987106</b>	<b>KC999004</b>	<b>KC998966</b> <b>KC987182</b>
<b><i>Acremonium</i> sp. A108</b>		1,2	<b>KC987221</b>	<b>KC987145</b>	<b>KC987107</b>	<b>KC999005</b>	<b>KC998967</b> <b>KC987183</b>
<b><i>Acremonium</i> sp. A109</b>		1	<b>KC987222</b>	<b>KC987146</b>	<b>KC987108</b>	<b>KC999006</b>	<b>KC998968</b> <b>KC987184</b>
<b><i>Acremonium</i> sp. A110</b>		1,2	<b>KC987223</b>	<b>KC987147</b>	<b>KC987109</b>	<b>KC999007</b>	<b>KC998969</b> <b>KC987185</b>
<b><i>Acremonium</i> sp. A111</b>		1,2	<b>KC987224</b>	<b>KC987148</b>	<b>KC987110</b>	<b>KC999008</b>	<b>KC998970</b> <b>KC987186</b>
<b><i>Acremonium</i> sp. E102</b>		1,2	<b>KC987248</b>	<b>KC987172</b>	<b>KC987134</b>	<b>KC999030</b>	<b>KC998994</b> <b>KC987210</b>
<i>Acremonium tubakii</i> T	CBS 790.69	1	HQ232148				
<i>Acremonium tubakii</i>	CBS 111360	2		AY632654	AY632689	-	-
" <i>Acremonium tubakii</i> "	CBS 824.69	1	HQ232149				
<i>Acremonium verruculosum</i> T	CBS 989.69	1	HQ232150				
" <i>Cephalosporium malorum</i> " T	CBS 117.25	1	HQ232015				
" <i>Cephalosporium purpurascens</i> " T	CBS 149.62	1	HQ232071				

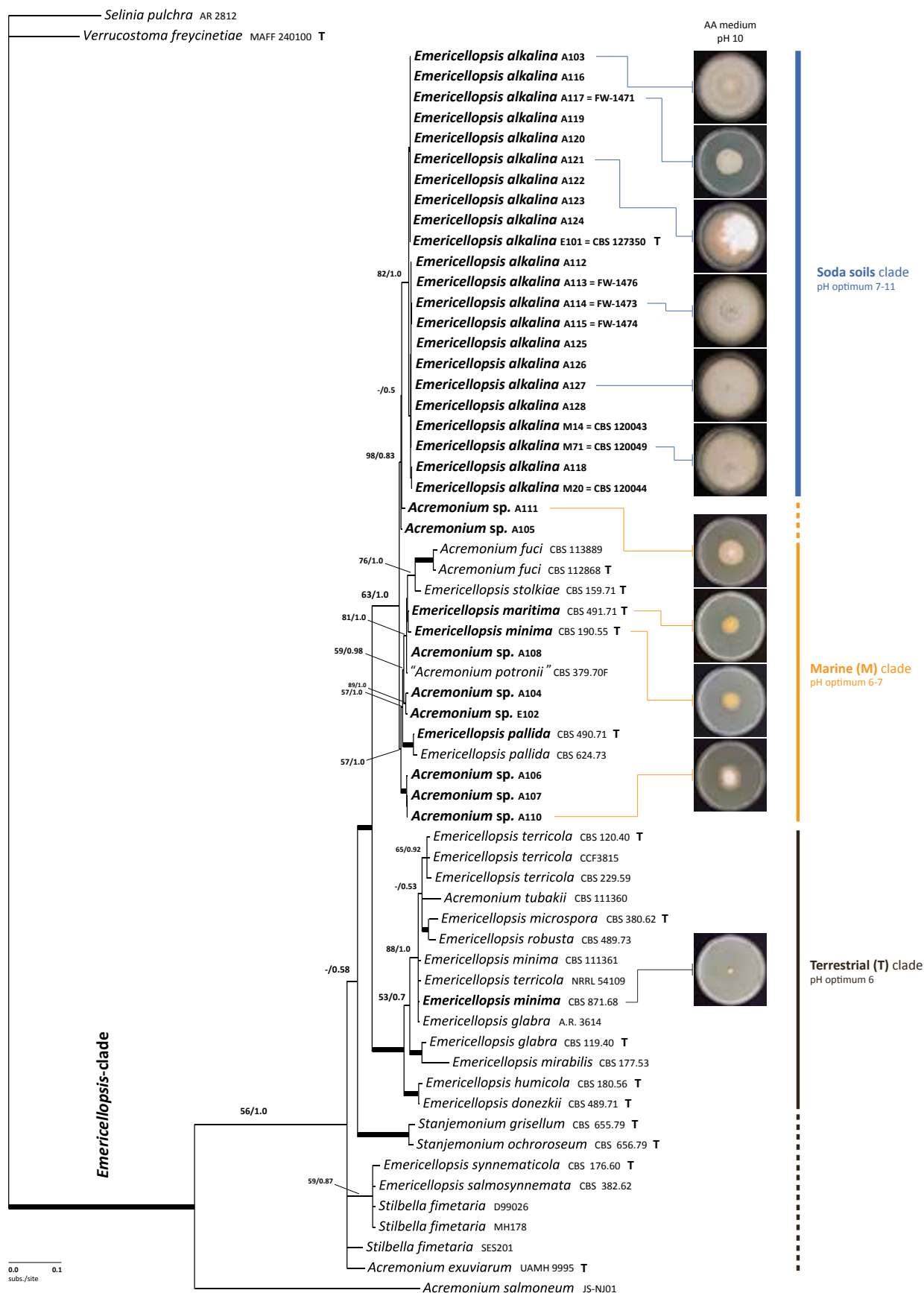
Table 3. (Continued).

