

## New acremonium-like taxa in the Bionectriaceae and Plectosphaerellaceae

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**Abstract** Several molecular studies have demonstrated that species traditionally assigned to the form genus *Acremonium* are polyphyletic, while *Acremonium sensu stricto* is a central element of the Bionectriaceae (Hypocreales). Based on phenotypic characters and molecular phylogenetic analyses, two new *Acremonium* species, *A. moniliforme* and *A. dimorphosporum*, are described. The former is related to *Emericellopsis* and is characterized by cylindrical conidia, acicular phialides and abundant moniliform hyphae. *Acremonium dimorphosporum* resembles *Acremonium borodinense*. It produces cylindrical, smooth-walled and ellipsoidal, rough-walled conidia. The new genus *Brunneomyces* is proposed based on three species, including *B. brunnescens* (formerly *A. brunnescens*), *B. europaeus* and *B. hominis*. All of them are characterized by brown hyphae, sympodial conidiophores and chains of ovoidal to ellipsoidal conidia. Also, the proposed new species *Chordomyces albus* is characterized by its light-coloured colonies, simple or branched conidiophores, phialides with percurrent proliferations and cylindrical collarettes, and ellipsoidal to cylindrical conidia. The combined analysis of the LSU, ITS, *RPB2* and *TEF1- $\alpha$*  loci supports the inclusion of *B. brunnescens*, *B. europaeus*, *B. hominis* and *C. albus* in Plectosphaerellaceae.

**Key words** *Emericellopsis*, Hypocreales, Plectosphaerellaceae, Phylogeny, Taxonomy

## Introduction

*Acremonium* accommodates saprobic species that can colonize diverse substrates (Gams 1971, 1975; Domsch et al. 2007), important plant pathogens (Alfaro-García et al. 1996; Lin et al. 2004) or agents of opportunistic infections in humans (Summerbell 2003; Guarro 2012; de Hoog et al. 2015). Species-level identifications in *Acremonium* is difficult on the basis of morphological characters because their asexual structures are poorly differentiated. Molecular phylogenetic analyses have demonstrated that the genus is polyphyletic. Recent phylogenetic studies have shown that *Acremonium* species cluster in different lineages throughout the Ascomycota, mainly in the Sordariomycetes (Glenn et al. 1996; Zare et al. 2007; Schoch et al. 2009; Gräfenhan et al. 2011; Perdomo et al. 2011; Summerbell et al. 2011; Giraldo et al. 2012, 2015). Epitypification of the type species of the genus, *A. alternatum*, linked *Acremonium sensu stricto* to the Bionectriaceae (Hypocreales) (Summerbell et al. 2011), which also accommodates several other, partly teleomorphically typified genera with an *acremonium*-like anamorph, such as *Emericellopsis*, *Hapsidospora*, *Nigrosabulum*, *Bulbithecium* and *Mycoarachis* (Gams 1971; Summerbell et al. 2011). Other species of *Acremonium* are distantly related to the type species of the genus and belong to the Glomerellales or other families of the Hypocreales (Zare et al. 2007; Gräfenhan et al. 2011; Carlucci et al. 2012; Giraldo et al. 2012; Grum-Grzhimaylo 2013a; Maharachchikumbura et al. 2015, 2016; Lombard et al. 2015).

In a previous study on *Acremonium* species from clinical samples in USA (Perdomo et al. 2011), some of the isolates distributed in different groups within the Hypocreales (informally named groups J and N) and Plectosphaerellaceae (groups Q and R), could not be identified. The taxonomy of those isolates and of other *Acremonium* species in Plectosphaerellaceae was resolved in the present study by using multilocus DNA sequence analyses and phenotypic methods.

## Materials and methods

### Fungal isolates and sequences

The fungi included in this study are shown in Table 1. Six clinical isolates provided by the Fungus Testing Laboratory at the University of Texas Health Science Center (UTHSC) were tentatively identified as *A. hyalinulum* and previously linked to Hypocreales or Plectosphaerellaceae (Perdomo et al. 2011). In addition, one *Acremonium* isolate (FMR 11785) obtained from soil with the procedure described in Giraldo et al. (2012), and five ex-type or reference strains provided by the CBS-KNAW Fungal Biodiversity Centre (CBS) were also included in our study. Numerous DNA sequences of *Acremonium* species and related genera reported in different studies (Sigler et al. 2004; Zuccaro et al. 2004; Zare et al. 2007; Summerbell et al. 2011; Carlucci et al. 2012; Grum-Grzhimaylo et al. 2013a,b, 2016; Giraldo et al. 2014) were retrieved from public databases (Table 2) and included in the phylogenetic analyses.

### Phenotypic studies

Morphological features were examined on potato dextrose agar (PDA; Pronadisa, Madrid, Spain) and oatmeal agar (OA; filtered oat flakes after 1 h of simmering, 30 g; agar, 20 g; distilled water to final volume of 1 000 mL). Cultures were incubated at 25 °C in the dark for 4 wk. Colony diameters were measured after 14 days of incubation and the colony colour rated after Kornerup and Wanscher (1978). Microscopic features were examined and measured from cultures grown on OA under an Olympus CH-2 light microscope (Olympus Corporation, Tokyo, Japan) from direct wet mounts with either 85 % lactic acid or Shear's solution, or from slide cultures. At least 30 randomly selected elements were measured for each structure using an ocular micrometer. Photomicrographs were obtained with a Zeiss Axio-Imager M1 light microscope (Zeiss, Oberkochen, Germany), using phase contrast and Nomarski differential interference. The ability of the fungi to grow at 4, 12, 15, 20, 25, 30, 32, 35, 37 and 40 °C was determined on PDA in duplicate.

**Table 1** Isolates included in this study

Species	Strain <sup>a</sup>	Origin <sup>b</sup>	Previous identification <sup>c</sup>	GenBank accession number <sup>d</sup>					Reference
				LSU	ITS	BT2	TEF1- $\alpha$	RPB2	
<i>Acremonium dimorphosporum</i> (= <i>Acremonium</i> sp. II)	UTHSC 08-3639 <sup>T</sup> (= CBS 139050, FMR 10548)	BAL, USA	<i>Acremonium</i> sp. (Group J)	<b>LN810506</b>	<b>LN810515</b>	–	–	–	This study
<i>Acremonium moniliforme</i> (= <i>Acremonium</i> sp. I)	FMR 11785 <sup>T</sup> (= CBS 139051)	Soil, Spain	<i>Acremonium</i> sp.	<b>LN810507</b>	<b>LN810516</b>	<b>LN810523</b>	<b>LN810531</b>	<b>LN810525</b>	This study
	UTHSC 08-2284 (= FMR 10363)	Toe nail, USA	<i>Acremonium</i> sp. (Group N)	<b>LN810508</b>	<b>LN810517</b>	<b>LN810524</b>	<b>LN810532</b>	<b>LN810526</b>	This study
<i>Acremonium stromaticum</i>	CBS 863.73 <sup>T</sup>	Root and rhizome of <i>Musa sapientum</i> , Honduras	<i>A. stromaticum</i>	HQ232143	DQ825969	–	<b>LN810533</b>	–	Summerbell et al. (2011), this study
<i>Brunneomyces brunnescens</i>	CBS 559.73 <sup>T</sup>	On dead stem of <i>Dendrocalamus giganteus</i> , Sri Lanka	<i>A. brunnescens</i>	HQ231966	<b>LN810520</b>	–	<b>LN810534</b>	–	Summerbell et al. (2011), this study
<i>Brunneomyces hominis</i>	UTHSC 06-415 <sup>T</sup> (= CBS 139053, FMR 10429)	Sputum, USA	<i>A. hyalinulum</i> (Group Q)	<b>LN810509</b>	KP131517	–	<b>LN810535</b>	–	Irinyi et al. (2015), this study
	UTHSC R-3853 (= CBS 139054, FMR 10437)	Sputum, USA	<i>A. hyalinulum</i> (Group Q)	<b>LN810510</b>	KP131518	–	<b>LN810536</b>	–	Irinyi et al. (2015), this study
<i>Brunneomyces europaeus</i>	CBS 560.86	Leaf of <i>Bambusa</i> sp., France	<i>A. hyalinulum</i> (Group Q)	<b>LN810511</b>	<b>LN810518</b>	–	<b>LN810537</b>	<b>LN810527</b>	This study
	CBS 652.96 <sup>T</sup>	River sediment, Spain	<i>A. hyalinulum</i>	<b>LN810512</b>	<b>LN810519</b>	–	<b>LN810538</b>	<b>LN810528</b>	This study
<i>Chordomyces albus</i>	CBS 987.87 <sup>T</sup>	On <i>Hypogymnia physodes</i> , Luxembourg	<i>A. antarcticum</i>	JX158444	DQ825970	–	JX158400	JX158466	Grum-Grzhimaylo et al. (2013a), Zare et al. (2007), this study
	UTHSC 06-874 (= FMR 10433)	Sputum, USA	<i>Acremonium</i> sp. (Group R)	<b>LN810513</b>	<b>LN810521</b>	–	<b>LN810539</b>	<b>LN810529</b>	This study
<i>Chordomyces antarcticus</i>	UTHSC 08-3693 (= CBS 139055; FMR 10549)	Nail, USA	<i>Acremonium</i> sp. (Group R)	<b>LN810514</b>	<b>LN810522</b>	–	<b>LN810540</b>	<b>LN810530</b>	This study

<sup>a</sup> CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; FMR, Faculty of Medicine Reus, Spain; UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas, USA; <sup>T</sup> Type strain. <sup>b</sup> BAL, Bronchoalveolar lavage fluid. <sup>c</sup> Identification in Perdomo et al. (2011). <sup>d</sup> Accession numbers of sequences newly generated in this study are indicated in boldface. LSU large subunit of the nrDNA; ITS internal transcribed spacer regions of the nrDNA and intervening 5.8S nrDNA; BT2 beta-tubulin gene; TEF1- $\alpha$  transcription elongation factor 1-alpha; RPB2 RNA polymerase II second largest subunit.

