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A new *Acremonium* species associated with *Fucus* spp., and its affinity with a phylogenetically distinct marine *Emericellopsis* clade

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Abstract: We investigated the evolutionary relationships of terrestrial and marine *Emericellopsis* species, as well as related *Stanjemonium* species, to newly obtained *Emericellopsis* and *Acremonium* isolates from thalli of phaeophyte algae in the genus *Fucus*. Sequences of the internal transcribed spacer regions, the 5.8S rRNA gene and intron 3 of the β-tubulin gene from twenty-one species were analysed using maximum parsimony and quartet puzzling with maximum likelihood. The monophyly of the group containing *Emericellopsis*, *Stanjemonium* and some marine-derived *Acremonium* species was supported. The group consisted of four clades, one of which contained only isolates originating from marine sources or saline lakes. A new species, described here as *Acremonium fuci* sp. nov., was included within this clade. It was isolated independently from *Fucus serratus* in Europe and *F. distichus* in western North America. The germination of its conidia occurred in the presence of *F. serratus* tissue or aqueous tissue homogenates, but not in seawater alone. Enzyme activity and carbohydrate utilisation profiles were done for the species studied; results were found to correlate with clades and habitats. Specifically, polyphenol oxidase activity was found only in clades associated with terrestrial habitats, while assimilation of fucoidan and fucose, compounds commonly associated with phaeophytes, was found only in members of the marine clade.

Taxonomic novelty: *Acremonium fuci* Summerbell, Zuccaro & W. Gams sp. nov.
Key words: *Acremonium fuci*, anamorph, conidial germination, *Emericellopsis*, enzymes, marine clade.

INTRODUCTION

The genus *Emericellopsis* J.F.H. Beyma was established in 1940 to accommodate a soil isolate of *E. terricola* J.F.H. Beyma. It houses, currently, fifteen species of primarily soil-derived fungi that are distinguished, principally, by differences in ascospore size and in the shape and size of wing-like ascospore appendages (Davidson & Christensen 1971, Gams 1971, Belyakova 1974, Udagawa & Furuya 1988). All species have more or less conspicuous *Acremonium*-like conidial states; one species forms *Stilbella*-like synnemata (Gams 1971). Species of the anamorph genus *Stanjemonium* W. Gams, O'Donnell, Schroers & M. Christensen, for which no corresponding teleomorph is known, also have a close relationship with *Emericellopsis* (Gams *et al.* 1998b). Molecular phylogenetic studies based on small and large ribosomal RNA and β-tubulin gene sequences placed *Emericellopsis* and *Stanjemonium* within the *Hypocreales* as sister groups to *Bionectria* Speg. (Glenn *et al.* 1996, Gams *et al.* 1998b, Rossman *et al.* 2001).

Emericellopsis species may be isolated from various environments world-wide, including agricultural and forest soils, peat, rhizomes, prairies and freshwater-, estuarine- and marine-mud sediments (Domsch *et al.* 1980). Aquatic systems prove to be a rich source of isolates belonging to this genus. Grosklags & Swift (1957) noted that periodically waterlogged soils appear to be preferred by many *Emericellopsis* species. Tubaki (1973), during a survey of fungi associated with mud in marine and brackish estuarine habitats, noted that *E. humicola* (Cain) J.C. Gilman, *E. stolkiae* D.E. Davidson & M. Christensen and *E. minima* Stolk were isolated frequently; he stated that aquatic sediments might be the characteristic habitats of these species. It is unclear, however, whether any of these species are active in these habitats.

The presence of a species in marine environments does not necessarily mean that it forms part of the active community structure (Kohlmeyer & Volkmann-Kohlmeyer 2003). Many terrestrial species, while able to withstand saline conditions, cannot complete their life cycles in marine systems (Hyde *et al.* 2000). Similar and related species, however, may have become adapted to marine substrates (Geiser *et al.* 1998, Alker *et al.* 2001, Duncan *et al.* 2002) and it may be difficult to distinguish between native and transitory species recovered from maritime environments.

Table 1. List of fungal strains used in the molecular analyses during this study. CBS, Centraalbureau voor Schimmelcultures, Utrecht; T.U.B., Technische Universität Braunschweig.

Species	ID number	Source	Localities	Clade in Fig. 4
A. potronii Vuillemin	CBS 379.70F	Skin lesion in dolphin	Belgium	B (marine)
Acremonium fuci	T.U.B. 264 = CBS 112868 T	*Fucus serratus*	North Sea, Germany	B (marine)
	UAMH 6508 = CBS 113889	*Fucus distichus*	Vancouver, Canada	B (marine)
A. tubakii W. Gams	T.U.B. K90 = CBS 111360	*Fucus serratus*	North Sea, Germany	A (terrestrial)
E. donezkii Beljakova	CBS 489.71 T	River water	Severnyi Donets R. , Ukraine	A (terrestrial)
E. glabra (J.F.H. Beyma) Backus & Orpurt	CBS 119.40 T	Soil	Netherlands	A (terrestrial)
E. humicola (Cain) J.C. Gilman	CBS 180.56 T	Peat soil	Canada	A (terrestrial)
E. maritima Beljakova	CBS 491.71 T	Coastal seawater	Ukraine, Black Sea	B (marine)
E. microspora Backus & Orpurt	CBS 380.62 T	Wet prairie soil	U.S.A., Wisconsin	A (terrestrial)
E. mirabilis (Malan) Stolk	CBS 177.53 T	Damp soil	Italy	A (terrestrial)
E. minima Stolk	CBS 190.55 T	Mangrove soil	Mozambique	B (marine)
	CBS 871.68	Wheat field soil	Germany	A (terrestrial)
	T.U.B. Em = CBS 111361	*Fucus serratus*	North Sea, Germany	A (terrestrial)
E. pallida Beljakova	CBS 624.73	Soil	Manitoba, Canada	B (marine)
E. robusta van Emden & W. Gams	CBS 489.73	Agricultural soil	Netherlands	A (terrestrial)
E. salmosynnemata Grosklags & Swift	CBS 382.62	Soil	Belgium	D (synnematous)
E. stolkiae D.E. Davidson & M. Christensen	CBS 159.71 T	Mud in saline lake	U.S.A., Wyoming	B (marine)
E. synnematicola P.N.Mathur & Thirumalachar	CBS 176.60 T	Soil	India	D (synnematous)
E. terricola J.F.H. Beyma	CBS 229.59	Soil	Netherlands	A (terrestrial)
Stanjemonium grisellum W.Gams, Schroers & M. Christensen	CBS 655.79 T	Soil	U.S.A., Wyoming	C (*Stanjemonium*)
Stanjemonium ochroroseum W. Gams & M. Christensen	CBS 656.79 T	Soil	U.S.A., Wyoming	C (*Stanjemonium*)

T = ex-type cultures.

The common recovery of *Emericellopsis*-like rDNA sequences (Zuccaro *et al.* 2003) and unusual *Acremonium* Link isolates from healthy and decaying thalli of *Fucus* L. species suggested that unidentified members of these genera might be adapted to the marine environment. The present paper describes a new *Acremonium* species, phylogenetically related to *Emericellopsis,* that was isolated from *Fucus* spp., and investigates the phylogenetic relationship, as well as physiological differences, between terrestrial and saline-derived *Emericellopsis* species and related anamorphs.

MATERIALS AND METHODS

Fungal isolates

The fungal strains used in the molecular analyses during this study are listed in Table 1. Seventeen isolates were obtained from the CBS fungal collection, and three strains were freshly collected from *Fucus serratus* L. [T.U.B. Em = CBS 111361, T.U.B. K90 = CBS 111360 and T.U.B. 264 = CBS 112868; note that isolate designations beginning with T.U.B. (Technische Universität Braunschweig) do not imply a connection with the standard acronym TUB, referring to the herbarium of the Eberhard-Karls-Universität, Tübingen]. One of twenty isolates of the undescribed species (T.U.B. 264 = CBS 112868), collected by A. Z. during a one-year survey of fungi from *F. serratus* in Heligoland, North Sea, Germany, was among the strains included in phylogenetic analysis, while the others were included in a morphological study. Additional isolates of this species had been collected by R.C.S. over a 20-year period from *Fucus distichus* L. in Vancouver, British Columbia, Canada, and were noted as "*Acremonium* sp." by Summerbell *et al.* (1989). These isolates were revived from the University of Alberta Microfungus Collection and Herbarium (UAMH; Edmonton, Alberta, Canada) and the Cen-

traalbureau voor Schimmelcultures (CBS; Utrecht, the Netherlands); one of them (UAMH 6508 = CBS 113889) was included in a molecular analysis while the other, identical isolates were included along with it in morphological studies. The strains studied morphologically but not included in the phylogenetic analysis are not listed in Table 1; they are listed under "Additional specimens and cultures examined" under the species description below.