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

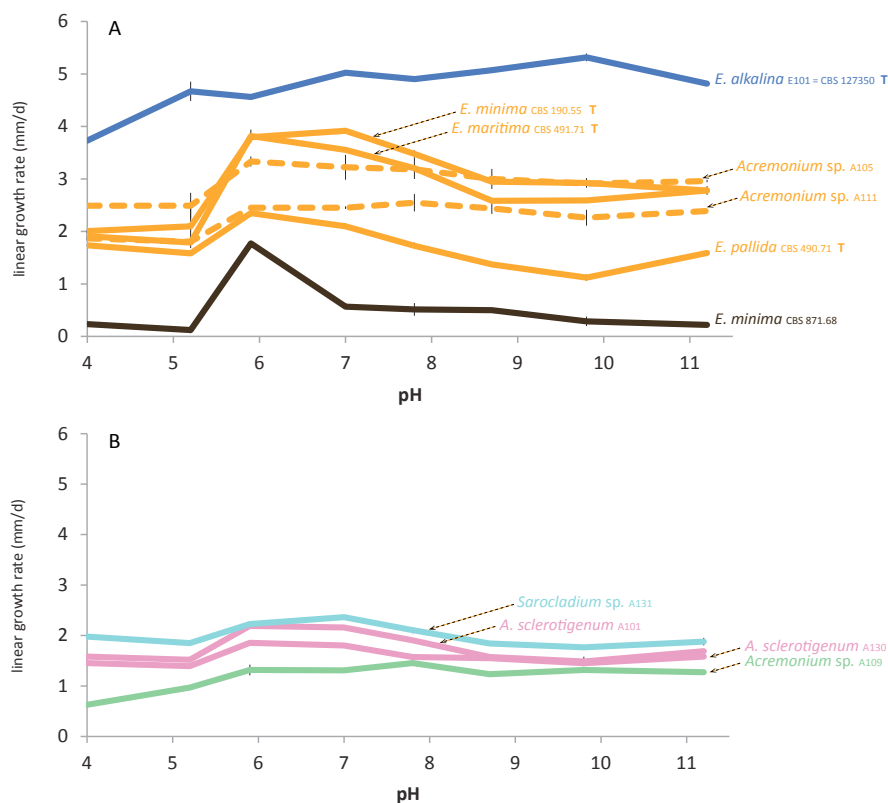
Table 3. (Continued).

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





**Fig. 2.** Four-gene phylogeny of the new alkali-tolerant isolates within the *Emericellopsis*-clade based on partial sequences for ITS (including 5.8S rDNA),  $\beta$ -tub, RPB2 and TEF1- $\alpha$  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* Bilanenکو & 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<sup>-1</sup>, 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