**Table 2** Taxa used in the phylogenetic analyses with their GenBank accession numbers

Species	Strain <sup>a</sup>	GenBank accession number <sup>b</sup>					Reference
		LSU	ITS	BT2	TEF1- $\alpha$	RPB2	
<i>Acremonium acutatum</i>	CBS 682.71 <sup>T</sup>	HQ231965	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium alcalophilum</i>	CBS 114.92	JX158443	DQ825967	–	JX158399	JX158465	Zare et al. (2007), Grum-Grzhimaylo et al. (2013a)
<i>Acremonium alternatum</i>	CBS 407.66 <sup>I</sup>	HQ231988	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium bisseptum</i>	CBS 750.69 <sup>T</sup>	HQ231998	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium borodinense</i>	CBS 101148 <sup>T</sup>	HQ232003	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium brachyphenium</i>	CBS 866.73 <sup>I</sup>	HQ232004	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium breve</i>	CBS 150.62 <sup>I</sup>	HQ232005	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium camptosporum</i>	CBS 677.74	HQ232007	–	–	–	–	Summerbell et al. (2011)
	CBS 756.69 <sup>I</sup>	HQ232008	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium cerealis</i>	CBS 207.65	HQ232013	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium charticola</i>	CBS 117.25 <sup>T</sup>	HQ232016	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium citrinum</i>	CBS 384.96 <sup>T</sup>	HF680217	HF680236	–	–	–	Giraldo et al. (2014)
	CBS 758.69	HQ232012	HF680222	–	–	–	Summerbell et al. (2011), Giraldo et al. (2014)
<i>Acremonium collariferum</i>	CBS 124585	FJ765364	–	–	–	–	Weisenborn et al. (2010)
	CBS 124586 <sup>T</sup>	FJ765366	–	–	–	–	Weisenborn et al. (2010)
<i>Acremonium chrysogenum</i>	CBS 144.62 <sup>T</sup>	HQ232017	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium curvulum</i>	CBS 430.66 <sup>T</sup>	HQ232026	–	–	–	–	Summerbell et al. (2011)
	CBS 229.75	HQ232021	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium exuviarum</i>	UAMH 9995 <sup>T</sup>	HQ232036	AY882946	AY882947	–	–	Sigler et al. (2004), Summerbell et al. (2011)
<i>Acremonium flavum</i>	CBS 596.70 <sup>T</sup>	HQ232037	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium fuci</i>	CBS 113889	HQ232038	AY632652	–	–	–	Zuccaro et al. (2004), Summerbell et al. (2011)
	CBS 112868 <sup>I</sup>	–	AY632653	AY632690	–	–	Zuccaro et al. (2004)
<i>Acremonium furcatum</i>	CBS 122.42 <sup>T</sup>	EF543831	AY378154	–	–	–	Zare et al. (2007)
<i>Acremonium fusidioides</i>	CBS 705.86	HF680218	HF680237	–	–	–	Giraldo et al. (2014)
	CBS 840.68 <sup>T</sup>	HQ232039	FN706542	–	–	–	Perdomo et al. (2011), Summerbell et al. (2011)
	UTHSC 08-1455	HF680216	HF680235	–	–	–	Giraldo et al. (2014)
<i>Acremonium gamsii</i>	CBS 726.71 <sup>I</sup>	HQ232040	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium guillematii</i>	CBS 766.69 <sup>T</sup>	HQ232042	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium hennebertii</i>	CBS 768.69 <sup>T</sup>	HQ232044	HF680238	–	–	–	Summerbell et al. (2011), Giraldo et al. (2014)
“ <i>Acremonium hyalinulum</i> ”	CBS 271.36	HQ232045	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium incrustatum</i>	CBS 159.70 <sup>T</sup>	HQ232049	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium inflatum</i>	CBS 212.69 <sup>I</sup>	HQ232050	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium minutisporum</i>	CBS 147.62 <sup>T</sup>	HQ232061	–	–	–	–	Summerbell et al. (2011)
	det267B*	HQ232062	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium nepalense</i>	CBS 971.72 <sup>I</sup>	HQ231970	DQ825971	–	–	–	Zare et al. (2007), Summerbell et al. (2011)

<i>Acremonium nigrosclerotium</i>	CBS 154.72 <sup>I</sup>	HQ232069	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium parvum</i>	CBS 381.70A <sup>T</sup>	HQ231986	HF680219	–	–	–	Summerbell et al. (2011), Giraldo et al. (2014)
	CBS 831.97	HQ231989	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium persicinum</i>	CBS 310.59 <sup>T</sup>	HQ232077	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium pilosum</i>	CBS 124.70 <sup>T</sup>	HF680209	HF680228	–	–	–	Giraldo et al. (2014)
	CBS 125.70	HF680210	HF680229	–	–	–	Giraldo et al. (2014)
	CBS 511.82	HF680207	HF680226	–	–	–	Giraldo et al. (2014)
<i>Acremonium pinkertoniae</i>	CBS 157.70 <sup>T</sup>	HQ232089	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium potronii</i>	CBS 379.70F	–	AY632655	AY632691	–	–	Zuccaro et al. (2004)
<i>Acremonium pseudozeylanicum</i>	CBS 560.73 <sup>T</sup>	HQ232101	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium pteridii</i>	CBS 782.69 <sup>T</sup>	HQ232102	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium radiatum</i>	CBS 142.62 <sup>I</sup>	HQ232104	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium recifei</i>	CBS 137.35 <sup>T</sup>	HQ232106	–	–	–	–	Summerbell et al. (2011)
	CBS 362.76	HQ232108	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium restrictum</i>	CBS 178.40 <sup>I</sup>	HQ232119	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium roseolum</i>	CBS 289.62 <sup>T</sup>	HQ232123	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium rutilum</i>	CBS 396.66 <sup>T</sup>	HQ232124	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium salmoneum</i>	CBS 721.71 <sup>I</sup>	HQ232125	–	–	–	–	Summerbell et al. (2011)
	JS-NJ01 *	–	HM747162	–	–	–	Unpublished
<i>Acremonium sclerotigenum</i>	CBS 124.42 <sup>T</sup>	HQ232126	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium sordidulum</i>	CBS 385.73 <sup>I</sup>	HQ232136	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium spinosum</i>	CBS 136.33 <sup>T</sup>	HQ232137	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium tectonae</i>	CBS 725.87 <sup>T</sup>	HQ232144	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium thermophilum</i>	CBS 734.71 <sup>I</sup>	HQ232145	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium tubakii</i>	CBS 790.69 <sup>T</sup>	HQ232148	–	–	–	–	Summerbell et al. (2011)
	CBS 111360	–	AY632654	AY632689	–	–	Zuccaro et al. (2004)
<i>Acremonium verruculosum</i>	CBS 989.69 <sup>I</sup>	HQ232150	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium vitellinum</i>	CBS 792.69 <sup>T</sup>	HQ232151	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium zonatum</i>	CBS 565.67	HQ232155	–	–	–	–	Summerbell et al. (2011)
<i>Acremonium</i> sp.	A104 *	–	KC987141	KC987103	KC998963	KC999001	Grum-Grzhimaylo et al. (2013b)
	A105 *	–	KC987142	KC987104	KC998964	KC999002	Grum-Grzhimaylo et al. (2013b)
	A106 *	–	KC987143	KC987105	KC998965	KC999003	Grum-Grzhimaylo et al. (2013b)
	A107 *	–	KC987144	KC987106	KC998966	KC999004	Grum-Grzhimaylo et al. (2013b)
	A108 *	–	KC987145	KC987107	KC998967	KC999005	Grum-Grzhimaylo et al. (2013b)
	A110 *	–	KC987147	KC987109	KC998969	KC999007	Grum-Grzhimaylo et al. (2013b)
	A111 *	–	KC987148	KC987110	KC998970	KC999008	Grum-Grzhimaylo et al. (2013b)
<i>Acremonium</i> sp.	E102*	–	KC987172	KC987134	KC998994	KC999030	Grum-Grzhimaylo et al. (2013b)
	FMR 11780	KJ807179	–	–	–	–	Crous et al. (2015)
<i>Acrostalagmus annulatus</i>	DAOM 212126	GU180646	GU180632	–	–	GU180662	Réblová et al. (2011)
<i>Acrostalagmus luteoalbus</i>	CBS 194.87	EF543826	–	–	–	–	Zare et al. (2007)
<i>Chordomyces antarcticus</i>	CBS 120042	KJ443108	KJ443240	–	KJ443196	KJ443156	Grum-Grzhimaylo et al. (2016)
	CBS 120045 <sup>I</sup>	KJ443109	KJ443241	–	KJ443197	KJ443157	Grum-Grzhimaylo et al. (2016)
	CBS 137610	KJ443106	KJ443238	–	KJ443194	KJ443154	Grum-Grzhimaylo et al. (2016)
<i>Emericellopsis alkalina</i>	A124 *	–	KC987161	KC987123	KC998983	KC999020	Grum-Grzhimaylo et al. (2013b)
	A118 *	–	KC987155	KC987117	KC998977	KC999014	Grum-Grzhimaylo et al. (2013b)

	CBS 127350 <sup>1</sup>	KC987247	KC987171	KC987133	KC998993	KC999029	Grum-Grzhimaylo et al. (2013b)
	CBS 120049	KC987246	KC987170	KC987132	KC998992	KC999028	Grum-Grzhimaylo et al. (2013b)
	CBS 120043	–	KC987168	KC987130	KC998990	KC999026	Grum-Grzhimaylo et al. (2013b)
	CBS 120044	–	KC987169	KC987131	KC998991	KC999027	Grum-Grzhimaylo et al. (2013b)
<i>Emericellopsis donezkii</i>	CBS 489.71 <sup>T</sup>	–	AY632658	AY632674	–	–	Zuccaro et al. (2004)
<i>Emericellopsis glabra</i>	CBS 119.40 <sup>T</sup>	–	AY632657	AY632673	–	–	Zuccaro et al. (2004)
<i>Emericellopsis humicola</i>	CBS 180.56 <sup>1</sup>	–	AY632659	AY632675	–	–	Zuccaro et al. (2004)
<i>Emericellopsis maritima</i>	CBS 491.71 <sup>T</sup>	–	AY632670	AY632686	FJ238393	KC999033	Zuccaro et al. (2004), Schoch et al. (2009), Grum-Grzhimaylo et al. (2013b)
<i>Emericellopsis microspora</i>	CBS 380.62 <sup>T</sup>	–	AY632663	AY632679	–	–	Zuccaro et al. (2004)
<i>Emericellopsis minima</i>	CBS 190.55 <sup>T</sup>	–	AY632669	AY632685	KC998995	KC999031	Zuccaro et al. (2004), Grum-Grzhimaylo et al. (2013b)
	CBS 871.68	–	AY632660	AY632676	KC998996	KC999032	Zuccaro et al. (2004), Grum-Grzhimaylo et al. (2013b)
	CBS 111361	–	AY632661	AY632677	–	–	Zuccaro et al. (2004)
<i>Emericellopsis mirabilis</i>	CBS 177.53	–	AY632656	–	–	–	Zuccaro et al. (2004)
<i>Emericellopsis pallida</i>	CBS 490.71 <sup>T</sup>	–	KC987176	KC987138	KC998998	KC999034	Grum-Grzhimaylo et al. (2013b)
	CBS 624.73	–	AY632667	AY632683	–	–	Zuccaro et al. (2004)
<i>Emericellopsis robusta</i>	CBS 489.73	–	AY632664	AY632680	–	–	Zuccaro et al. (2004)
<i>Emericellopsis salmosynnemata</i>	CBS 382.62	–	AY632666	AY632682	–	–	Zuccaro et al. (2004)
<i>Emericellopsis stolckiae</i>	CBS 159.71 <sup>1</sup>	–	AY632668	AY632684	–	–	Zuccaro et al. (2004)
<i>Emericellopsis synnematicola</i>	CBS 176.60 <sup>T</sup>	–	AY632665	AY632681	–	–	Zuccaro et al. (2004)
<i>Emericellopsis terricola</i>	CBS 120.40 <sup>T</sup>	U57082	U57676	–	–	–	Glenn et al. (1996)
	CBS 229.59	–	AY632662	AY632678	–	–	Zuccaro et al. (2004)
	CCF 3815	–	FJ430737	–	–	–	Hujslóvá et al. (2009)
	NRRL 54109	–	HQ698592	–	–	–	Unpublished
<i>Gibellulopsis piscis</i>	CBS 892.70 <sup>1</sup>	EF543835	DQ825985	–	–	–	Zare et al. (2007)
<i>Gibellulopsis nigrescens</i>	CBS 101221	EF543840	EF543848	–	EF543797	–	Zare et al. (2007)
<i>Gliocladium cibotii</i>	CBS 109240 <sup>T</sup>	EF543842	DQ825980	–	–	–	Zare et al. (2007)
<i>Gliomastix polychromum</i>	CBS 181.27 <sup>1</sup>	HQ232091	–	–	–	–	Summerbell et al. (2011)
<i>Gliomastix roseogrisea</i>	CBS 134.56 <sup>T</sup>	HQ232121	–	–	–	–	Summerbell et al. (2011)
<i>Leucosphaerina arxii</i>	CBS 737.84 <sup>T</sup>	HQ232159	–	–	–	–	Summerbell et al. (2011)
<i>Linkosia fusiformis</i>	HKUCC 10824	DQ408571	–	–	–	–	Shenoy et al. (2006)
<i>Musicillium theobromae</i>	CBS 385.32	EF543836	–	–	–	–	Zare et al. (2007)
	CBS 458.51	EF543837	EF543858	–	–	–	Zare et al. (2007)
	CBS 968.72 <sup>1</sup>	EF543838	EF543859	–	–	–	Zare et al. (2007)
<i>Niesslia exilis</i>	CBS 357.70	AY489718	–	–	–	–	Castlebury et al. (2004)
<i>Phialemonium atrogriseum</i>	CBS 604.67 <sup>T</sup>	HQ231981	–	–	–	–	Summerbell et al. (2011)
<i>Plectosphaerella cucumerina</i>	CBS 137.37 <sup>1</sup>	JF780520	JF780522	–	–	–	Carlucci et al. (2012)
	DAOM 226828	GU180647	GU180630	–	–	GU180663	Réblová et al. (2011)
	Plect 170 *	HQ239032	HQ238991	–	–	–	Summerbell et al. (2011), Carlucci et al. (2012)
<i>Sarocladium bacillisporum</i>	CBS 425.67 <sup>T</sup>	HE608658	–	–	–	–	Giraldo et al. (2012)
<i>Sarocladium bactrocephalum</i>	CBS 749.69 <sup>T</sup>	HQ231994	–	–	–	–	Summerbell et al. (2011)
<i>Sarocladium glaucum</i>	CBS 796.69 <sup>1</sup>	HE608657	–	–	–	–	Giraldo et al. (2012)