Isolation

Isolation techniques used by A.Z. are described in Zuccaro *et al.* (2003). R.C.S. collected detached but undecayed-looking *F. distichus* thalli along beaches, as well as some attached thalli from adjacent rocks, and incubated them nearly immersed in sterile distilled water at room temperature (19–22.5 ºC). After approximately 14 d, when a thin layer of conidiophores was apparent on many areas of the thalli breaking through the water line, a fine needle with a minute block of agar on its tip (i.e., an approx. 0.25 mm wide block cut from the medium in the plate to be used for isolation, see media detailed hereafter) was used to pull away conidia without touching the algal surface. The conidial masses were streaked onto malt extract agar (MEA) or Sabouraud glucose agar (Gams *et al.* 1998a) with various combinations of antibacterials.

Fungal cultivation, DNA extraction, PCR amplification and sequencing

For molecular and some of the morphological analyses, the fungi were grown on potato-carrot agar (PCA; Gams *et al.* 1998a) at 25°C except for strains T.U.B. 264, T.U.B. K90 and T.U.B. Em, which were grown on PCA containing 33 g/L marine salt (Meersalz, Wiegandt GmbH, Krefeld, Germany). Fresh mycelium removed from the agar surface with a sterile loop was extracted using the Ultra Clean Soil DNA isolation kit (MoBio Laboratories, Inc., Solana Beach, CA, U.S.A). Primers used in PCR were ITS1-F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) to amplify the single gene region spanning the nuclear 5.8S rRNA and internal transcribed spacer (ITS1 and ITS2) loci (referred to hereafter as the "ITS region") and primers T10 (O'Donnell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995) for intron 3 of the β-tubulin gene (hereafter abbreviated "β-tubulin region").

All PCR amplifications were performed in a reaction buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.1 % Triton X-100) with 200 µM of dNTP mix, standard 1.25 enzyme-units/bead TaqBead Hot Start Polymerase beads (Promega GmbH, Mannheim, Germany) at one per 50 µL amplification reaction volume, 2.5 µM $MgCl_2$, and 0.5 µM of each primer. Amplification was performed on a Biometra T personal PCR system (Biometra biomedizinische Analytik GmbH, Göttingen, Germany), using the following parameters: 5 min at 94 ºC followed by 35 cycles of 1 min at 94 ºC, 1 min at 55 ºC, 1 min at 72 ºC and a final extension for 7 min at 72 ºC. Negative controls were included for each set of reactions. The PCR samples were purified using GENECLEAN III Kit (Qbiogene, Irvine, CA, U.S.A.), and run on agarose gel together with a self-made standard to check DNA concentration. Cleaned PCR products were sequenced using the fluorescent method in a Li-COR 4200 DNA sequencer (Amodia Bioservice GmbH, Braunschweig, Germany). Complementary sequences were obtained for each isolate using primers ITS1 and ITS4 for the ITS region and primers T10 and Bt2b for the β-tubulin region.

Phylogenetic analysis

All sequences were aligned manually in GENEDOC (Nicholas *et al.* 1997) and in the text editor of PAUP 4.0b10 [March 2, 2002, update of PAUP (Swofford 2000)]. The alignment contained data for 26 taxa including ITS and β-tubulin region sequences from *Bionectria samuelsii* Schroers CBS 699.97, *B. aureofulvella* Schroers & Samuels CBS 195.93 and *B. oblongispora* Schroers CBS 100285, which were used as the outgroup. Sequence accession numbers are given in Table 2. The combined data matrix consisted of 911 characters that were partitioned into sets representing the different genetic regions. These regions were analysed separately and in combination after performing partition homogeneity tests implemented by PAUP for pair-wise comparisons of permuted characters (Farris *et al.* 1994, Swofford 2000). Typical settings for these analyses were 1000 replicates using a heuristic search with random sequence addition for 500 replicates. Maximum parsimony analysis was performed using the ITS (561 characters), β-tubulin (350 characters) and combined matrices. Heuristic searches were performed using random sequence addition (up to 50 replicates) and the tree-bisection reconnection (TBS) branch-swapping algorithm with and without successive weighting (Farris 1969). Bootstrap analyses were performed using up to 1000 replicates in heuristic searches with random sequence addition (10 replicates). Decay indices (Bremer 1988) were calculated using TreeRot (Sorenson 1996). Maximum likelihood analysis was performed using the combined matrix and quartet puzzling where the characters were partitioned to account for rate heterogeneity. Substitution rate-matrix parameters and nucleotide frequencies were estimated from the data, and site-specific rates were calculated according to the "general time-reversible, site-specific" (GTR + SS) model implemented in PAUP.

Enzyme assays

Seventeen isolates belonging to the genus *Emericellopsis* and related anamorphic species were tested for the production of the extracellular enzymes cellulase, β-glucosidase, polyphenol oxidase, lipase, amylase and protease.

Table 2. GenBank accession numbers of sequences used in this study. CBS, Centraalbureau voor Schimmelcultures, Utrecht.

Species	CBS accession numer	NCBI gene accession no.	
		ITS-5.8S rDNA	β-tubulin
Emericellopsis mirabilis	177.53	AY632656	
E. glabra	119.40	AY632657	AY632673
E. donezkii	489.71	AY632658	AY632674
E. humicola	180.56	AY632659	AY632675
E. minima	871.68	AY632660	AY632676
E. minima	111361	AY632661	AY632677
E. terricola	229.59	AY632662	AY632678
E. terricola	120.40	U57676	
E. microspora	380.62	AY632663	AY632679
E. robusta	489.73	AY632664	AY632680
E. synnematicola	176.60	AY632665	AY632681
E. salmosynnemta	382.62	AY632666	AY632682
E. pallida	624.73	AY632667	AY632683
E. stolkiae	159.71	AY632668	AY632684
E. minima	190.55	AY632669	AY632685
E. maritima	491.71	AY632670	AY632686
Stanjemonium grisellum	655.79	AY632671	AY632687
S. ochroroseum	656.79	AY632672	AY632688
Acremonium fuci	113889	AY632652	
A. fuci	112868	AY632653	AY632690
A. tubakii	111360	AY632654	AY632689
A. potronii	379.70F	AY632655	AY632691
Bionectria samuelsii	699.97	AF358236	AF358190
B. aureofulvella	195.93	AF358226	AF358181
B. oblongispora	100285	AF358248	AF358169

The tests were carried out by incorporating the corresponding substrates into the media (semi-quantitative direct test) or by the addition of reagents after a certain period of growth (semi-quantitative indirect test). After incubation, the tubes or plates were checked for the appearance of colour reactions or clearance zones. Colour reactions were rated for intensity in arbitrary units; diameters of clearance zones were measured in mm. Three replicates of each test were incubated in the dark at 20 °C.

Cellulase activity (Kreisel & Schauer 1987): The cellulose medium used in this test contained, per litre water, 25 g Biomalt (Gesundprodukte GmbH, Kirn, Austria), 10 g cellulose microcrystalline (Fluka Bio-Chemika AG, Buchs, Switzerland), 33 g sea salt ("Meersalz," Wiegandt) and 20 g agar (Fluka), with final pH adjusted to 6.2. Plates were evaluated after 2 wk by flooding them with Lugol's iodine-potassium iodide solution, 1 % (Merck, Darmstadt, Germany). Cellulose degradation was indicated by a clear zone around the colony in medium otherwise opaquely stained purple.

β-*Glucosidase activity*: The medium used contained, per litre water, 5 g arbutin, 20 g agar, 0.5 g peptone from soya meal, 1 g yeast extract, 1 g glucose and 33 g sea salt, with final pH adjusted to 6.8. In positive reactions, dark brown pigment formed around the point of inoculation, usually within 4 d.

Polyphenol oxidase activity (Bavendamm 1928): Tests were carried out with tannic acid medium (per litre water, 15 g biomalt, 5 g tannic acid, 20 g agar, and 33 g sea salt, pH 6.4). In positive reactions, a clear zone formed around the inoculum in approximately 1 wk.