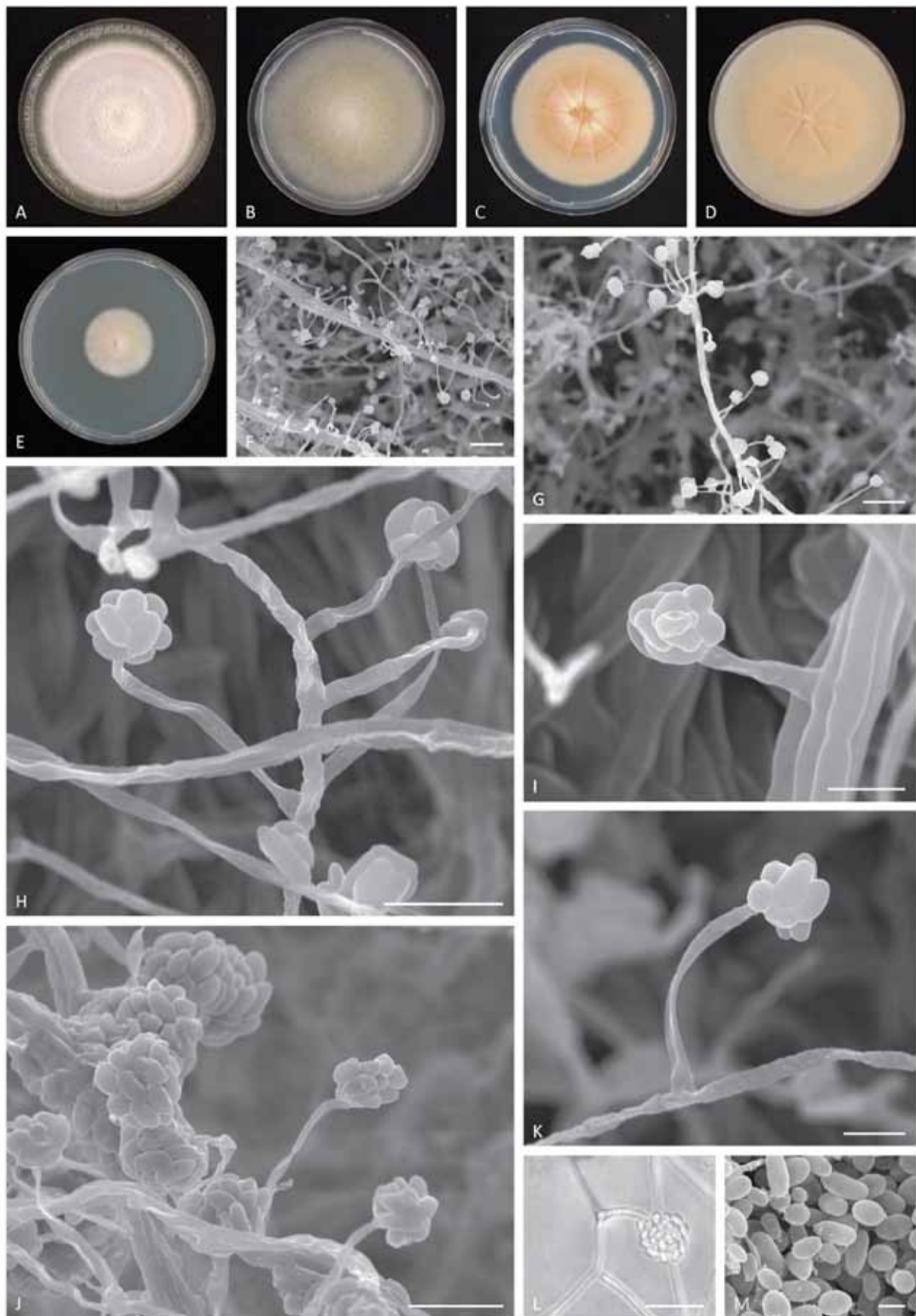
a multilayered peridium, composed mostly of five layers of flattened cells. The peridium of *E. pallida* ex-type isolate CBS 490.71 is thinner, 1–2 layered. The ascospore morphology of the type of *E. alkalina* (CBS 127350) looks similar to that of *E. pallida* and *E. minima*. However, *E. alkalina* 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).