<i>Sarocladium kiliense</i>	CBS 122.29 <sup>I</sup>	HQ232052	–	–	–	–	Summerbell et al. (2011)
<i>Sarocladium ochraceum</i>	CBS 428.67 <sup>T</sup>	HQ232070	–	–	–	–	Summerbell et al. (2011)
<i>Sarocladium oryzae</i>	CBS 180.74 <sup>T</sup>	HQ232166	–	–	–	–	Summerbell et al. (2011)
<i>Sarocladium strictum</i>	CBS 346.70 <sup>T</sup>	HQ232141	–	–	–	–	Summerbell et al. (2011)
<i>Sarocladium terricola</i>	CBS 243.59 <sup>T</sup>	HQ232046	–	–	–	–	Summerbell et al. (2011)
<i>Sarocladium zeae</i>	CBS 801.69 <sup>T</sup>	HQ232152	–	–	–	–	Summerbell et al. (2011)
<i>Selinia pulchra</i>	AR 2812 *	–	HM484859	HM484884	HM484841	–	Chaverri et al. (2011)
<i>Sodiomyces alkalinus</i>	CBS 110278 <sup>T</sup>	JX158427	JX158405	–	JX158383	JX158449	Grum-Grzhimaylo et al. (2913a)
	CBS 132731	JX158428	JX158406	–	JX158384	JX158450	Grum-Grzhimaylo et al. (2913a)
<i>Sodiomyces magadii</i>	CBS 137619 <sup>T</sup>	KJ443148	KJ443278	–	–	–	Grum-Grzhimaylo et al. (2016)
<i>Sodiomyces tronii</i>	CBS 137618 <sup>T</sup>	KJ443147	KJ443277	–	–	–	Grum-Grzhimaylo et al. (2016)
	CBS137620	KJ443149	KJ443279	–	–	–	Grum-Grzhimaylo et al. (2016)
<i>Stanjemonium grisellum</i>	CBS 655.79 <sup>I</sup>	–	AY632671	AY632687	–	–	Zuccaro et al. (2004)
<i>Stanjemonium ochroroseum</i>	CBS 656.79 <sup>T</sup>	–	AY632672	AY632688	–	–	Zuccaro et al. (2004)
<i>Stilbella fimetaria</i>	DAOM 229279	HQ232176	–	–	–	–	Summerbell et al. (2011)
	D99026 *	–	AY952467	–	–	–	Lehr et al. (2006)
	MH178 *	–	FJ430712	–	–	–	Hujšlová et al. (2009)
	SES201 *	–	FJ939394	–	–	–	Mazzaferro et al. (2010)
<i>Verrucostoma freycinetiae</i>	MAFF 240100	–	HM484866	HM484885	HM484853	–	Chaverri et al. (2011)
<i>Verticillium albo-atrum</i>	CBS 130.51 <sup>T</sup>	HQ231976	DQ825977	–	–	–	Zare et al. (2007), Summerbell et al. (2011)
<i>Verticillium dahliae</i>	AFTOL-ID 237	DQ470945	–	–	–	–	Spatafora et al. (2006)
	ATCC 16535	U17425	–	–	AY489632	DQ522468	Rehner & Samuels (1995), Castlebury et al. (2004), Spatafora et al. (2007)

<sup>a</sup> AFTOL, Assembling the Fungal Tree of Life project; ATCC, American Type Culture Collection, Manassas, Virginia, USA; CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; CCF, Culture Collection of Fungi, Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic; DAOM, Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; FMR, Faculty of Medicine Reus, Spain; HKUCC, Hong Kong University Culture Collection, Department of Ecology and Biodiversity, Hong Kong, China; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, US Department of Agriculture, Peoria, Illinois, USA; MAFF, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan; UAMH, University of Alberta Microfungus Collection and Herbarium; Edmonton, Canada; UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas, USA; <sup>T</sup> Type strain; \* from GenBank. <sup>b</sup> LSU large subunit of the nrDNA; ITS internal transcribed spacer regions of the nrDNA and intervening 5.8S nrDNA; *BT2* beta-tubulin gene; *TEF1-α* transcription elongation factor 1-alpha; *RPB2* RNA polymerase II second largest subunit.



### **DNA extraction, amplification and sequencing**

Total genomic DNA was extracted from fresh colonies using PrepMan Ultra Sample Preparation Reagent (Applied Biosystems, Foster City, CA, USA), following the manufacturer's protocol. The DNA was quantified using a NanoDrop 3000 fluorospectrometer (Thermo Scientific, Asheville, NC, USA). The internal transcribed spacer (ITS) regions and the 5' end of the 28S nrDNA gene (LSU) were amplified and sequenced with the primer pairs ITS5/ITS4 (White et al. 1990) and LR0R/LR5 (Vilgalys and Hester 1990; Vilgalys and Sun 1994), respectively. Fragments of the translation elongation factor 1-alpha (*TEF1- $\alpha$* ), RNA polymerase II second largest subunit (*RPB2*) and  $\beta$ -tubulin (*BT2*) genes were amplified with the following primer sets: EF 983F/EF 2218R (Rehner and Buckley 2005) for *TEF1- $\alpha$* , RPB2-5F/RPB2-7R (Liu et al. 1999) for *RPB2* and Bt1a/Bt1b (Glass and Donaldson 1995) for *BT2* using PCR protocols described elsewhere (Zuccaro et al. 2004; Grum-Grhimaylo et al. 2013a). PCR products were purified and sequenced at MacroGen Europe (Amsterdam, The Netherlands) with the same primers used for amplification. The program SeqMan v. 7.0.0 (DNASTAR, Madison, WI, USA) was used to obtain consensus sequences of each isolate.

### **Phylogenetic analysis**

Phylogenetic analyses based on LSU sequences determined relatedness of taxa to either the "J and N" groups of the Hypocreales or to the "Q and R" groups of the Plectosphaerellaceae. Subsequently, several multilocus sequence analyses for each particular clade were performed to confirm the results obtained with the LSU data. ITS, *BT2*, *RPB2* and *TEF1- $\alpha$*  loci were used for the isolate of the group N; ITS and LSU for the group J; and ITS, LSU, *RPB2* and *TEF1- $\alpha$*  for the isolates included in groups Q and R. Sequences were aligned and concatenated in MEGA v. 6.06 (Tamura et al. 2013) using the Clustal W and MUSCLE applications (Thompson et al. 1994; Edgar 2004). Manual corrections of the alignments, selection of the best-fit nucleotide substitution models for each locus and for the combined dataset, and Maximum Composite Likelihood (ML) phylogenetic analyses were performed in MEGA 6.06. Gaps or missing data were treated as partial deletion with a site coverage cut-off of 95 % and Nearest-Neighbor-Interchange (NNI). The internal branch support was

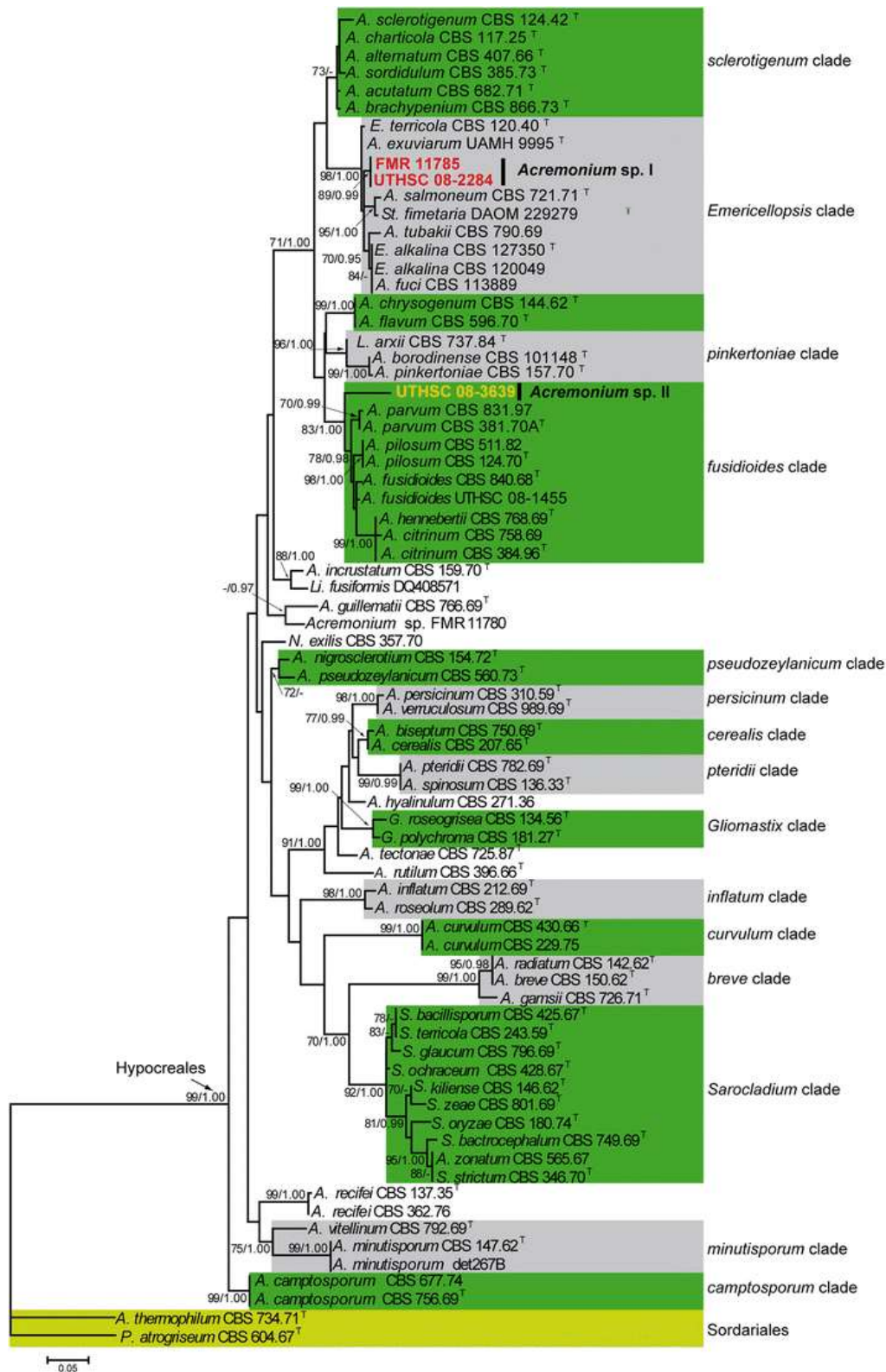
assessed by a search of 1000 bootstrapped data sets. A bootstrap support (BS)  $\geq 70$  was considered as statistically significant. Phylogenetic distance values among isolates were estimated with Kimura 2-parameter as nucleotide substitution model under the same software. A second phylogenetic reconstruction via Bayesian inference (BI) was done using MrBayes v. 3.2.1 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012). Markov chain Monte Carlo (MCMC) sampling was performed with two simultaneous runs for 3 million generations, with samples taken every 100 generations. Bayesian posterior probabilities (PP) were obtained from the 50 % majority-rule consensus tree after removing the first 25 % of the collected trees. A PP value  $\geq 0.95$  was considered statistically significant. The best nucleotide substitution model for each gene in the Bayesian analysis (GTR+G+I) was determined using MrModelTest v. 2.3 (Nylander 2004). Congruency of the sequence datasets for the separate loci were determined using tree topologies of 70 % reciprocal Neighbour-Joining (NJ) bootstrap trees with Maximum Likelihood distances for identifying topology conflict visually (Gueidan et al. 2007). Because no incongruence was observed, the different matrices were combined in the final phylogenetic analyses. All novel DNA sequences were deposited in GenBank (Table 1), the alignments and the resulting trees in TreeBASE (<http://www.treebase.org>), and taxonomic novelties in MycoBank (<http://www.MycoBank.org>; Crous et al. 2004).