Lipase activity (Carroll & Petrini 1983): This test was carried out in test tubes in which were combined, after separate autoclaving, 1 mL of solution B (per litre water, 4.4 g $ZnSO_4 \cdot 7H_2O$, 5 g $MnSO_4 \cdot H_2O$, 1 g $CuSO_4 \cdot 5H_2O$, 5 g iron citrate, 0.5 g $Na_2MoO_4 \cdot 2H_2O$ and 1 g $Na_2B_4O_2$) and 3 mL solution C (per litre water, 0.01 g thiamine, 0.1 g nicotinic acid, 1 g inositol and 0.1 g folic acid) per litre of medium A (per litre water, 3 g glucose, 2 g KH_2PO_4, 1.4 g $(NH_4)_2SO_4$, 0.3 g $CaCl_2$, 0.3 g $MgSO_4 \cdot 7H_2O$, 1 g yeast extract, 10 g Tween 20 and 17 g agar). After 5 wk the inoculated test tubes were checked for two indicators of lipase activity. One was the production of precipitate crystals, as described in the original method; the other was the production of a translucent area under the inoculum in an otherwise semi-opaque, milky medium. Measurements were made along the length of the tube of the depth to which precipitate formation could be seen or the depth (mm) of the cleared zone.

Amylase activity: Medium contained, per litre water, 20 g biomalt, 1 g yeast extract, 2 g soluble starch and 12 g agar. Starch degradation was tested after 2 wk by flooding the plates with iodine solution for 5 min and measuring clear zones.

Protease activity. Test tubes used contained medium consisting, per litre water, of 167 g Nutrient Gelatin Special Grade (Fluka) and 33 g sea salt, pH 6.8. Protease activity was measured based on the ability of the fungi to liquefy the solid media. The test was read after 2 wk growth. To ascertain that gelatin had been broken down, not melted, liquefied media were kept for up to 1 d at 4 °C to check if they would solidify again.

Utilisation of different algal carbon sources

The ability of terrestrial and marine isolates to grow on laminarin extracted from *Laminaria digitata* (Hudson) Lamouroux, fucoidan from *Fucus vesiculosus* L., or L-(–)-fucose (all supplied by Fluka) as sole carbon sources (C sources) was tested. For 4 wk prior to inoculation of test media, fungi were pre-cultured on an induction medium containing, per litre water, 3 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g KCl, 0.01 g $FeSO_4 \cdot 7H_2O$,

33 g sea salt, 15 g agar, pH 7 and 1 g/L of the C source to be tested (laminarin, fucoidan or fucose). Inoculum for the subsequent tests was obtained by cutting 3 mm discs from colonies by means of a cork borer. Tests for C source utilization were conducted using a liquid mineral-salts medium (Hornei *et al.* 1972) containing, per litre double-distilled (dd) water, 5 g $NH_4H_2PO_4$, 2.5 g KH_2PO_4, 1 g $MgSO_4 \cdot 7H_2O$, 20 mg $Ca(NO_3)_2 \cdot 4H_2O$, 2 mg $FeCl_3 \cdot 6H_2O$, and 15 g sea salt. D-glucose was used as a positive control. After autoclaving the salts solution, 10 mL each of solution A and B were added to it using sterile filtration. Solution A (Fritsche 1968) contained, per litre dd water, 50 mg H_3BO_3, 40 mg $MnSO_4 \cdot 4H_2O$, 40 mg $ZnSO_4 \cdot 7H_2O$, 20 mg $NaMoO_4$, 10 mg $CuSO_4 \cdot 5H_2O$, 10 mg $CoCl_2$, and 10 mg potassium iodide. Solution B (van der Walt & van Kerken 1961) contained, per litre dd water, 20 mg 4-aminobenzoic acid, 0.2 mg biotin, 0.2 mg folic acid, 200 mg *myo*-inositol, 40 mg nicotinic acid, 40 mg pantothenic acid (hemicalcium salt), 40 mg pyridoxine hydrochloride, 20 mg riboflavin, and 100 mg thiamin hydrochloride. Soluble sugar substrates were prepared in concentrated solutions, filter-sterilised and added to the culture media at a final concentration of 2 g/L. The negative control medium contained no C source. Two parallel replicates were inoculated for each C source tested and for the negative control; a third control was left uninoculated (sterile control). The test cultures were cultivated for 12 d at 130 rpm on a shaker (Infors AG, Bottmingen, Switzerland) at 20 °C. Detection of free glucose was carried out for each replicate, inoculated or sterile, at the beginning and end of the incubation period, using an enzymatic test described by Huggett & Nixon (1957) and Bergmeyer (1988). Detection of reducing sugars was carried out following the Anthron method described by Roe (1955) and Herbert *et al*. (1971).

Conidial germination test

The germination of conidia in the presence and absence of *F. serratus* thalli or aqueous homogenate of this seaweed was assessed for two isolates. The algal isolate *Acremonium* sp. T.U.B. 264 and a terrestrial strain of *Emericellopsis terricola* CBS 229.59 were cultured on cellulose medium (recipe given above). Conidia from each strain were collected using sterile artificial sea water [ASW; consisting of distilled water amended with 33 g/L Meersalz (Wiegandt)] and used to inoculate (1 mL of conidial suspension) Petri dishes containing sterile ASW with and without an intact, young thallus of *F. serratus* or 1 mL of an aqueous homogenate of the alga. Homogenates of *F. serratus* were obtained using 80 g of frozen material mixed using a chopper (type 4142, Braun GmbH, Kronberg at Frankfurt-am-Main, Germany) for 5 min in the presence of 500 mL ASW at 70 °C. Conidial germination was observed using inverse microscopy (Axiovert 35M, Zeiss AG, Göttingen, Germany) at time intervals of 24 h over the course of one wk.

Morphological studies

Isolates of the undescribed species were grown on MEA and oatmeal agar (OA; Gams *et al.* 1998a) at 20 °C and examined after 10 d. Growth at temperatures ranging from 6 to 40 °C was examined at 7, 10, 14 and 21 d on MEA, starting from a 3 mm inoculum plug aseptically cut from a MEA plate with 7-day colony growth.

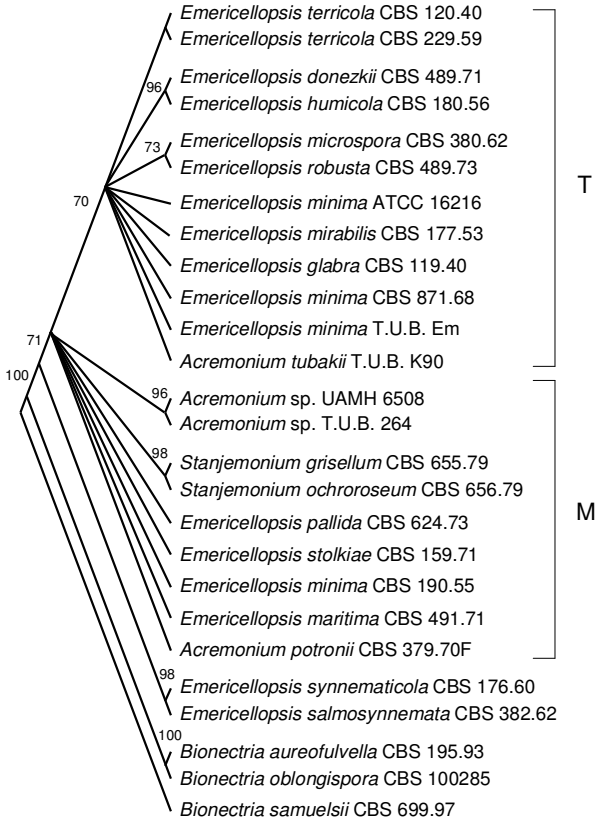

Fig. 1. Relationship between *Emericellopsis* species determined using sequences from the internal transcribed spacer (ITS) region (ITS1, ITS2 and 5.8S rDNA). Majority consensus cladogram produced after a bootstrap analysis using maximum parsimony. Bootstrap values above 70 % are shown at the nodes supported.