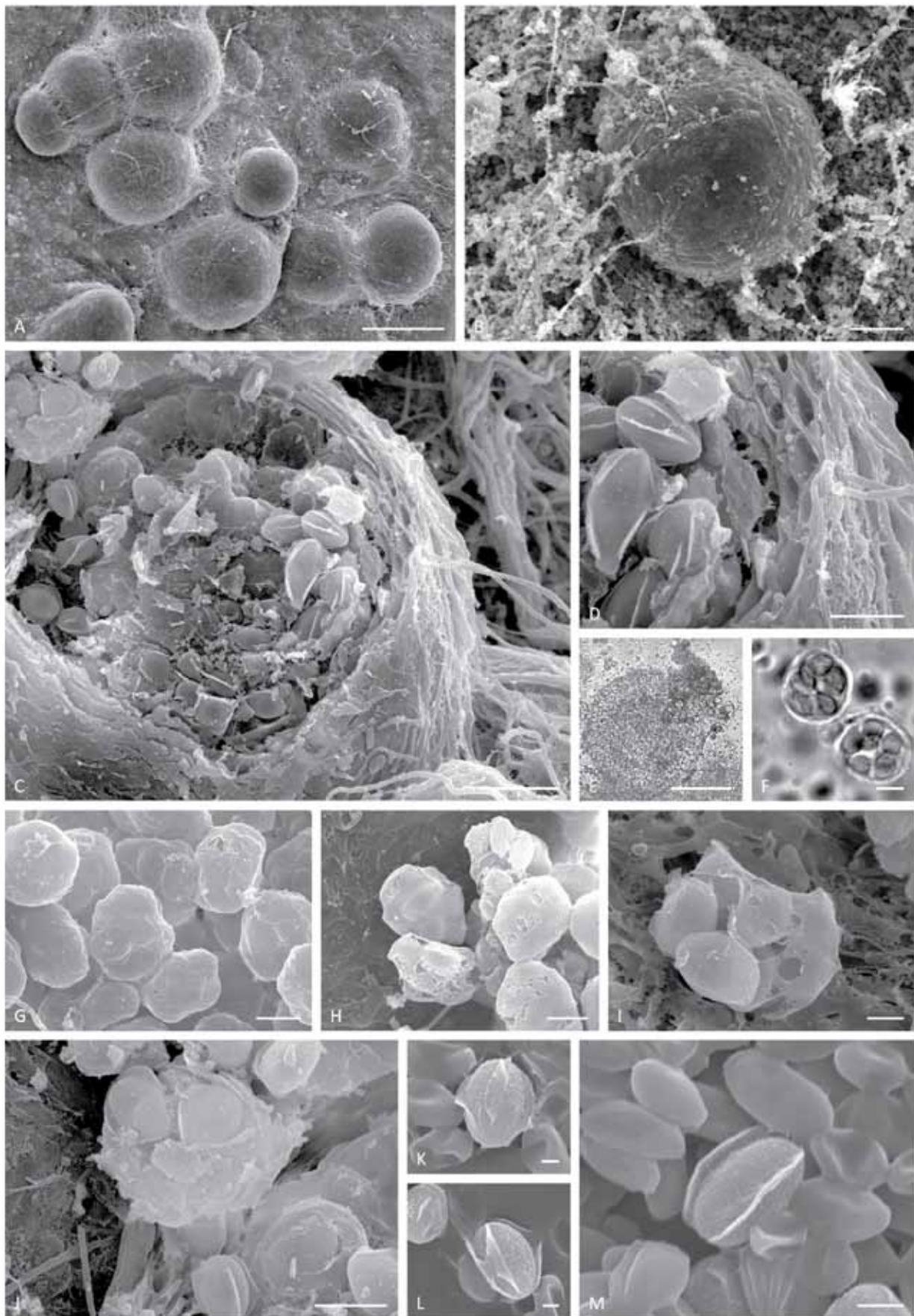
The beginning of the 1970s marked a new period in the study of *Emericellopsis* with the establishment of marine mycology. New *Emericellopsis* species were discovered in the sediments of soda lakes and along the seacoasts. *Emericellopsis stolkiae*, for instance, was isolated from the soil on the edge 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 acromonium-like conidiation (SEM). **H.** Conidiogenous 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  $\mu$ m; H, J and L = 10  $\mu$ m; I and K = 5  $\mu$ m; and M = 2  $\mu$ m.





**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  $\mu\text{m}$ ; B = 20  $\mu\text{m}$ ; C = 10  $\mu\text{m}$ ; D, F–H, and J = 5  $\mu\text{m}$ ; I and M = 2  $\mu\text{m}$ ; and K–L = 1  $\mu\text{m}$ .



At the moment, *Emericellopsis* comprises homothallic saprobic cleistothecial species with acremonium-like conidiation; one species, *E. synnematacola*, 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, Hujsová *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 (Eliades *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  $\beta$ -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<sup>+</sup>-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

We are grateful to Dmitry Sorokin for providing soil samples, Bertha Koopmanschap and Marijke Slakhorst for technical assistance, and Denis Landin for processing the images. The work was funded by the Laboratory of Genetics at Wageningen University (The Netherlands) and an RFBR grant No. 11-04-01576 (Russia).