## Results

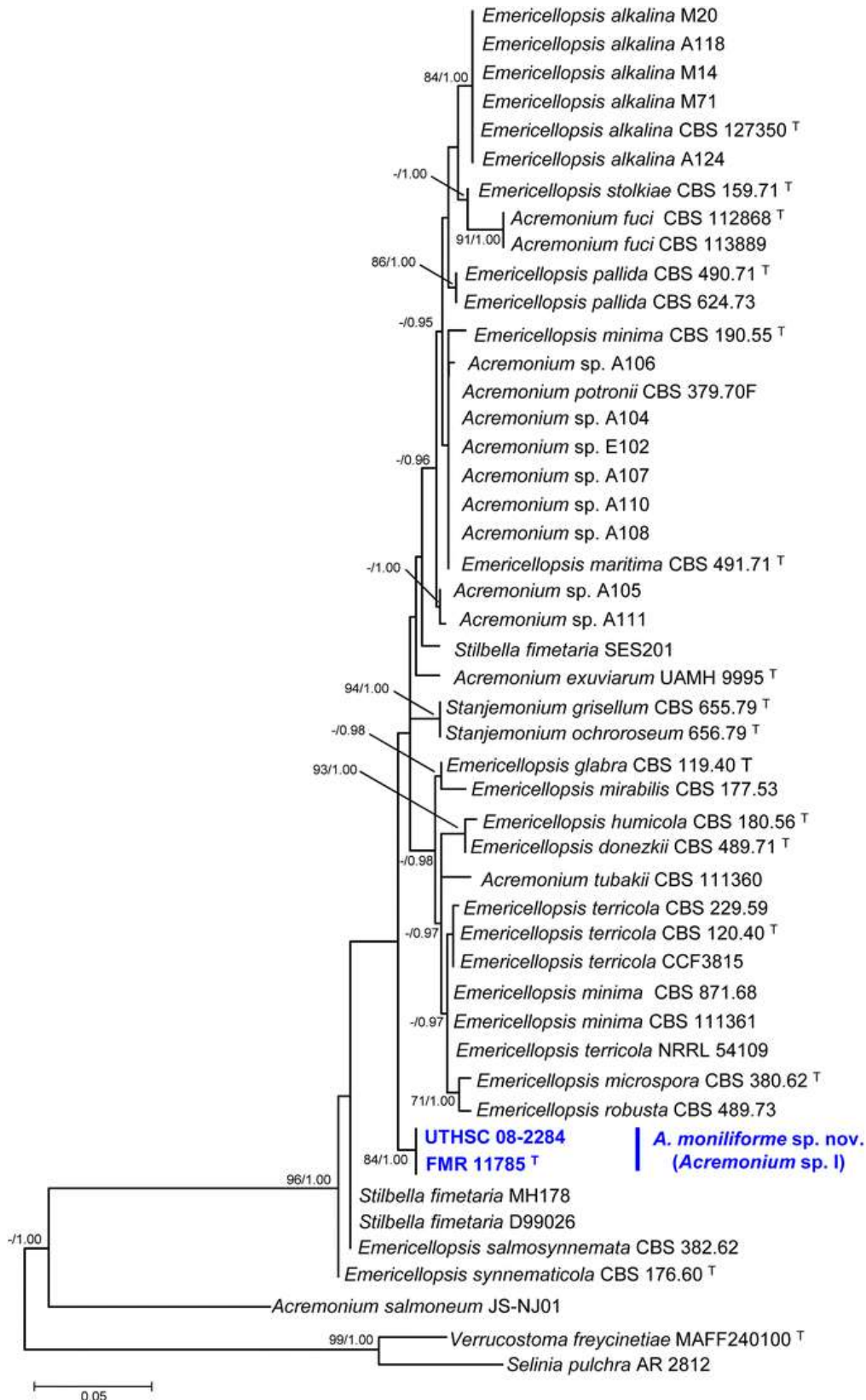
The phylogenetic analysis based on LSU sequences of the isolates UTHSC 08-2284 (group N), UTHSC 08-3639 (group J) and FMR 11785 together with hypocrealean *Acremonium* species and related genera reported by Summerbell et al. (2011) is shown in the Figure 1. The final tree is based on 75 aligned sequences, 847 characters including gaps, of which 580 were conserved, 267 were variable and 195 were phylogenetically informative. Tamura-Nei with gamma distribution (TN+G) and the general time reversible with gamma distribution and a portion of invariable sites (GTR+G+I) were found as the best-fit nucleotide substitution models for ML and BI, respectively. The phylogenetic

tree revealed that the isolates clustered in the “*Emericellopsis*” and “*fusidioides*” clades (Bionectriaceae) as defined by Summerbell et al. (2011). The isolates UTHSC 08-2284 and FMR 11785 fell into the *Emericellopsis* clade (BS = 98 %, PP = 1.00) together with the type species of *Emericellopsis*, *E. terricola* (CBS 120.40), *A. exuviarum* (UAMH 9995), *A. fuci* (CBS 113889) and *A. salmoneum* (CBS 721.71). The two mentioned unidentified isolates showed identical sequences and were grouped in a highly supported subclade (*Acremonium* sp. I). The isolate UTHSC 08-3639 (*Acremonium* sp. II) was represented by a single branch, phylogenetically related (BS = 83 %, PP = 1.0) to *A. fusidioides*, *A. hennebertii* and the recently described species *A. citrinum*, *A. parvum* and *A. pilosum* (Giraldo et al. 2014), all belonging to the *fusidioides* clade (Fig. 1).

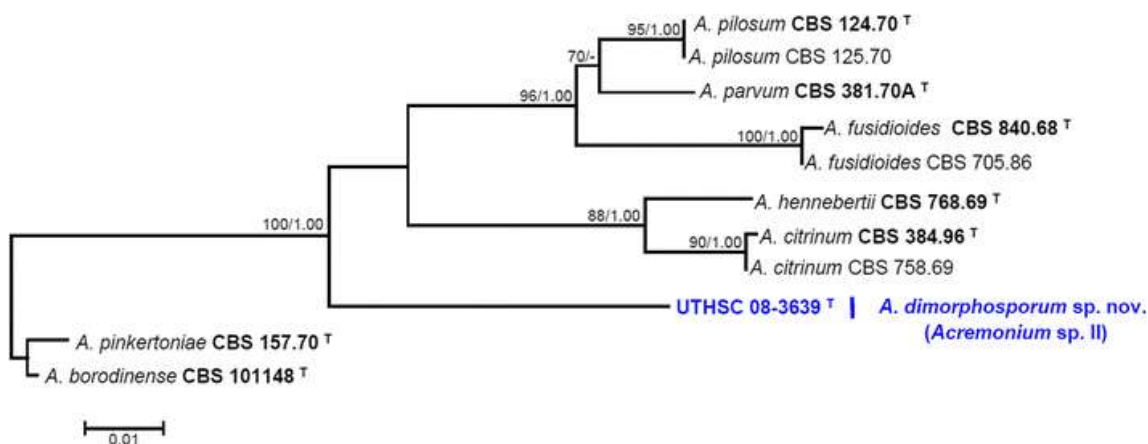
To better resolve the phylogenetic relationships obtained from the LSU analyses of *Acremonium* sp. I and *Acremonium* sp. II, a multilocus study was performed for each of the unidentified species and their respective closely related species. The first one (Fig. 2) was based on ITS, *BT2*, *RPB2* and *TEF1- $\alpha$*  sequences and targeted the two isolates of *Acremonium* sp. I, members of the *Emericellopsis* clade, and additional species previously reported to be related to *Emericellopsis* (Sigler et al. 2004; Zuccaro et al. 2004, Grum-Grzhimaylo et al. 2013b) such as *Stanjemonium grisellum*, *S. ochroroseum* and *Acremonium potronii*. The data set included 48 sequences of different strains and 2807 characters (2004 conserved, 803 variables and 604 phylogenetically informative). *Verrucostoma freycinetiae* and *Selinia pulchra* were used as outgroup. Tamura 3-parameter with gamma distribution (T92+G) and GTR+G+I were found to be the best nucleotide substitution models for ML and BI, respectively. The trees generated by using ML and BI had a similar topology. The phylogenetic tree was consistent with previously reported phylogenies (Sigler et al. 2004; Zuccaro et al. 2004; Grum-Grzhimaylo et al. 2013b). The two isolates of *Acremonium* sp. I formed a highly supported basal clade (BS = 84 %, PP = 1.00), distant from the species of *Acremonium*, *Emericellopsis* and *Stanjemonium*. *Acremonium* sp. I is described below as a new species, named *Acremonium moniliforme*.