RESULTS

Phylogenetic analysis based on the ITS region

The sequences of the ITS region from species of *Emericellopsis*, *Acremonium* and *Stanjemonium* were similar, lacking major repeat units or indels, and were thus easily aligned. Maximum parsimony analysis using a heuristic search with random sequence addition (50 replicates) of 26 species generated 131 equally parsimonious trees of 193 steps (confidence index (CI) = 0.788, retention index (RI) = 0.875, rescaled consistency index (RC) = 0.689, and homoplasy index (HI) = 0.212, with parsimony-informative characters (PIC) = 107) from five tree islands of

different length. A majority consensus cladogram produced after bootstrap analysis (100 replicates) is shown in Fig. 1 where the taxa resolved into two main polytomies, T and M, along with a strongly supported basal clade consisting of the ex-type isolate of *E. synnematicola* P. N. Mathur & Thirumalachar and an isolate identified as *E. salmosynnemata* Grosklags & Swift. Clade T comprised primarily terrestrial isolates with the exception of *Acremonium tubakii* W. Gams T.U.B. K90 and *Emericellopsis minima* T.U.B. Em, which were recovered from *F. serratus*. Two other species falling into clade T, *E. humicola* and *E. microspora* Backus & Orpurt, have been reported as being isolated from marine environments (Tubaki 1973) but the ex-type strains used in this study were recovered from soil.

Clade M represented species recovered mainly from marine or saline environments, though the *E. pallida* Belyakova isolate analysed was from soil. The species of *Stanjemonium* also joined this polytomy. Within the M clade, two *Acremonium* isolates, strains T.U.B. 264 and UAMH 6508, were found to have identical ITS region sequences. These isolates were also morphologically identical and were typical of strains repeatedly isolated from species of *Fucus.* One of them, *Acremonium* sp. T.U.B. 264, was typical of *Acremonium* isolates frequently obtained from *F. serratus* in Heligoland, Germany, while the other, *Acremonium* sp. UAMH 6508, was representative of a number of identical strains from *F. distichus* in Canada. Both isolates differed from the majority of other *Acremonium* species by having relatively large, ovate conidia that possessed a hilum and often had a slightly truncate apex. In addition, the colonies, when in pristine condition, had a characteristic low growth with zonate production (in light) of orange coloured mucoid masses of conidia on conidiogenous cells more or less divided into shorter, undulate lateral phialides and longer terminal phialides. These isolates were considered new to science and are described here below as *Acremonium fuci* sp. nov.

Acremonium fuci Summerbell, Zuccaro & W. Gams, **sp. nov.** MycoBank MB500075.

Coloniae 10 diebus 23–30 mm diam, copiose sporulantes, madidae, pallide roseae vel aurantiae, interdum zonatae, reverso concolori, sporulatio phalacrogena–nematogena. Mycelium tenerum. *Phialides* simplices, raro unum vel paucos ramos formantes, saepe flexuosae, basi non chromophila, plerumque 7.5–23.5 × 1.0–2.2 µm. Conidia capitulis mucidis connexa, obovoidea vel late ellipsoidea, nonnumquam late clavata, ad basim truncata, plerumque 5–8 × 3.2–5 µm. Chlamydosporae absentes, cellulae inflatae saepe formatae.

Typus: Colonia exsiccata ex CBS 112868 (Zuccaro T.U.B. 264), isolatus e thallo *Fuci serrate*, Helgolandiae in Germania, iin herb. CBS.

Colonies after 10 d on malt extract agar at 20 °C 23–30 mm diam, flat or, at colony centre, folded into low wrinkles, overall pasty in texture due to heavy conidial production; in cultures grown in daylight, conidial masses forming conspicuous concentric zones. Surface colour pale pinkish orange [ranging from colours 5A3 to 6A3 in Kornerup & Wanscher (1978)], reverse concolorous. Degenerate sectors developing readily, paler in colour, faster growing, poorly conidiating, and covered with a moist, velvety to funiculose nap. Colonies on oatmeal agar similar to those on MEA but with a mucoid surface bearing no perceptible diurnal zonation. Sporulation mostly phalacrogenous (minimal aerial mycelium; only conidiophores extending above agar surface) varying to nematogenous [conidiophores arising from agar surface level and from short, recumbent aerial hyphae; terminology according to Gams (1971)]. *Hyphae* 1.5–2.5 µm wide, uncommonly with swellings to 4.8 µm. *Phialides* (Figs 2, 3) attached singly to subtending hyphae or less commonly borne in small numbers (usually two or three) basitonously or acrotonously on otherwise undifferentiated conidiophores; when lateral, frequently borne in closely spaced series along subsurface hyphae, 7.5–23.5 × 1–2.2 µm, robust in appearance, often somewhat swollen near the base and sometimes constricted at the point of attachment, tapering towards the apex, frequently undulate, curving, or slightly irregular in outline, with a small, distinct, cylindrical or slightly flared collarette. Terminal phialides relatively long (–42 µm) and in combination with 2–4 contiguous basal cells infrequently forming erect structures to 57 µm. Adelophialides infrequent, 5–12 × 1–1.5 µm. *Conidia* (Figs 2, 3) 5–8(–15) × 3.2–5(–6.0) µm obovoid, broadly ellipsoidal, with a short, truncate basal hilum and often a slightly flattened or bilaterally expanded apex, i.e., with overall shapes suggestive of pumpkin seeds in face view; exceptionally cylindrical to obclavate with base abruptly tapering towards the hilum, 10–15 × 3–6 µm. Differentiated *chlamydospores* absent, but small numbers of rounded hyphal swellings present.

Holotype: dried culture of isolate from blade of *F. serratus*, Heligoland, **Germany** in herb. CBS, culture ex-type CBS 112868 (= Zuccaro T.U.B. 264)

Additional specimens and cultures examined: **Canada**, British Columbia, Vancouver, English Bay, *Fucus distichus,* littoral, date not recorded, R.C. Summerbell: UAMH 5950, UAMH 6506 (= CBS 113887), UAMH 6507 (= CBS 113888), UAMH 6508 (= CBS 113889), UAMH 6509, and CBS 550.86. **Germany**, Heligoland, *Fucus serratus*, date not recorded, A. Zuccaro: T.U.B. 238, T.U.B. 239, T.U.B. 243, T.U.B. 254, T.U.B. 262, T.U.B. 263, T.U.B. 264 (= CBS 112868), T.U.B. 265, T.U.B. 272, T.U.B. 274, T.U.B. 275, T.U.B. 281, T.U.B. 284, T.U.B. 285, T.U.B. 286, T.U.B. 308, T.U.B. 314, T.U.B. 371, T.U.B. 375 and T.U.B. 379.

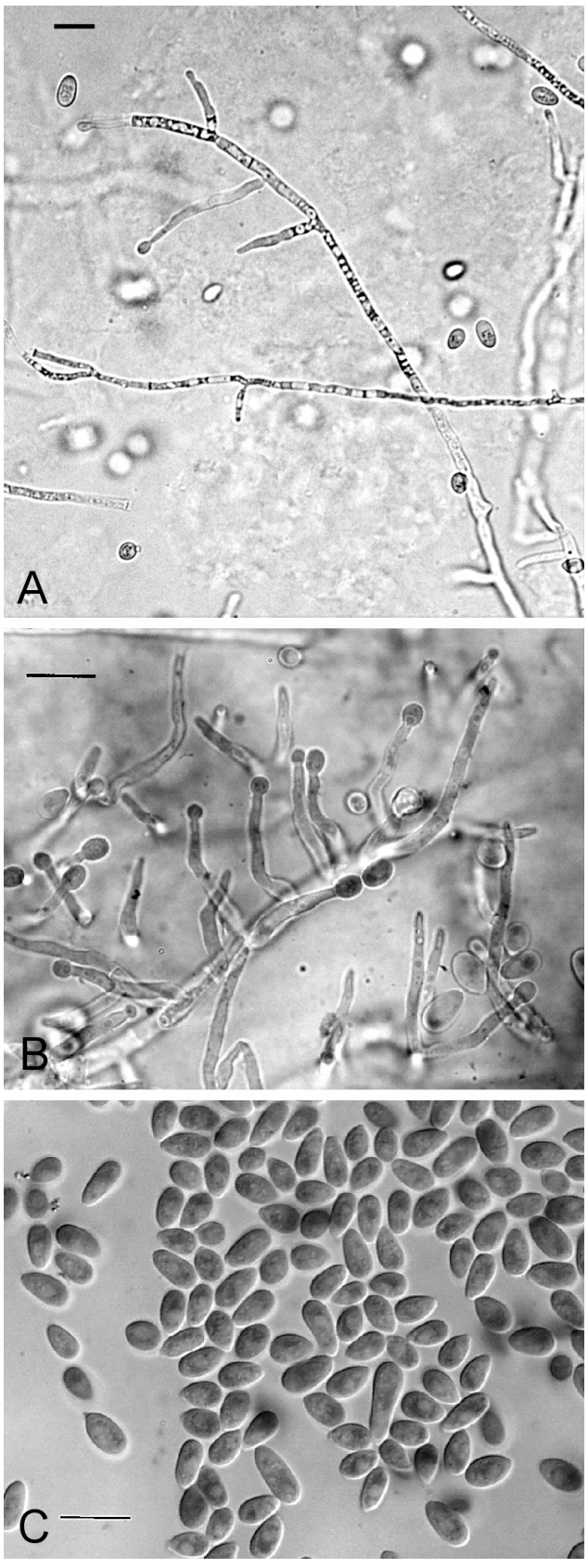

Fig. 2 A–C. *Acremonium fuci*. A, B. Conidiophores and conidia. C. Conidia. Scale bars = 10 µm.