## REFERENCES

- Backus MP, Orpurt PA (1961) A new *Emericellopsis* from Wisconsin, with notes on other species. *Mycologia* **53**(1): 64–83.
- Batra LR, Batra SWT, Bohart GE (1973) The mycoflora of domesticated and wild bees (*Apoidea*). *Mycopathologia et Mycologia applicata* **49**: 13–44.
- Belyakova LA (1970) Novij vid roda *Emericellopsis* (*Euroticiaeae*). *Mikologiya i Fitopatologiya* **4**: 530–531. [In Russian.]
- Belyakova LA (1974) Rod *Emericellopsis* van Beyma (*Euroticiaeae*). *Mikologiya i Fitopatologiya* **8**: 385–395. [In Russian.]
- Buchalo AS, Wasser SP, Nevo E (2009) Fungi in saline water bodies with special attention to the hypersaline Dead Sea mycobiota. In: *Fungi from Different Environments* (Misra JK, Deshmukh SK, eds): 56–80. Enfield, NH: Science Publishers.
- Cain RF (1956) Studies of soil fungi. *Saturnomyces* a new genus of the *Aspergillaceae*. *Canadian Journal of Botany* **34**: 135–141.
- Caracuel Z, Casanova C, Roncero MIG, Di Pietro A, Ramos J (2003) pH response transcription factor PacC controls salt stress tolerance and expression of the P-type Na<sup>+</sup>-ATPase *ena1* in *Fusarium oxysporum*. *Eukaryot Cell* **2**: 1246–1252.
- Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004) MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* **50**: 19–22.
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.
- Davidson DE, Christensen M (1971) *Emericellopsis stolckiae* sp. nov. from saline soils in Wyoming. *Transactions of the British Mycological Society* **57**: 385–391.
- Domsch KH, Gams W, Anderson T-H (2007) *Compendium of Soil Fungi*. 2<sup>nd</sup> edn. Eching: IHW-Verlag.
- Duckworth AW, Grant WD, Jones BE, Steenberg RV (1996) Phylogenetic diversity of soda lake alkaliphiles. *FEMS Microbiology Ecology* **19**: 181–191.
- Durrell LW (1959) Some studies of *Emericellopsis*. *Mycologia* **51**(1): 31–43.
- Elíades LA, Cabello MN, Voget CE (2006) Contribution to the study of alkaliphilic and alkali-tolerant *Ascomycota* from Argentina. *Darwiniana* **44**: 64–73.
- Gilman JC (1957) *A Manual of Soil Fungi*. 2<sup>nd</sup> edn. Ames, IA: Iowa State College Press.
- Glenn AE, Bacon CW, Price R, Hanlin RT (1996) Molecular phylogeny of *Acremonium* and its taxonomic implications. *Mycologia* **88**: 369–383.
- Grum-Grzhimaylo AA, Debets AJM, van Diepeningen AD, Georgieva ML, Bilanenko EN (2013) *Sodiomyces alkalinus*, a new holomorphic alkaliphilic ascomycete within the *Plectosphaerellaceae*. *Persoonia* **31**: 147–158.
- Guindon S, Gascuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* **52**: 696–704.
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.
- Huelsenbeck JP, Ronquist F (2001) MrBayes: bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- Hujsová M, Kubátová A, Chudíčková M, Kolařík M (2010) Diversity of fungal communities in saline and acidic soils in the Soos National Natural Reserve, Czech Republic. *Mycological Progress* **9**: 1–15.
- Jones BE, Grant WD, Duckworth AW, Owenson GG (1998) Microbial diversity of soda lakes. *Extremophiles* **2**: 191–200.
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.
- Kladwang W, Bhumirattana A, Hywel-Jones N (2003) Alkali-tolerant fungi from Thailand. *Fungal Diversity* **13**: 69–83.
- Kohlmeyer J, Volkmann-Kohlmeyer B (2003) Fungi from coral reefs: a commentary. *Mycological Research* **107**: 386–387.
- Maddison WP, Maddison DR (2011) *Mesquite: a modular system for evolutionary analysis*. Version 2.75. <http://mesquiteproject.org>
- Mathur PN, Thirumalachar MJ (1960) A new *Emericellopsis* species with *Stilbella*-type of conidia. *Mycologia* **52**: 694–697.