**Fig. 1** Maximum composite likelihood tree based on analyses of partial LSU sequences of selected genera of the Hypocreales including *Acremonium* (abbreviated as *A.*), *Emericellopsis* (*E.*), *Gliomastix* (*G.*), *Leucosphaerina* (*L.*), *Linkosia* (*Li.*), *Niesslia* (*N.*), *Phialemonium* (*P.*), *Sarocladium* (*S.*) and *Stilbella* (*St.*). Clade names are based on Summerbell et al. (2011). Bootstrap support values above 70%/ Bayesian posterior probability values above 0.95 are shown at the nodes. <sup>T</sup>, Type strain. *Acremonium* sp. I represents newly described *A. moniliforme* and *Acremonium* sp. II, *A. dimorphosporum*



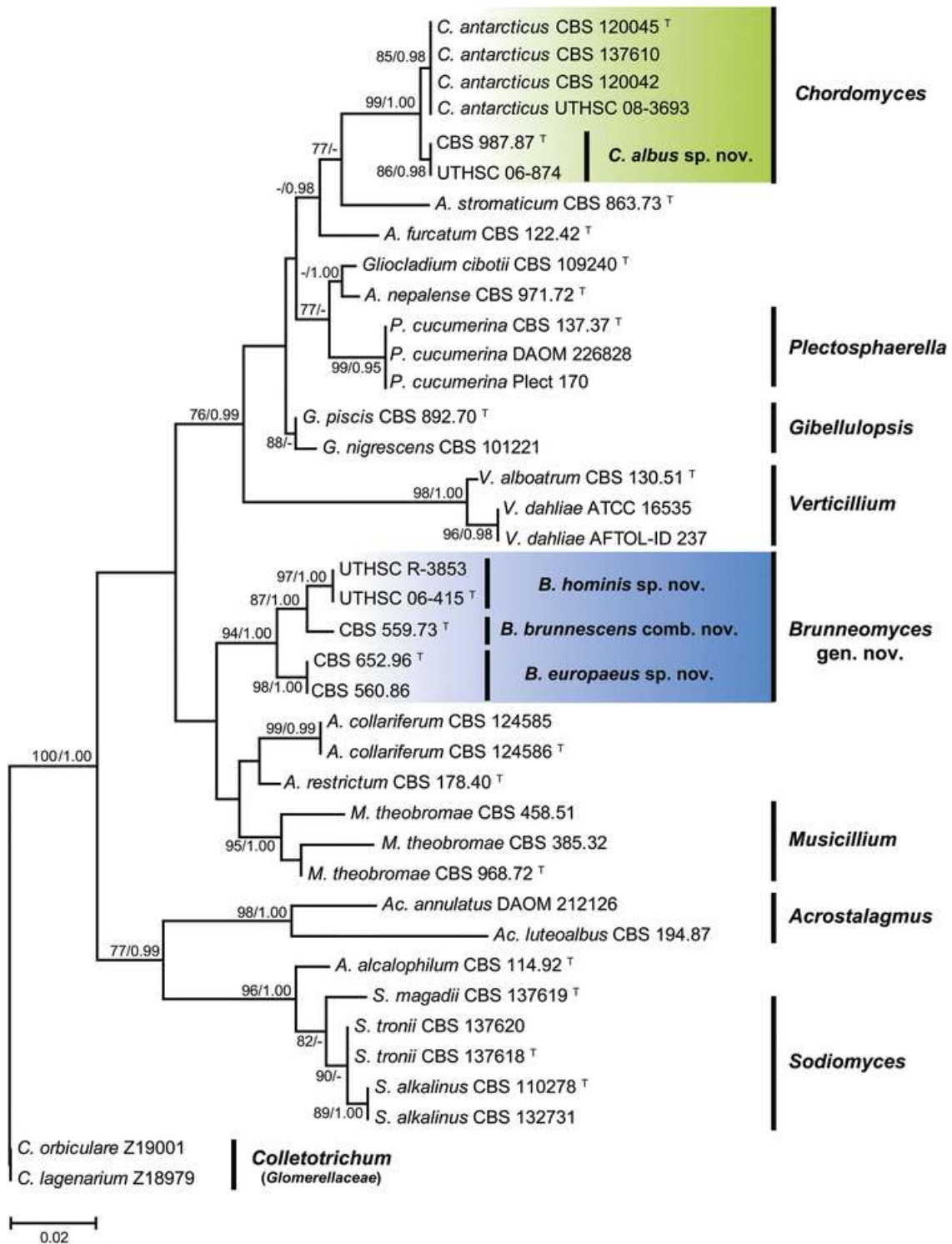
**Fig. 2** Maximum composite likelihood tree based on sequences of ITS and partial protein encoding genes (*BT2*, *RPB2* and *TEF1-α*) of selected acremonium-like taxa of the Bionectriaceae. Bootstrap support values above 70 % / Bayesian posterior probability values above 0.95, are shown at the nodes. <sup>T</sup>, Type strain



**Fig. 3** Maximum composite likelihood tree based on sequences of ITS and partial LSU genes from *A. dimorphosporum* and species of the *fusidioides* clade. Bootstrap support values above 70 % / Bayesian posterior probability values above 0.95, are shown at the nodes. <sup>T</sup>, Type strain

The other analysis (Fig. 3) included a combination of the ITS and LSU sequences of *Acremonium* sp. II and the ex-type and reference strains of the *Acremonium* species from the *fusidioides* clade. The data set consisted of 11 sequences of different strains and 915 characters (731 conserved, 184 variable and 135 phylogenetically informative). The ex-type strains of *Acremonium pinkertoniae* and *A. borodinense* were used as outgroup. ML/BI analyses were done with Kimura-two parameter with gamma distribution (K2+G) as the best-fit nucleotide substitution model. In this analysis, *Acremonium* sp. II clustered distantly from *Acremonium* species forming dimorphic conidia (*A. fusidioides*, *A. pilosum* and *A. borodinense*) and other species with elongate conidia in chains (*A. hennebertii*, *A. parvum* and *A. citrinum*). *Acremonium* sp. II is proposed as a new species, *A. dimorphosporum*.

The phylogenetic reconstruction using the LSU, ITS, *TEF1-α* and *RPB2* loci from the clinical isolates of the groups Q (UTHSC 06-415 and UTHSC R-3853) and R (UTHSC 06-874 and UTHSC 08-3693), and other representative species of the Plectosphaerellaceae (Fig. 4) is based on a combined dataset consisting of 3271 characters, including 805 phylogenetically informative positions (135 LSU, 151 ITS, 158 *TEF1-α* and 361 *RPB2*), and 39 strains or taxa including the outgroup *Colletotrichum orbiculare* and *C. lagerarium*. The best-fit nucleotide substitution model for ML and BI analysis was K2+G. The phylogenetic tree showed that the isolates UTHSC 06-874 and UTHSC 08-3693 clustered in *Chordomyces* (Grum-Grzhimaylo et al. 2016). While UTHSC 08-3693 was



**Fig. 4** Maximum composite likelihood tree based on analysis of ITS and partial LSU, *RPB2* and *TEF1- $\alpha$*  sequences of genera of the Plectosphaerellaceae and related genera. Bootstrap support values above 70 % / Bayesian posterior probability values above 0.95, are shown at the nodes. <sup>T</sup>, Type strain

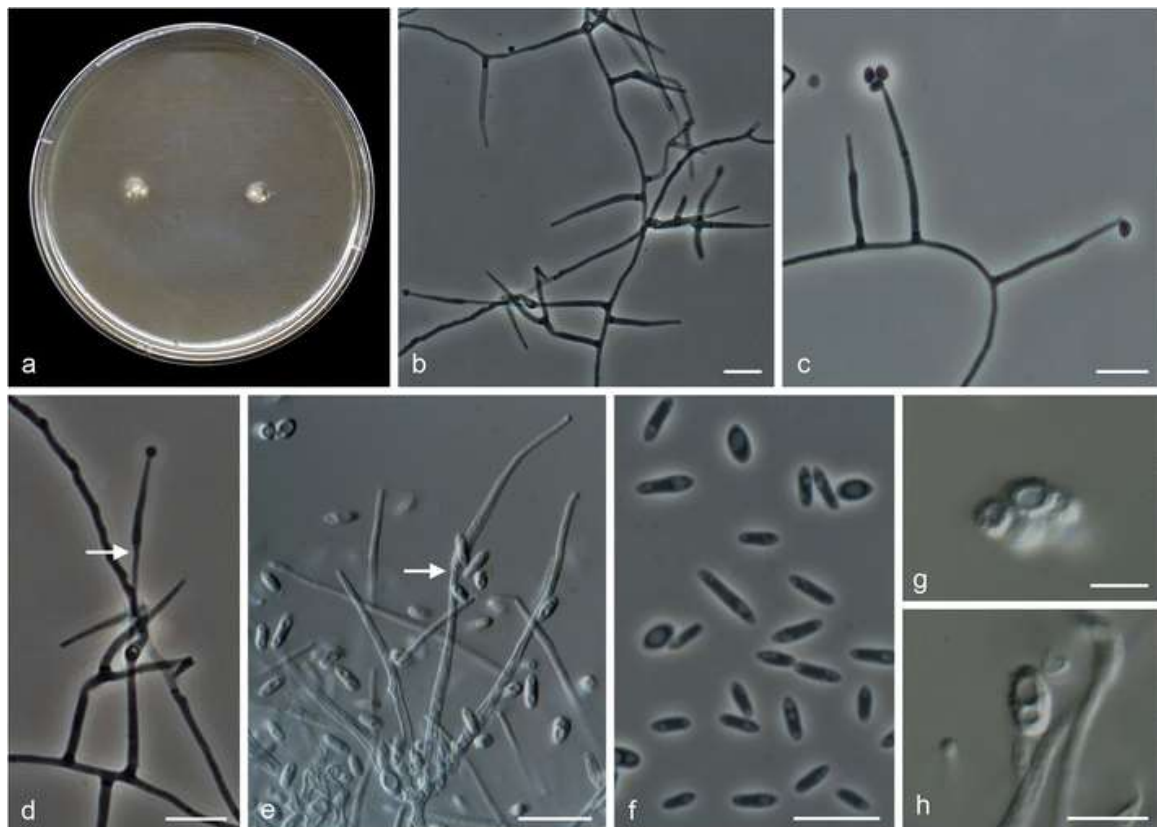
grouped with the ex-type strain of *C. antarcticus* (BS = 85 %; PP = 0.98), UTHSC 06-874 clustered with strain CBS 987.87 in a separated subclade (BS = 86 %; PP = 0.98). Because the last two isolates were phylogenetically distant

from the clade of *C. antarcticum*, the new species *Chordomyces albus* is proposed. In the same analysis, a monophyletic group (BS = 94 %; PP = 1.00) was formed by all isolates of the group R, two reference strains of *A. hyalinulum* formed a well-supported clade (BS = 98 %; PP = 1.00), two clinical isolates (UTHSC R-3853 and UTHSC 06-415) clustered together with high support (BS = 97 %; PP = 1.00), while the ex-type strain of *A. brunnescens* formed a single branch. This novel lineage of acremonium-like fungi within the Plectosphaerellaceae is proposed as a new genus, *Brunneomyces* based on *A. brunnescens*, and the two new species, *B. hominis* and *B. europaeus*.

## Taxonomy

***Acremonium dimorphosporum*** Giraldo, Deanna A. Sutton & Gené, **sp. nov.**

[MB 811461] Fig. 5



**Fig. 5** *Acremonium dimorphosporum* UTHSC 08-3639. **a** Colonies on OA, after 21 days at 25 °C. **b, c** Simple conidiophores and conidia forming heads. **d, e** Phialides with percurrent proliferation (arrow). **f–h** Smooth-walled, ellipsoidal (**f**) and thick-walled, verrucose, ovoidal conidia (**h**). *Scale bars*: **b–f** = 10 µm, **g, h** = 5 µm



Etymology. The name refers to the dimorphic conidia produced by the species.