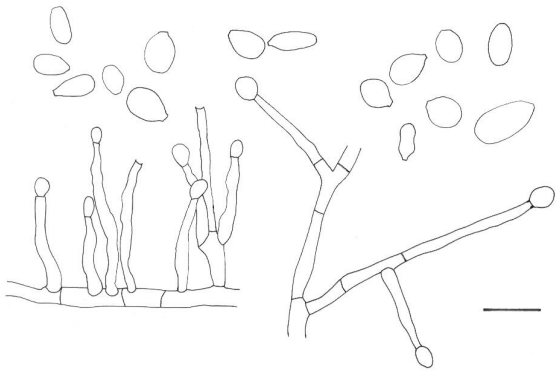

Fig 3. Various conidiophores and conidia. Scale bar = 10 µm.

Notes: The optimal growth temperature for T.U.B. 264 is 24 °C, where it attains colony diameters of 20, 30 and 40 mm at 7, 10 and 14 d, respectively on MEA. Maximum temperature for growth is 33 °C and the minimum below 6 °C. Growth at 6 and 9 °C is slow, with diameters of 5 and 6 mm, respectively, after 14 d on MEA. Growth was different on biomalt media with or without salt. Colonies on salt media grew 26 to 30 mm diam in 10 d at 20 °C in the dark, and were compact with little aerial mycelium and abundant conidia. On media without salt, colonies attained diameters of only 14 to 20 mm at 10 d and had reduced conidial production.

Conidia of *A. fuci* T.U.B. 264 germinated promptly in the presence of *F. serratus*, or of aqueous homogenates of the alga, but not in the absence of these materials (Fig. 4; Table 4). Conidia from *E. terricola* CBS 229.59, in contrast, germinated in seawater alone, but seemed to be inhibited by the presence of *Fucus* thalli or aqueous homogenates of thalli, in that germinating hyphae exposed to thalli or homogenates were shorter than those observed in the absence of the brown alga (Fig. 4).

Phylogenetic analysis based on combined ITS and β-tubulin sequences

In order to resolve the relationships among *A. fuci, Emericellopsis* spp., and related species, sequences of the ITS region and the β-tubulin region were combined after partition homogeneity tests had been performed. The two main character partitions (ITS and β-tubulin region sequences) were compared using a heuristic search with random sequence addition (50 replicates) and 1000 bootstrap replications. Differences in tree length distributions between the two partitions were judged insignificant (p = 0.65), and the data sets were combined for further analysis.

Maximum parsimony analysis of 22 taxa, including *Bionectria samuelsii* as the outgroup, generated 19 trees (tree length = 408; CI = 0.767; RI = 0.854; RC = 0.655; HI = 0.233; PIC = 188) from a heuristic search with random sequence addition. A strict consensus of these trees is shown in Fig. 5 revealing the presence of four strongly supported clades (A to D).

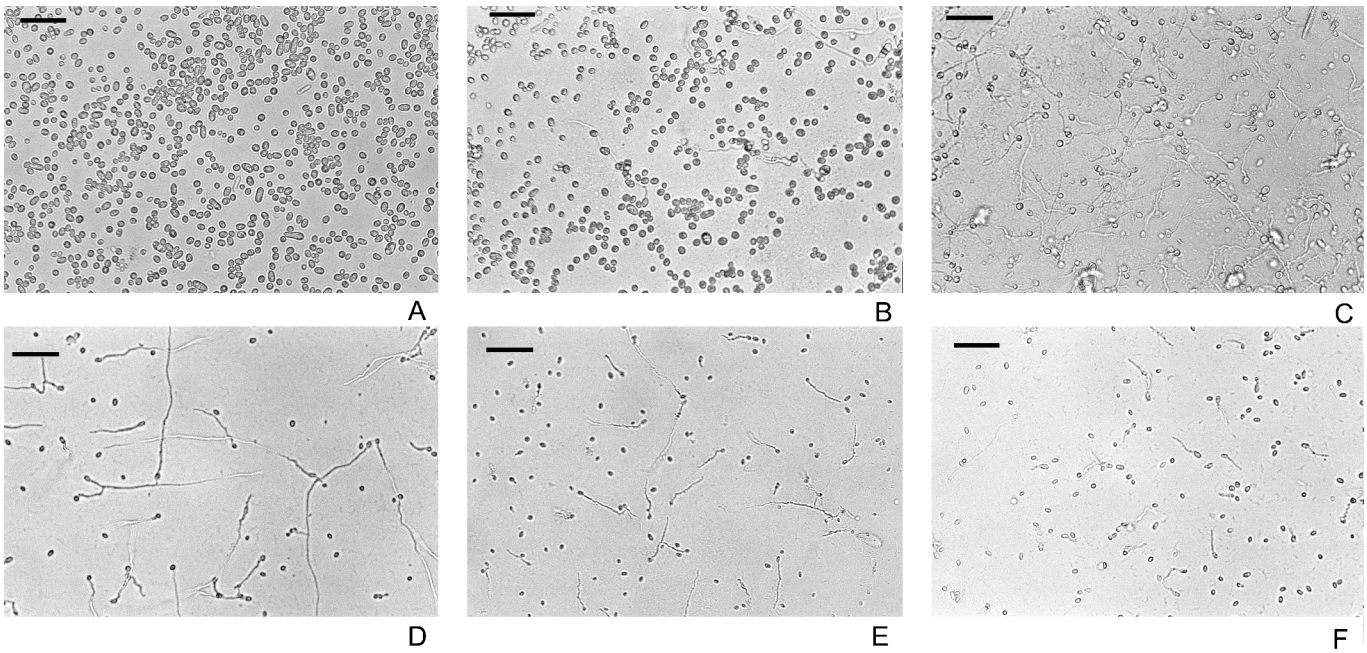

Fig. 4. Germination test of conidia from *Acremonium fuci* isolate T.U.B. 264 (top pictures), originally isolated from a *Fucus serratus* thallus, and the soil isolate *Emericellopsis terricola* CBS 229.59 (bottom pictures) in sea water (A, D), in sea water with an *F. serratus* thallus at a distance of 2 cm (B, E) and in sea water 0.1 cm from *F. serratus* (C, F). Photos taken 24 h after inoculation. Scale bars = 24 µm.

To account for any distortion in this phylogeny resulting from a disparity in rate heterogeneity between the partitions, maximum likelihood analysis using quartet puzzling was performed where the relative rates of the two partitions (ITS and β-tubulin) were estimated from the data. Likelihood distances were calculated by the least squares method using estimated base frequencies of A = 0.2, C = 0.28, G = 0.28 and T = 0.24 and an estimated rate matrix. The relative rates were 0.75 for the ITS partition and 1.34 for β-tubulin. Quartet puzzling of 10 000 steps, examining 7315 quartets and 284 distinct data patterns, generated a tree of similar topology to that shown in Fig. 5 with four supported clades (A–D). Clade A included, predominantly but not exclusively, species from terrestrial environments, whereas the members of clade B were principally marine or, in one case, derived from a saline lake. These clades are hereunder referred to as the "terrestrial" and "marine" clades, respectively. Within clade A, three supported groups were observed, one comprising isolates identified as *E. minima* (CBS 871.68 and T.U.B. Em), another for *E. microspora* and *E. robusta* Emden & W. Gams, and the third for *E. donezkii* Belyakova and *E. humicola*.

Clade B1 consisted of species that were exclusively derived from marine or other saline sources but that were isolated from a variety of substrates (Table 1). The well-supported clade B also contained an *E. pallida* isolate that was of terrestrial origin. Of the remaining clades, clade C comprised the species of *Stanjemonium,* while clade D contained the ex-type isolate of *E. synnematicola* and an isolate identified as *E. salmosynnemata*.