- Mathur PN, Thirumalachar MJ (1962) Studies on some Indian soil fungi-II. On some new or interesting *Ascomycetes*. *Sydwia* **16**: 1–6.
- Mueller GM, Bills GF, Foster MS (eds) (2004) *Biodiversity of Fungi: inventory and monitoring methods*. Amsterdam: Elsevier Academic Press.
- Nagai K, Sakai T, Rantiatmodjo R, Suzuki K, Gams W, Okada G (1995) Studies on the distribution of alkaliphilic and alkali-tolerant Soil Fungi I. *Mycoscience* **36**: 247–256.
- Nagai K, Suzuki K, Okada G (1998) Studies on the distribution of alkaliphilic and alkali-tolerant soil fungi II: fungal flora in two limestone caves in Japan. *Mycoscience* **39**: 293–298.
- Ogawa H, Yoshimura A, Sugiyama J (1997) Polyphyletic origins of species of the anamorphic genus *Geosmithia* and the relationships of the cleistothecial genera: evidence from 18S, 5S and 28S rDNA sequence analyses. *Mycologia* **89**: 756–771.
- Okada G, Niimura Y, Sakata T, Uchimura T, Ohara N, Suzuki H, Kozaki M (1993) *Acremonium alcalophilum*, a new alkaliphilic cellulolytic hyphomycete. *Transactions of the Mycological Society of Japan* **34**: 171–185.
- Rambaut A, Drummond AJ (2007) *Tracer v. 1.5*. Computer program and documentation distributed by the authors: <http://beast.bio.ed.ac.uk/Tracer>
- Rossmann AY, McKemy JM, Pardo-Schultheiss RA, Schroers HJ (2001) Molecular studies of the *Bionectriaceae* using large subunit rDNA sequences. *Mycologia* **93**: 100–110.
- Rossmann AY, Samuels GJ, Rogerson CT, Lowen R (1999) Genera of *Bionectriaceae*, *Hypocreaceae* and *Nectriaceae* (*Hypocreales*, *Ascomycetes*). *Studies in Mycology* **42**: 1–248.
- Sigler L, Zuccaro A, Summerbell RC, Mitchell J, Paré JA (2004) *Acremonium exuviarum* sp. nov., a lizard-associated fungus with affinity to *Emericellopsis*. *Studies in Mycology* **50**: 409–413
- Sorokin ID, Kravchenko IK, Tourova TP, Kolganova TV, Boulygina ES, Sorokin DY (2008) *Bacillus alkalidiazotrophicus* sp. nov., a diazotrophic, low salt-tolerant alkaliphile isolated from Mongolian soda soil. *International Journal of Systematic and Evolutionary Microbiology* **58**: 2459–2464.
- Steiman R, Ford L, Ducros V, Lafond JL, Guiraud P (2004) First survey of fungi in hypersaline soil and water of Mono Lake area (California). *Antonie van Leeuwenhoek* **85**: 69–83.
- Stolk AC (1955) *Emericellopsis minima* sp. nov. and *Westerdykella ornata* gen. nov. sp. nov. *Transactions of the British Mycological Society* **38**: 419–424.
- Stöver BC, Müller KF (2010) TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* **11**: 7.
- Sukumaran J, Holder MT (2010) DendroPy: A Python library for phylogenetic computing. *Bioinformatics* **26**: 1569–1571.

- Summerbell RC, Gueidan C, Schroers HJ, De Hoog GS, Starink M, Rosete YA, Guarro J, Scott JA (2011) *Acremonium* phylogenetic overview and revision of *Gliomastix*, *Sarocladium*, and *Trichothecium*. *Studies in Mycology* **68**: 139–162.
- Tubaki K (1973) Aquatic sediment as a habitat of *Emericellopsis*, with a description of an undescribed species of *Cephalosporium*. *Mycologia* **65**: 938–941.
- van Beyma thoe Kingma FH (1939-40) Beschreibung einiger neuer pilzarten aus dem Centraalbureau voor Schimmelcultures, Baarn (Nederland). *Antonie van Leeuwenhoek* **6**: 263–290.
- Zuccaro A, Summerbell RC, Gams W, Schroers HJ and Mitchell JI (2004) A new *Acremonium* species associated with *Fucus* spp., and its affinity with a phylogenetically distinct marine *Emericellopsis* clade. *Studies in Mycology* **50**: 283–297.
- Zwickl DJ (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. PhD dissertation, University of Texas at Austin.