*Colonies* at 25 °C after 14 days on OA reaching 10–11 mm diam, white (1A1) to yellowish white (4A2), flat, with scarce aerial mycelium, reverse colourless; at 25 °C after 14 days on PDA reaching 14–15 mm diam, pinkish white (7A2), flat, cottony, reverse orange (6A6). *Mycelium* consisting of hyaline, smooth- and thin-walled, 1.5–2 µm wide hyphae. *Conidiophores* erect, usually reduced to single phialides emerging from vegetative hyphae, occasionally basitonously branched and then bearing 2–4 phialides, straight, up to 60 µm long, hyaline, smooth, with cell walls usually thicker than those of the vegetative hyphae. *Phialides* subulate, 17–30(45) µm long, 1–1.5 µm wide at the base, hyaline, thick- and smooth-walled, often borne on short cylindrical subtending cells; percurrently proliferating phialides are occasionally present. *Conidia* arranged in slimy heads, 1-celled, hyaline, of two types: i) cylindrical with more or less rounded ends, 3–7 × 1–1.5 µm, thin- and smooth-walled; ii) ellipsoidal, 3–4 × 2–3 µm, thick- and rough-walled. *Chlamydospores* and *sexual morph* not observed.

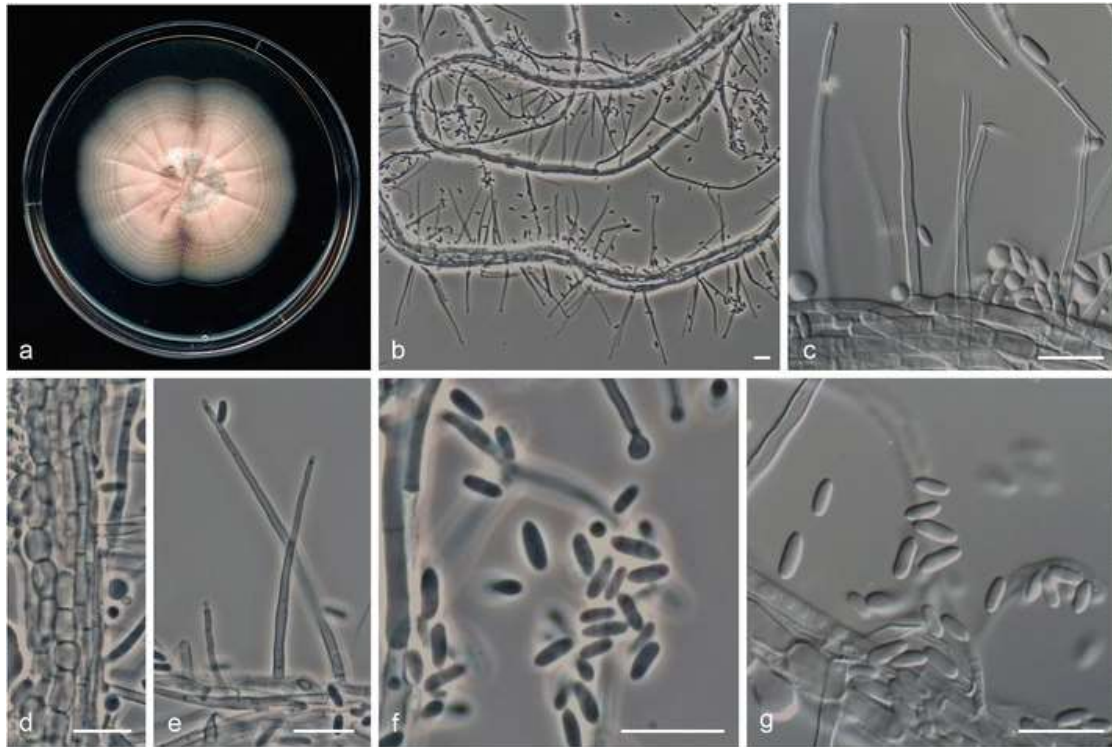
*Cardinal temperatures for growth*: Optimum 20–25 °C, maximum 30 °C, minimum 15 °C.

*Specimen examined*. **USA**, Texas, from bronchoalveolar lavage fluid, 2008, D.A. Sutton (holotype CBS H-22021, dried culture on OA; cultures ex-type CBS 139050 = FMR 10548 = UTHSC 08-3639).

*Notes*: Although *A. dimorphosporum* is phylogenetically distant to *A. borodinense*, it is morphologically similar to that species in producing both ellipsoidal rough-walled and cylindrical smooth-walled conidia (Ito et al. 2000). However, *A. borodinense* differs from *A. dimorphosporum* by its faster growth at 25 °C (27–29 mm diam. after 10 d) and by its ability to grow at 37 °C. Furthermore, its cylindrical conidia are slightly curved and smaller (4.5–5.5 µm long), and its ellipsoidal and rough-walled conidia are larger (4.2–5.5 × 3–4 µm) than those of *A. dimorphosporum*.

***Acremonium moniliforme*** Giraldo, Deanna A. Sutton & Guarro, **sp. nov.**

[MB 811462] Fig. 6



**Fig. 6** *Acremonium moniliforme* FMR 11785. **a** Colonies on PDA after 14 days at 25 °C. **b, c** Simple conidiophores arising laterally from ropes of hyphae. **d** Moniliform hyphae. **e** Phialide with periclinal thickening at the apex. **f, g** Conidia. Scale bars = 10 µm

**Etymology.** The name refers to the presence of moniliform hyphae.

**Colonies** at 25 °C after 14 days on OA reaching 45–60 mm diam, yellowish white (4A2), flat, glabrous; reverse colourless; at 25 °C after 14 days on PDA reaching 36–50 mm diam, pinkish white (7A2), radially folded, zonate towards the periphery, felty, greyish red (7B4); reverse salmon (6A4). **Mycelium** consisting of branched, septate, hyaline, smooth- and thick-walled hyphae, initially 2–2.5 µm wide, often swelling at maturity, becoming barrel-shaped, up to 7 µm wide. **Conidiophores** reduced to conidiogenous cells, emerging laterally from hyphae. **Phialides** acicular with a slightly flexuose apex, 30–50 µm long, 1.5–2 µm wide at the base, with a distinct periclinal thickening, thick- and smooth-walled, hyaline. **Conidia** arranged in slimy heads, 1-celled, cylindrical with rounded ends, 3–5(6) × 1–2 µm, hyaline, thick- and smooth-walled. **Chlamydospores** and **sexual morph** not observed.

**Cardinal temperatures for growth:** Optimum 25–30 °C, maximum 37 °C, minimum below 4 °C.

*Specimens examined:* **Spain**, Aragón, Huesca province, Ordesa y Monte Perdido National Park, from forest soil, 2011, coll. A. Giraldo, M. Hernández, & J. Capilla, isol. A. Giraldo (holotype CBS H-22022, dried culture on OA; cultures ex-type CBS 139051 = FMR 11785). **USA**, Utah, from toe nail, 2008, D.A. Sutton (FMR 10363 = UTHSC 08-2284).

*Notes:* *Acremonium moniliforme* is phylogenetically distant from the species of the *Emericellopsis* clade. It can be morphologically differentiated from other *Acremonium* species by the production of abundant moniliform hyphae. *Acremonium fuci* occasionally produces small rounded hyphal swellings, similar to the moniliform hyphae of *A. moniliforme*. However, both species can be distinguished by the conidial shape, which is obovoid or broadly ellipsoidal in the former, and cylindrical in the latter. Additionally, the maximum temperature for growth in *A. fuci* is 33 °C (Zuccaro et al. 2004), while in *A. moniliforme* it is 37 °C.

***Brunneomyces*** Giraldo, Gené & Guarro, **gen. nov.** [MB 811471]

*Type species.* *Brunneomyces brunnescens* (W. Gams) Giraldo, Gené & Guarro

*Etymology.* The name refers to brownish pigmented hyphae formed by the type species of this genus.

*Mycelium* consisting of branched, septate, hyaline and thin-walled hyphae, often becoming dark brown, verrucose and thick-walled with age. *Conidiophores* erect, unbranched or poorly branched, often proliferating sympodially, showing conidiogenous cells as short lateral and cylindrical projections. *Conidiogenous cells* enteroblastic, mono- and polyphialidic, hyaline, terminal, lateral or intercalary (adelophialides), subulate, lageniform or cylindrical, usually with short cylindrical collarettes, often subhyaline or pale brown, and with a distinct periclinal thickening at the conidiogenous locus. *Conidia* arranged in chains, 1-celled, pyriform or ellipsoidal, hyaline or brown. *Sexual morph* unknown.

***Brunneomyces brunnescens*** (W. Gams) Giraldo, Gené & Guarro, **comb. nov.** [MB 811472] Fig. 7



**Fig. 7** *Brunneomyces brunnescens* CBS 559.73. **a** Colonies on OA after 14 days at 25 °C. **b** Brown pigmented hyphae. **c** Discrete phialides. **d** Phialides with slightly pigmented collarettes and conidial chains collapsing in slimy heads. **e** Sympodial conidiophore. **f** Conidia. Scale bars = 10 µm

Basionym. *Acremonium brunnescens* W. Gams, Trans. Br. Mycol. Soc., 64: 398. 1975.

*Specimen examined.* **Sri Lanka**, Hakgala Bot. Gardens, from dead stem of *Dendrocalamus giganteus*, Jan. 1973, W. Gams (holotype CBS H-6641, dried plant material; cultures ex-type CBS 559.73 = ATCC 32180 = IMI 185378).

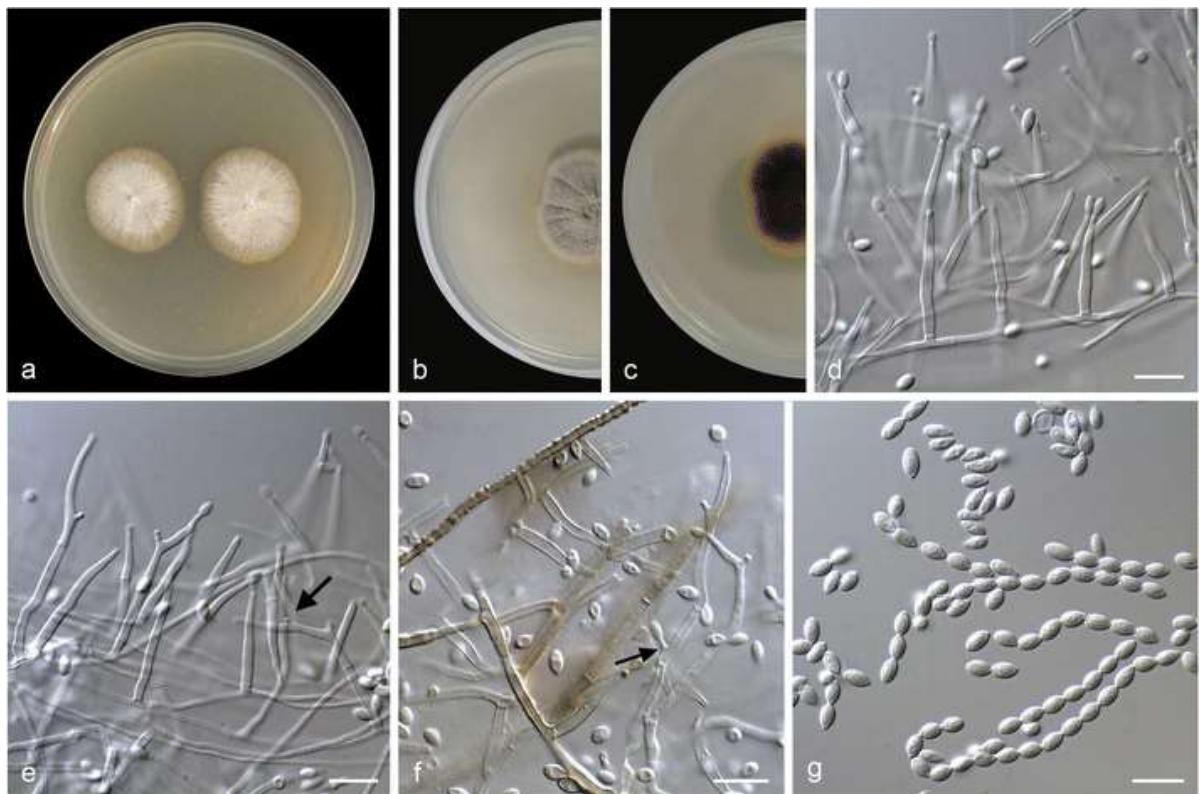
*Notes:* Although the three species of *Brunneomyces* show a similar conidiogenous apparatus to that of the genus *Acremonium*, they can be distinguished by the presence of sympodial conidiophores and dark brown, verrucose, thick-walled hyphae. The combination of these morphological features are usually absent in the species of *Acremonium* and other genera of plectosphaerellaceous fungi. In addition, *Brunneomyces* is the only genus of the Plectosphaerellaceae with conidial chains.