Two clearly discernible subclades were present within the mostly terrestrial clade A (Fig. 5). Subclade A1 included: a) two isolates identified as *E. minima* (not conspecific with the ex-type isolate) making up one closely related group; b) *E. microspora* and *E. robusta*, two species morphologically very different from one another, forming a second well-supported group; and c) *E. terricola* and *Acremonium tubakii*, both positioned near the base of the clade. Subclade A2 consisted of *E. donezkii*, *E. humicola* and the very distinct *E. glabra* (J.F.H. Beyma) Backus & Orpurt.

The marine clade contained the isotype strain of *E. stolkiae*, originally from a saline lake, as a relatively close relative of *A. fuci*, as well as the ex-type isolate of *E. minima* from Mozambican mangrove mud and the closely related, morphologically similar *E. maritima* Belyakova. An isolate identified as *Acremonium potronii* Vuillemin from a dolphin skin wound, CBS 379.70F, not known to have caused infection in the wound, was also associated with this clade. Thus all members found to belong to the clade are known to have originated from a saline habitat except the most basal member, CBS 624.73, identified as *E. pallida*, which is listed in the CBS database only as being from "soil". The ex-isotype isolate of *E. pallida*, CBS 490.71 (not sequenced here), is indeed from seawater.

Members of the genus *Stanjemonium*, an anamorph genus quite different from *Acremonium* and not known to be associated with a teleomorph, were positioned still more distally basal than *E. pallida* to the marine clade. *Stanjemonium* spp. are typically associated with semidesert and desert soils; they have no marine association (Gams *et al.* 1998b). The most basal clade of the entire group of fungi analysed

contained the two *Emericellopsis* species potentially producing synnematous anamorphs, *E. salmosynnemata* and *E. synnematicola*, both known only from soil or as airborne contaminants. Though *E. salmosynnemata* has not been mentioned as producing synnemata in recent literature and has been described as having an *Acremonium*-like anamorph, its ex-type culture, when freshly isolated, produced indeterminate synnemata to 900 µm tall (Roberts 1952).

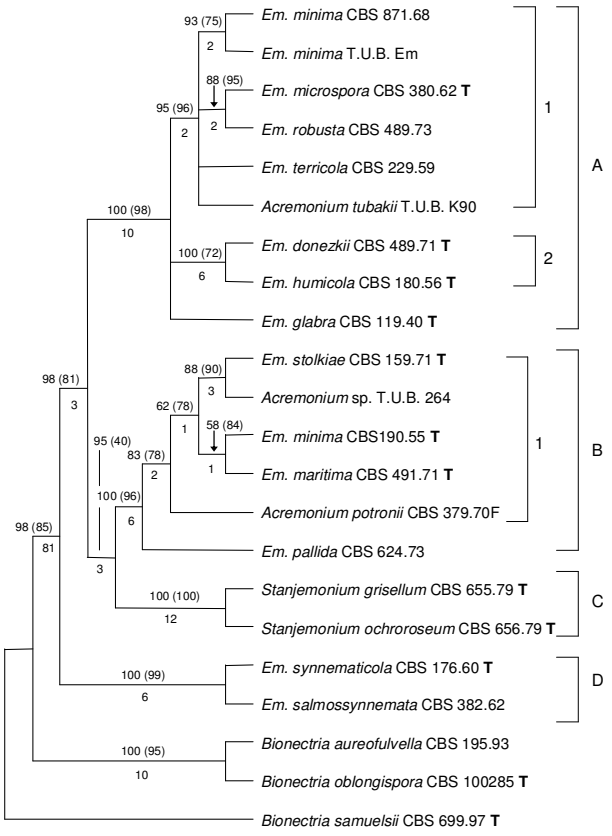

Fig. 5. Strict consensus cladogram of 19 most-parsimonious trees of length 408 (for statistics see text) showing the relationships between species of *Emericellopsis*, *Acremonium* and *Stanjemonium* based upon ITS region and β-tubulin sequences. Bootstrap values are shown above the branches and quartet puzzling supports based on maximum likelihood estimates are shown in brackets. Decay indices are shown below the branches (T = ex-type isolate).

With regard to morphological features, it was noted that all *Emericellopsis* species known to have distinctly brown ascospore wings sorted either into the terrestrial clade [*E. robusta, E. mirabilis* (Malan) Stolk*, E. glabra*] or the terrestrially-associated synnematous clade (*E. synnematicola*). *E. robusta, E. mirabilis* and *E. glabra* are also the only *Emericellopsis* species with all or most ascospores regularly exceeding 9 µm in length, associating this character with the terrestrial habitat. It should be noted that CBS 489.73, the *E. robusta* isolate studied by us, was not the ex-type isolate, though it was isolated from a comparable habitat and was identified by an author of the species description (W. Gams). *Emericellopsis stolkiae* and *E. maritima*, the two *Emericellopsis* species with olivaceous ascospores bearing very long (to 10 µm or more), pointed extensions on their wings, both fell into the marine clade and derived from saline habitats. Besides differing by approximately 2 % in β-tubulin sequence, they also differed in conidial size [3.5–8.4 × 2.2–5 µm but mostly 4–6 × 3–4 µm in the former, 6.5–8(–9) × 2.5–3.3(–4) µm in the latter] and are here confirmed as specifically distinct.

The similar *E. minima ss. str*., which in its original description was also noted as having olive-brown ascospores with wings extending as far as 6 µm from the main spore wall, differed from the most closely related species, *E. maritima,* by 4 % in ITS sequence and 1 % in tubulin. The only isolates among those studied that could be conclusively identified as conspecific were the representative European and Canadian *A. fuci* isolates and the incorrectly identified *E. minima* isolates CBS 871.78 and Zuccaro T.U.B. Em (which were morphologically similar and also 99 % similar in both ITS and tubulin sequences).

Enzyme profiles, carbohydrate utilisation and salt tolerance

The enzyme profiles and carbohydrate utilisation of 17 species of *Emericellopsis* were compared to identify patterns supportive of life in marine environments for the taxa belonging to the marine clade (Table 3). In general, all of the *Emericellopsis* species tested proved to have strong cellulase, protease and laminarinase activities. Differences in profiles for the remaining enzymes agreed mostly with the distribution of species into clades determined phylogenetically. All of the isolates forming marine clade B and sub-clade B1 (Fig. 5) exhibited greater growth on media containing salt than was seen with isolates from soil; in addition, no marine clade isolate showed polyphenol oxidase activity. The salt-adapted isolates exhibited an ability to metabolise fucose and occasionally also fucoidan, carbohydrates that are normally present in brown algae; members of the terrestrial clade did not detectably utilize these compounds.

Table 3. Enzyme profiles and carbohydrate utilisation of selected fungi. Details of test methods are given in the main text. Unless otherwise stated in a footnote, + indicates a positive reaction and – indicates a negative one.

Strain	ID number	Growth with salt[a]	Polyphenol-oxidase	Fucoidanase[b]	Growth with fucose[b]	ß-glucosidase	Amylase	Lipase	Protease	Cellulase	Laminarinase[b]	Growth with glucose[b]
Emericellopsis minima	CBS 190.55	++	–	+/–	++	–	+	+/–	++	nt	+	++
E. stolkiae	CBS 159.71	++	–	+/–	+	+	–	+	+/–	nt	+	++
Acremonium fuci	T.U.B. 264	++	–	–	++	–	+	+	+/–	++	++	++
Emericellopsis maritima	CBS 491.71	++	–	–	+	+	+	+	++	nt	+	++
E. pallida	CBS 624.73	++	–	+/–	+	+	+	+	+	nt	++	++
E. synnematicola	CBS 176.60	+	–	+/–	+	–	++	+	+	++	++	++
E. salmosynnemata	CBS 382.62	+	–	+/–	+/–	–	++	+	+	++	++	++
E. donezkii	CBS 489.71	+	+	+/–	+/–	++	+	+	+	+	++	++
E. humicola	CBS 180.56	+	+	–	–	++	++	+	+	++	++	++
E. terricola	CBS 229.59	+	+	–	–	++	–	–	++	+/–	++	++
E. minima	T.U.B. Em	+	+	–	–	+	–	–	++	+	++	++
Acremonium tubakii	T.U.B. K90	+	+	–	–	–	+	–	++	++	++	++
Emericellopsis minima	CBS 871.68	+	+	–	–	++	–	+/–	+/–	+	++	++
E. mirabilis	CBS 177.53	+	+	–	–	+/–	+	–	++	+	++	++
E. glabra	CBS 119.40	+	+	–	–	–	–	–	+	+	+	++
E. microspora	CBS 380.62	+	+	–	–	–	–	–	++	+	++	++
E. robusta	CBS 489.73	+	+	–	–	–	+	+/–	++	++	++	++

[a]Colony diameters after 12 d at 20 °C on biomalt medium with 33 g/L marine salt: '+' = 2–3.5 cm; '++' = 5–7 cm.[b]Symbols for uptake of C-source/d: '+/–' = < 0.08 g; '+' = 0.08–0.15 g ; '++' = > 0.16 g.

Table 4. Percentage *Acremonium fuci* conidia germinated in variously amended sterile artificial seawater (containing 33 g/L marine salt) and in a sterile double-distilled water control.

	Conidia germinated (mean % ± standard deviation)		
Medium	9h	12h	24h
Fucus serratus intact thallus	6±2	75±10	80±10
F. serratus aqueous tissue homogenate	6±2	60±2	80±10
Mannitol 1 g/L + yeast extract 0.1 g/L	2±1	26±5	80±10
Glucose 1 g/L + yeast extract 0.1 g/L	2±1	32±5	80±10
Fucose 1 g/L + yeast extract 0.1 g/L	1±1	27±5	70±10
Artificial seawater	0–1*	0–1	0–1
Double-distilled water	0–1	0–1	0–1

*0–1 = no conidial germination or few germinating conidia, but not more than 1% of conidia germinating.

DISCUSSION

The genus *Emericellopsis* was originally placed within the *Eurotiales* because of the formation of cleistothecia, globose asci and winged ascospores (Domsch *et al.* 1980). Malloch (1981) moved it within the *Diaporthales* on the basis of centrum development; however the phialidic, *Acremonium*-like anamorph of these organisms was inconsistent with either of these classifications (Hawksworth 1994). Glenn *et al.* (1996) in a study of nuclear ribosomal small subunit (nrSSU) DNA, found that *E. terricola* and an isolate identified as *E. minima* [American Type Culture Collection (ATCC) 16216, seen here in Fig. 1 not to be a true *E. minima* isolate] clustered with *Nectria* (now *Cosmospora*) *vilior* Starbäck in the Hypocreales. Suh & Blackwell (1999), also studying nuSSU sequences, also found that *E. terricola* formed a strongly supported clade with *Leucosphaerina indica* (Arx, Mukerji & N. Singh) Arx within the *Hypocreales*.

Finally, Gams *et al.* (1998b) and Rossman *et al.* (2001) placed the genus within the *Bionectriaceae* based on nrLSU rDNA and β-tubulin sequence analysis, where it formed a strongly supported clade with species of *Stanjemonium*.

The phylogenetic analysis presented in this paper extends the molecular information available on *Emericellopsis* and supports its monophyly while providing evidence for three strongly supported clades containing named *Emericellopsis* species as well as the phylogenetic inclusion of the anamorph genus *Stanjemonium* within the group. Of the two largest *Emericellopsis* clades, A and B, one was composed predominantly of terrestrial isolates and the other of marine-derived isolates, a division that corresponded well with strain profiles for growth in the presence of salt, as well as enzyme production (presence or absence of polyphenol oxidase for tannic acid) and carbohydrate utilisation (ability to utilise fucose).

The present molecular study was intended to determine the relationships of the isolates we obtained in studies of *Fucus* spp., rather than to serve as a monographic study of *Emericellopsis*. Nonetheless, some conclusions about *Emericellopsis* taxonomy can be drawn from the limited range of ex-type and additional isolates studied. In particular, *E. minima ss. str.,* represented by its ex-type isolate CBS 190.55, can be identified as a marine *Emericellopsis* unrelated to the other isolates we studied that had been identified under this species name. It is also unrelated to *E. humicola, E. salmosynnemata* and *E. microspora*, three of four species tentatively synonymized with *E. minima* by Gams (1971) (the fourth synonymized species, *E. pusilla* Mathur, Sukapure & Thirumalachar, was not studied by us). Additional studies will need to be done to determine how this species can be morphologically distinguished. *Emericellopsis humicola* and *E. microspora,* for which ex-type isolates were sequenced, can be affirmed as distinct species. In the context of the phylogenetic association linking *E. minima ss. str.* with *E. stolkiae* and *E. maritima,* two species with long ascospore wing extensions, the relatively long wing extensions noted in the original description of *E. minima* by Stolk (1955), which were no longer conspicuous when the isolate was re-examined by Gams (1971), appear to be significant. The sequences available for three *E. minima* isolates other than the type showed that two isolates, CBS 871.68 and Zuccaro T.U.B. Em (= CBS 111361) were conspecific with one another, while another, ATCC 16216, was a separate member of the terrestrial clade. None of these *E. minima* isolates could be identified under an existing species name. Study of additional *E. terricola, E. robusta* and *E. pusilla* isolates, including the ex-type isolates of the last two species, is needed before any conclusions can be drawn about most members of the terrestrial clade. The ex-type isolate of *E. terricola*, CBS 120.40, is represented in Fig. 1, but no tubulin sequencing was done to place it in Fig. 5; the very similar CBS 229.59, deviating from CBS 120.40 in the ITS region at only two positions, represents *E. terricola* in the combined ITS-tubulin cladogram seen in Fig. 4.

The naming of anamorphs in the genus *Emericellopsis* is called into question by the presence of the recently described anamorph genus *Stanjemonium* as a subclade occurring within the broader *Emericellopsis* clade. *Stanjemonium* was erected to house isolates that, like some fungi in the genera *Aphanocladium* W. Gams, *Lecanicillium* W. Gams & Zare, *Verticimonosporium* Matsushima and *Sibirina* G.R.W. Arnold, produce phialide-like conidiogenous cells giving rise to only a single conidium (Gams *et al.* 1998b). As originally defined, *Stanjemonium* would thus seem inappropriate to contain other *Emericellopsis* anamorphs; however, as Zare & Gams (2001) have shown, such uniconidial blastic cells or aphanophialides are sometimes found along with normal phialides even within individual species such as *Lecanicillium dimorphum* (J.D. Chen) Zare & W. Gams, and may thus be conceived of as not especially distinctive at the generic level. Regardless of the degree of morphogenetic distinctiveness of such features, if Hennigian principles basing taxonomy purely on lineage histories were rigorously followed, *Stanjemonium* would need either to become the anamorph name for all members of the *Emericellopsis* clade, or to be synonymized with *Acremonium*, or to become one of four anamorph generic names, each corresponding to one of the four clades within *Emericellopsis.* The problem of what to name the anamorphs of clades A, B and D is exacerbated by the fact that *Acremonium* as a whole, as well as its type species *A. alternatum* Link, are as yet undefined in molecular phylogenetic terms. *Acremonium alternatum* has no living ex-type material (Gams 1971) and will need to be neotypified, before such analysis, leading to an anchoring of *Acremonium* in phylogenies, can proceed. Any consideration of placing *Emericellopsis* anamorphs into *Stanjemonium*, however, is precluded by the knowledge that a well-established older, valid anamorph name, not disclosed here, also falls within *Emericellopsis* (Seifert, pers. comm.). Like *Stanjemonium*, however, the name in question archetypically indicates a strongly apomorphic morphology not applicable to the *Acremonium*-like anamorphs seen in most *Emericellopsis* species. Cladistically heterodox discussion of whether to segregate such ecologically and morphologically distinctive apomorphs as saltatory genera arising from symplesiomorphic mother genera (Zare & Gams 2001, Bills *et al.* 2004), can be deferred in the present context until the missing data are made public. In the meantime, *Acremonium* suffices as a currently valid default placement for *A. fuci* with a reasonable probability of long-term endurance.