A detailed description of *B. brunnescens* was given in Gams (1975). Most relevant features of the ex-type strain of this species (CBS 559.73) studied here

were: slow growing colonies (6–8 mm and 21–22 mm diam. after 14 d on PDA and OA, respectively) with a mushroom-like odour, pigmented verrucose hyphae and dark brown conidia appearing after 21 days, phialides with short cylindrical and slightly pigmented collarettes, adelophialides of  $6\text{--}10 \times 1.5\text{--}2.5 \mu\text{m}$ , and conidial chains often collapsing soon in slimy heads. In addition, this fungus was unable to grow above  $32 \text{ }^\circ\text{C}$ .

***Brunneomyces hominis*** Giraldo, Deanna A. Sutton & Gené, **sp. nov.**

[MB811473] Fig. 8



**Fig. 8** *Brunneomyces hominis* a–c, f, g UTHSC 06-415; d, e UTHSC R-3853. **a** Colonies on OA after 14 days at  $25 \text{ }^\circ\text{C}$ . **b, c** Colonies on PDA after 21 days at  $25 \text{ }^\circ\text{C}$  obverse and reverse, respectively. **d** Unbranched conidiophores. **e** Unbranched conidiophores with terminal polyphialides and a sympodial conidiophore (arrow). **f** Pigmented verrucose hyphae and intercalary phialide (arrow). **g** Conidia. Scale bars =  $10 \mu\text{m}$

**Etymology:** The name refers to the isolation source of the type strain, human clinical samples.

**Colonies** at  $25 \text{ }^\circ\text{C}$  after 14 days on OA reaching 26–28 mm diam, orange white (6A2), flat, dusty; reverse colourless; at  $25 \text{ }^\circ\text{C}$  after 14 days on PDA reaching 17–18 mm diam, grey (5F1) at the centre, yellowish white (4A2) at the

periphery, crateriform and radially folded, felty; reverse grey (5F1). Strong mushroom-like (moist soil) odour. *Mycelium* consisting of septate, hyaline, smooth- and thin-walled hyphae, 1.5–2 µm wide at the beginning, becoming dark brown, verrucose and thick-walled, up to 3 µm wide with age. *Conidiophores* erect, mostly unbranched, occasionally with a few branches and proliferating sympodially, straight or slightly bent, up to 35 µm long, hyaline, smooth-walled. *Phialides* subulate, 12–20(30) µm long, 1.5–2 µm wide at the base, hyaline at first, dark brown in old cultures, thick- and smooth-walled, with conspicuous periclinal thickening and cylindrical collarettes; adelophialides sometimes present, up to 10 µm long; polyphialides with up to two conidiogenous loci commonly present. *Conidia* arranged in long dry chains, 1-celled, pyriform or ellipsoidal, 4–5(6) × 2–2.5 µm, with truncate base, subhyaline, thin- and smooth-walled. *Chlamydospores* and *sexual morph* not observed.

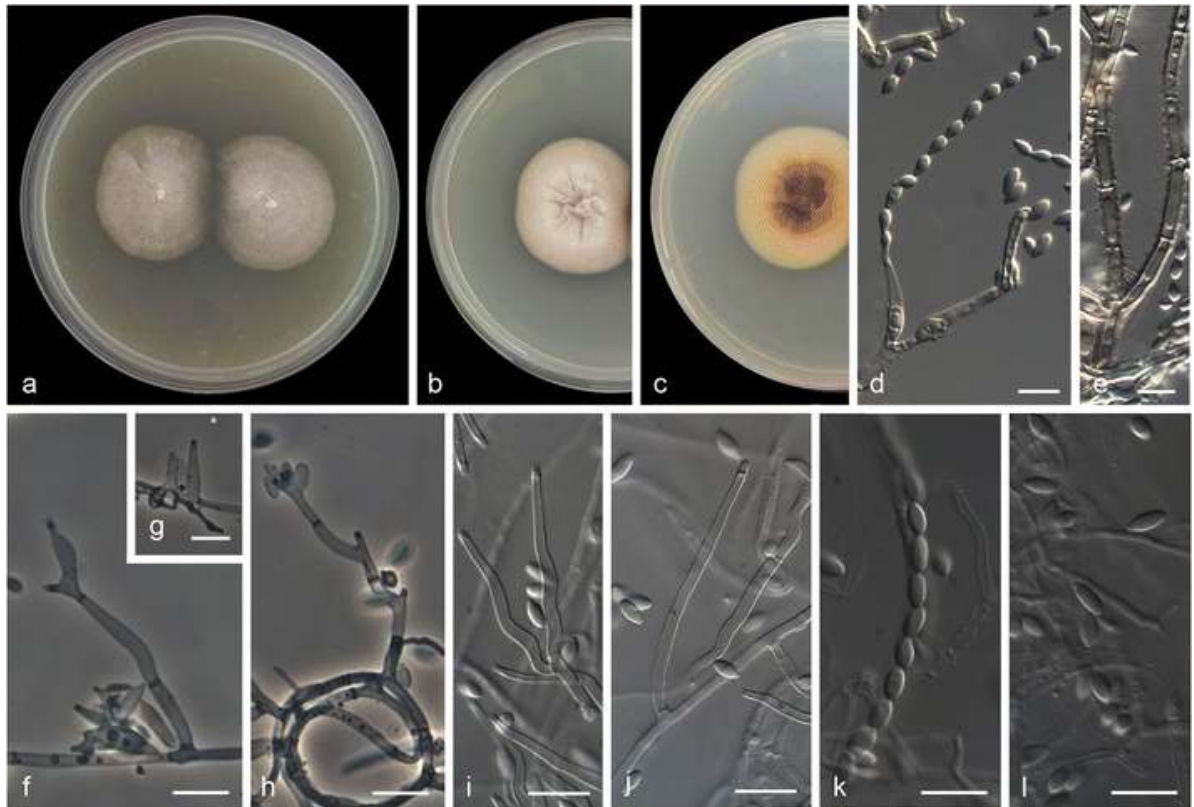
*Cardinal temperatures for growth*: Optimum 25–30 °C, maximum 35 °C, minimum below 4 °C.

*Specimens examined*. **USA**, Minnesota, from human sputum, 2006, D.A. Sutton (holotype CBS H-22023, dried culture on OA; cultures ex-type CBS 139053 = FMR 10429 = UTHSC 06-415). California, from human sputum, D.A. Sutton (CBS 139054 = FMR 10437 = UTHSC R-3853).

*Notes*: The colourless colony reverse on OA distinguishes *B. hominis* from the dark grey reverse in *B. brunnescens*. *Brunneomyces hominis* produces long, dry conidial chains, while in *B. europaeus* and *B. brunnescens* chains tend to collapse in slimy heads. It is the only *Brunneomyces* species able to grow at 35 °C.

***Brunneomyces europaeus*** Giraldo, Gené & Guarro, **sp. nov.** [MB811474]

Fig. 9



**Fig. 9** *Brunneomyces europaeus* a–c, f–h, k, l CBS 560.86; d, e, i, j CBS 652.96. **a** Colonies on OA after 14 days at 25 °C. **b, c** Colonies on PDA after 14 days at 25 °C obverse and reverse, respectively. **d.** Phialide producing a long conidial chain. **e** Pigmented verrucose hyphae. **f** Polyphialide. **g** Adelophialide. **h** Sympodial conidiophore. **i, j** Phialides with short cylindrical collarettes. **k, l** Conidia. Scale bars = 10 µm

**Etymology:** The name refers to the geographic origin of the isolates, Europe.

**Colonies** at 25 °C after 14 days on OA reaching 31–50 mm diam, yellowish white (4A2), flat, dusty; reverse colourless; at 25 °C after 14 days on PDA reaching 25–36 mm diam, greyish brown (6E2) at the centre, white (1A1) to orange-white (6A2) towards the periphery, radially folded, felty; reverse brown (6E2). Slight mushroom-like (moist soil) odour. **Mycelium** consisting of septate, hyaline, smooth- and thin-walled hyphae, 2–2.5 µm wide, becoming brownish, verrucose and thick-walled with age. **Conidiophores** erect, usually unbranched, some proliferating sympodially, up to 45 µm long, straight or slightly bent, hyaline to subhyaline, smooth-walled. **Phialides** subulate or somewhat cylindrical, 15–35(40) µm long, 2–3 µm wide at the base, hyaline, thick- and smooth-walled, with a distinct periclinal thickening at the conidiogenous locus and short cylindrical collarettes; adelophialides sometimes present, up to 15 µm

long; polyphialides with up to three conidiogenous loci commonly present. *Conidia* forming chains that soon collapse in slimy heads, 1-celled, ovoidal to ellipsoidal, 5–6(7) × 2–3 µm, with a distinctly truncate base, subhyaline, thin- and smooth-walled. *Chlamydospores* and *sexual morph* not observed.

*Cardinal temperatures for growth*: Optimum 20–25 °C, maximum 32 °C, minimum below 4 °C.

*Specimens examined*. **Spain**, Riumar, from sediments of Ebro River, 1991, coll. K. Ulfig, isol. J. Gené (holotype CBS H-22024, dried culture on OA; cultures ex-type CBS 652.96 = FMR 3962). **France**, Provence, from leaf of *Bambusa* sp., Dec. 1986, O. Petrini (CBS 560.86 = FMR 3406).

*Notes*: The two isolates of *B. europaeus* were previously identified wrongly as *A. hyalinulum* because they form brownish pigmented and verrucose hyphae, sympodially proliferating conidiophores and intercalary phialides that all are not mentioned in the protologue of *A. hyalinulum* (Gams 1971). The latter was described with hyaline smooth-walled hyphae, and lacking adelophialides. However, there is no ex-type strain of *A. hyalinulum* for a reliable comparison and, according to different studies, it seems to be a polyphyletic species (Perdomo et al. 2011; Summerbell et al. 2011).

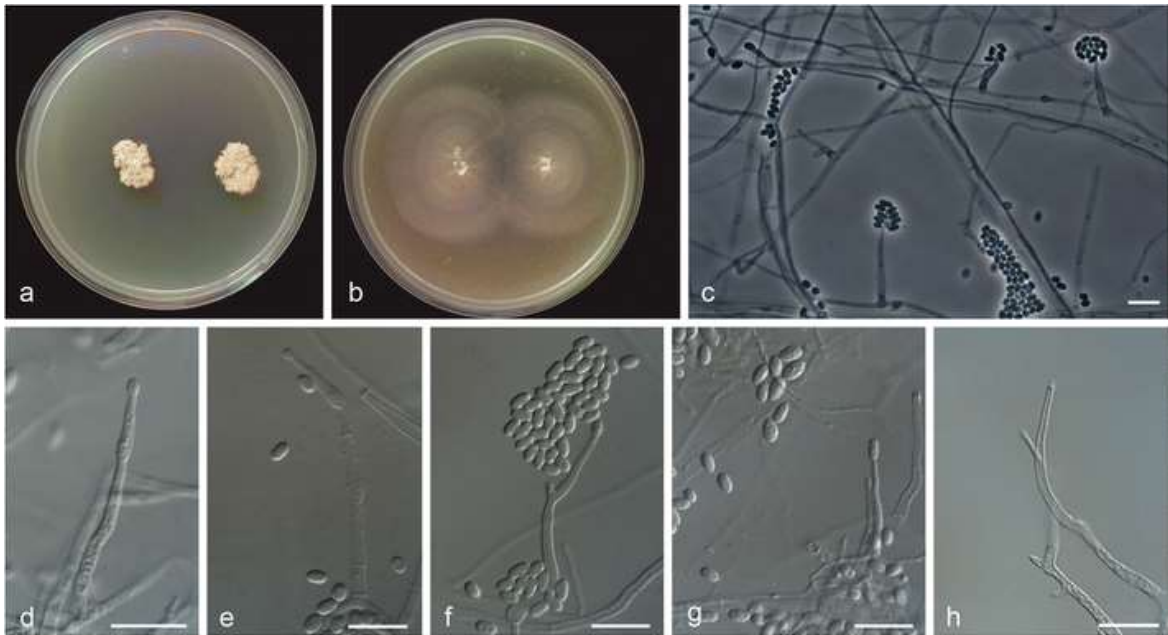
### ***Chordomyces*** Bilanenko, M.L. Georgieva & Grum-Grzhimaylo [Emmend]

*Type species*. *Chordomyces antarcticus* Bilanenko ML, Georgieva & Grum-Grzhimaylo

Modified from original description (Grum-Grzhimaylo et al. 2016): *Colonies* white, tufted, restricted to moderate fast growing. *Mycelium* superficial or immersed, consisting of septate, hyaline, thin- and smooth-walled hyphae. *Conidiophores* phalacrogenous, plectonematogenous or synnematomogenous, unbranched or branched, or consisting of single phialides. *Synnemata* when present, hyaline, without a differential sterile base, sometimes branched, appearing fimbriate due to radiating phialides. *Conidiogenous cells* mono- or polyphialidic, tapering to the apex, hyaline, often proliferating sympodially. *Conidia* arranged in slimy heads, 1(-2)-celled, subglobose, limoniform, ellipsoidal to cylindrical, rounded at the apex, sometimes with protuberant hilum, hyaline and smooth-walled. *Sexual morph* unknown.



***Chordomyces albus*** Giraldo, Deanna A. Sutton & Guarro, **sp. nov.** [MB 811476] Fig. 10



**Fig. 10** *Chordomyces albus* CBS 987.87. **a, b** Colonies on PDA and OA, respectively, after 14 days at 25 °C. **c** Unbranched conidiophores. **d, e** Phialides with percurrent proliferations. **f, g, h** Phialides with cylindrical collarettes and conidia. Scale bars =10 µm

**Etymology:** The name refers to light coloured colonies formed by the species.

**Colonies** at 25 °C after 14 days on OA reaching 40–41 mm diam, yellowish white (4A2), flat, dusty; reverse colourless; at 25 °C after 14 days on PDA reaching 10–11 mm diam, pale yellow (4A3), raised, cerebriform; reverse colourless. **Mycelium** consisting of septate, hyaline, smooth- and thin-walled hyphae, 1.5–2 µm wide. **Conidiophores** erect, unbranched, consisting of one or two cells, or with branches from near the middle bearing 3–4 phialides, up to 30 µm long, straight or slightly curved, hyaline to subhyaline, smooth-walled. **Synnemata** absent. **Phialides** cylindrical or subulate, 12–22 µm long, 2–2.5 µm wide at the base, with a distinct periclinal thickening at the conidiogenous locus and cylindrical collarettes, occasionally with a percurrent proliferation, hyaline, thick- and smooth-walled, sometimes with a second conidiogenous locus emerging laterally as an up to 5 µm long cylindrical projection near the basal septum. **Conidia** in slimy heads, 1-celled, ellipsoidal to near cylindrical, 3–4 × 2–2.5 µm, subhyaline, thick- and smooth-walled. **Chlamydospores** and **sexual morph** not observed.

*Cardinal temperature for growth:* Optimum 20–25 °C, maximum 32 °C, minimum below 4 °C.

*Specimens examined.* **Luxembourg**, Hautecharage, on *Hypogymnia physodes*, Dec. 1987, coll. G. Marson, isol. W. Gams (holotype CBS H-8083, dried plant material; cultures ex-type CBS 987.87 = FMR 10886). **USA**, Hawaii, from human sputum, 2006, D.A. Sutton (FMR 10433 = UTHSC 06-874).

Notes: The genus *Chordomyces* was recently proposed by Grum-Grzhimaylo et al. (2016) to accommodate *C. antarcticus*. Most isolates of *C. antarcticus* but CBS 987.87 derived from soda soil and were alkalitolerant. Strain CBS 987.87 was previously wrongly identified as *A. antarcticum*; however, it differs from the protologue of that species in the presence of schizophialides with conspicuous cylindrical collarettes and percurrent conidiophores (Spegazzini 1910; Hawksworth 1979). Furthermore, its DNA sequences differ significantly from *C. antarcticus* strains in Grum-Grzhimaylo et al. (2016). In the present study, CBS 987.87 and the clinical isolate UTHSC 06-874 have been found to be morphologically and genetically similar and, since they formed a novel lineage into the *Chordomyces* clade, they are proposed as a second species in the genus. *Chordomyces albus* morphologically differs from *C. antarcticus* in the absence of synnemata in culture, in having a faster growth on OA at 25 °C (40–41 mm vs. 22–28 mm in 14 days), shorter phialides (12–22 µm long vs. 28–30 µm long) and conidia without a protuberant hilum (Grum-Grzhimaylo et al. 2016).

## Discussion

Two bionectriaceous species are newly described. *Acremonium dimorphosporum* is phylogenetically related to species of the *fusidioides* clade (Summerbell et al. 2011; Giraldo et al. 2014). Conidial dimorphism seen also in other species of the *fusidioides* clade supports this phylogenetic inference, although no conidial chains but slimy masses were observed in *A. dimorphosporum* (Gams 1971; Ito et al. 2000; Giraldo et al. 2012, 2014). *Acremonium moniliforme* is phylogenetically closely related to species of the *Emericellopsis* clade, a well-defined monophyletic group within the Bionectriaceae accommodating also the type species of the synnematos genus *Stilbella*, *S. fimetaria* (Seifert 1985), the

type species of *Stanjemonium*, *S. grisellum* (Gams et al. 1998), and *Acremonium* species, such as *A. tubakii*, *A. fuci*, *A. exuviarum* and *A. salmoneum* (Sigler et al. 2004; Zuccaro et al. 2004; Summerbell et al. 2011; Grum-Grzhimaylo et al. 2013b). Members of this clade are commonly isolated from soil, dung, marine water, and occasionally from animal lesions (Sigler et al. 2004; de Hoog et al. 2015; Grum-Grzhimaylo et al. 2013b). The origin of the two strains of *A. moniliforme*, i.e. human nail from USA and soil from Spain, suggests that it is a widespread species as other species of this group.

Phylogenetic analyses of concatenated sequences from four loci support the monophyly of *Brunneomyces* and places the genus in the Plectosphaerellaceae. This family was introduced by Gams, Summerbell and Zare (Zare et al. 2007) and recently assigned to the Glomerellales (Maharachchikumbura et al. 2016). Currently, it comprises nine genera, i.e. *Acrostalagmus*, *Gibellulopsis*, *Lectera*, *Musicillium*, *Plectosphaerella*, *Stachylidium*, *Verticillium sensu stricto* and the recently described *Chordomyces* and *Sodiomyces* (Zare et al. 2007; Inderbitzin et al. 2011; Réblová et al. 2011; Cannon et al. 2012; Grum-Grzhimaylo et al. 2013a, 2016). In addition, *Gliocladium cibotii* and some *Acremonium* species, including *A. collariferum*, *A. furcatum*, *A. nepalense*, *A. restrictum* and *A. stromaticum*, belong to this family (Zare et al. 2007; Weisenborn et al. 2010; Carlucci et al. 2012). Morphologically, species of *Acrostalagmus*, *Gibellulopsis*, *Musicillium*, *Stachylidium* and *Verticillium* are mainly characterized by hyaline or light brown verticillate conidiophores (Hughes 1951; Zare et al. 2007; Inderbitzin et al. 2011; Réblová et al. 2011); *Lectera* produces brightly coloured sporodochia and brown setae (Cannon et al. 2012); the asexual morphs of *Sodiomyces* and *Plectosphaerella* have verticillate or penicillate conidiophores and septate conidia (Carlucci et al. 2012; Grum-Grzhimaylo et al. 2013a, 2016); *Chordomyces*, *G. cibotii* and above-mentioned *Acremonium* species form mostly cylindrical or ellipsoidal conidia arranged in slimy heads (Gams 1971, 1975; Zare et al. 2007; Weisenborn et al. 2010; Grum-Grzhimaylo et al. 2016). By contrast, the species of *Brunneomyces* are characterized by the production of sympodially proliferating conidiophores, ovoidal or ellipsoidal conidia arranged in chains and dark verruculose hyphae. Subglobose to oval conidia, conspicuous funnel-shaped collarette and olive-brown chlamydospores distinguishes *A. collariferum*

from *Brunneomyces* although it also forms verruculose hyphae (Weisenborn et al. 2010). In addition, *A. collariferum* did not produce conidial chains in any of the culture media tested here.

The genus *Phaeoacremonium* (Togniniaceae, Diaporthales) resembles *Brunneomyces* in having verruculose, pale brown hyphae and polyphialides. However, it is phylogenetically distant and its species produce conidia in slimy heads (Crous et al. 1996; Mostert et al. 2006).

Our phylogenetic analysis supports the monophyly of *Chordomyces* and the existence of a new species, *C. albus*. Based on morphological features of *C. antarcticus* the monotypic genus was restricted to fungi with cylindrical to ellipsoidal conidia (Grum-Grzhimaylo et al. 2016). However, UTHSC 08-3693 produced subglobose to limoniform conidia in all media tested. Therefore, the concept of *Chordomyces* is emended here accordingly.

The habitats of the members of Plectosphaerellaceae are quite diverse. *Verticillium*, *Musicillium*, *Plectosphaerella* and *Lectera* are well-known pathogens of different kinds of plants, including legumes, banana, cucurbits, potatoes, and others (Cannon et al. 2012; Carlucci et al. 2012; Masudi and Bonjar 2012; Hyde et al. 2014). *Acrostalagmus luteoalbus* has been reported also as a fungicolous species (Gams et al. 2004); *Gibellulopsis nigrescens* and most of the plectosphaerellaceous *Acremonium* species are soil-borne saprobes (Gams 1975; Domsch et al. 2007; Zare et al. 2007). *Gibellulopsis piscis* and *Plectosphaerella oratosquillae* have occasionally been reported as pathogens of fish and shrimp, respectively (Batista and da Silva 1959; Duc et al. 2009), and some species such as *Stachylidium bicolor*, *Acremonium alcalophilum*, *Chordomyces* and *Sodiomyces* species possess alkaliphilic or alkalitolerant abilities (Grum-Grzhimaylo et al. 2013a,b; 2016). The species of *Brunneomyces* and *Chordomyces* studied here seem to be saprotrophic; they are typically recovered from plant debris. Although, the isolates included in *B. hominis*, and some of *C. albus* and *C. antarcticus* are from human specimens (human nails and sputum), their human-related pathogenic role is unknown.

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