The remaining *Emericellopsis* clade comprised *E. synnematicola* and *E. salmosynnemata* (the latter represented only by a secondary collection), two species that may be related morphologically by the presence of synnematal conidiogenous structures at least in freshly collected isolates. In *E. salmosynnemata* this character has only been recorded for the ex-type isolate (Roberts 1952) and appears to have been lost on subculture (Gams 1971). *Emericellopsis salmosynnemata* has been reported to have been isolated from stagnant fresh water environments as an agent capable of lysing blue-green algae (Redhead & Wright 1978), though the relationship of the authors' voucher isolate, ATCC 36893, to *E. salmosynnemata* remains to be verified. In general, considerable sequence divergence was observed in our study between the synnematal clade and other members of the genus, but the cohesion of these isolates with *Emericellopsis* obtained strong bootstrap support in combined ITS region and tubulin analysis. Also, this group fits closely within *Emericellopsis* when the relevant ITS sequences were placed by one of us (R.C.S.) into an alignment with over 200 unpublished *Acremonium* sequences and over 100 additional Hypocrealean sequences.

Acremonium fuci appears to be the second *Acremonium* species with distinctively large conidia to have been described from marine environments. *Acremonium neocaledoniae* Roquebert & Dupont (Dupont *et al.* 2000) was not studied here in that no culture of this species, an antibiotic-producer, is known to be publicly available. Its partial 18S sequence, deposited in GenBank as AF217194, indicates that it is not a member of the *Emericellopsis* clade (data not shown). It can be distinguished from *A. fuci* by its brownish (described as "light beige to nutmeg") colonies, its 50–75 µm long phialides, all longer than the longest phialides seen in *A. fuci*, and its conidia measuring 7.5–11.5 × 5.6–7.5 µm, generally larger than those of *A. fuci*. Another large-conidial *Acremonium*, *A. hypholomatis* (Boedijn) D. Hawksworth (Hawksworth 1972) was neotypified based on an isolate from very damp decaying leaves; the ex-type isolate has died out at CBS and any inoculum remaining viable elsewhere has not yet been traced for further study. This species has conidia 12.5–18 × 4–7 µm, much bigger than those of *A. fuci* and approximated in the *Emericellopsis* clade only by the 13–17.5 × 4–4.5 µm conidia of *E. robusta.*

Data collected in the present study shed an interesting light on the differing ecologies of *Emericellopsis* species. Tubaki (1973) noted that the frequent recovery of *Emericellopsis* species from marine, brackish, lake and reservoir mud sediments indicated that aquatic sediments should be considered to be characteristic environments for some members of this genus. He also observed that all of the species isolated from aquatic systems were small-spored members of the genus, as had earlier been noted by Backus & Orput (1961). The species of clade B produced small ascospores (< 7.5 µm long); large-spored species (ascospores > 9 µm long) appeared to be excluded from marine and saline habitats. There was no converse exclusion of small-spored species from terrestrial habitats: some small-spored species, such as *E. microspora* and *E. humicola*, were found to belong to clade A. The small ascospore sizes found in members of the marine clade, combined with the presence of greatly extended ascospore wings in three of four species (*E. stolkiae, E. maritima, E. minima s. str.*) suggests adaptation for flotation and substrate surface attachment, as would be appropriate for fungal species competent to grow in aqueous habitats and in particular in habitats characterized by the presence of moving water. The relatively large conidia of *E. stolkiae* (3.5–8.4 × 2.2–5 µm), *E. maritima* [6.5–8 (–9) × 2.5–3.3 (–4) µm], and *E. minima s. str.* [4–10 × 2–3.5 µm *fide* Stolk (1955)], as well as the closely related *A. fuci* [recapitulated for comparison: 5–8 (–15) × 3–5 (–6) µm], may also confer an advantage in sedimentation onto substrates in moving waters of the littoral zone, particularly substrates such as *Fucus* thalli covered with a film of slime (which would tend to imbibe sedimented conidia and thus, in turbulent water, minimize extra shearing stress that might otherwise tear away such relatively large structures). It should be noted, however, that some *Emericellopsis* species typical of terrestrial habitats, such as *E. robusta*, also have large conidia, while *E. pallida*, a marine clade member originally described from Black Sea water, has conidia somewhat smaller than those of the other marine clade members [(3.2–)4–5.2(–7.8) × 1.5–2.5 (–3.3) µm].

The genetic and physiological relatedness of *Emericellopsis* species from marine and salt lake habitats supports the thesis of Anastasiou (1961, 1963) and Davidson (1974) that the mycota of inland salt lakes is indistinguishable from that of the oceans.

Physiological studies showed that species belonging to the marine clade B1 shared several unique characters. The finding that all members of clade B1 grew better than other *Emericellopsis* species on media containing marine salt supports the idea that members of this clade are products of an evolutionary radiation into saline environments. Members of clade B1 were also among the *Emericellopsis* species lacking polyphenol oxidase activity in tests done with tannic acid as a substrate. Enzymes within the broad group referred to as polyphenol oxidases are generally ecologically important as peroxidases, laccases, tyrosinases and catechol mono- and dioxygenases. Many variants of each of these enzyme types exists, each with its own substrate specificities for various aromatic and heterocyclic compounds. Tannins are a heterogeneous group of polyphenolic compounds that differ in their structural linkages (Khanbabee & van

Rees 2001). Commercial tannic acid, used as the substrate in the enzyme tests, consists of plant gallotannins (Salminen *et al*. 2002). The utilisation of this mixture of simple tannic acids is common among fungi, particularly those involved in wood decay. It is initially surprising that a tanninolytic ability could not be confirmed in the marine clade members, especially *A. fuci*, since phaeophyte algae including *Fucus* abundantly produce phlorotannins (Haug & Larsen 1958, Van Alstyne 1988, Schoenwaelder & Clayton 1998, Jormalainen *et al.* 2003). Our tests with commercial tannic acid, however, may not have been adequate to determine if *Emericellopsis* species can utilise this structurally different (Arnold & Targett 2002) group of tannins.

In phaeophytes, phlorotannins and other polyphenols are housed within specialised organelles called physodes in the algal thallus and are excreted mainly after tissue damage (Van Alstyne 1988, Schoenwaelder & Clayton 1998, Jormalainen *et al.* 2003) or, in small amounts, during adhesion (Vreeland *et al*. 1998). This compartmentalisation may restrict the access of algicolous fungi to these compounds and thus lessen their importance as a nutrient source. Since *Emericellopsis* marine clade members appear to have evolved from terrestrial ancestors capable of tannic acid utilisation, it appears that the transition to marine environments was accompanied by a change in the types of nutrients and substrates exploited by these fungi.

Clade B members all exhibited an ability to utilise fucose, a sugar that, though made in small quantities by many animals, plants and bacteria (Staudacher *et al.* 1999), is found in particularly high concentrations in brown algae. Clade A members showed little or no growth with fucose as sole C source. Interestingly the basal clade D cannot utilise tannic acid but can metabolise fucose, though it has no known marine connection.

Traditionally, marine fungi are defined ecologically, with *native* (obligate) species capable of completing their life cycle within the marine environment (Kohlmeyer & Kohlmeyer 1979, Hyde *et al.* 2000). Another criterion used, although less definitively, to distinguish between obligate and fortuitous marine-occurring fungi is the germination of conidia in the presence of seawater (Jones & Jennings 1964). *Emericellopsis terricola* CBS 229.59, a member of the terrestrial clade A and representative of a commonly collected species with no known marine habitat associations, could undergo conidial germination in seawater, seemingly confounding this test. This isolate proved, however, to be inhibited in conidial germination by intact *F. serratus* thalli or aqueous homogenates: germination tubes produced in the presence of these materials were shorter than those produced in their absence. *Acremonium fuci,* by contrast, germinated after 9 h in the presence of seawater + nutrients (glucose, mannitol or fucose) or seawater homogenates of *F. serratus*, and, via microcyclic conidiation, was able to produce abundant new conidia within 24 h. It was thus demonstrated to be able to complete at least the shortest version of its life cycle in marine substrata. Inhibition of fungal conidial germination by algae has been observed previously (Hellio *et al.* 2000), but a stimulation response has heretofore not been seen in this type of experiment. *Acremonium fuci* did not germinate in the presence of seawater alone. Other obligate marine fungi are also unable to undergo spore germination in seawater alone (Kirk 1980, Kohlmeyer 1981, Dix & Webster 1995). Evidence exists for a mycostatic effect of fresh seawater, but the addition of nutrients such as 0.1 % glucose, 0.1 % yeast extract or 0.1 % $(NH_4)_3PO_4$ nullifies this phenomenon (Jones & Jennings 1964. Kirk 1980). Conidia of *A. fuci* also failed to germinate in double-distilled water, demonstrating that this species possesses a self-inhibition mechanism related to the absence of nutrients rather than to the presence of materials conferring a mycostatic effect on seawater.

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