

1 **New acremonium-like taxa in the Bionectriaceae and Plectosphaerellaceae**

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17
18 **Abstract** Several molecular studies have demonstrated that species
19 traditionally assigned to the form genus *Acremonium* are polyphyletic, while
20 *Acremonium sensu stricto* is a central element of the Bionectriaceae
21 (Hypocreales). Based on phenotypic characters and molecular phylogenetic
22 analyses, two new *Acremonium* species, *A. moniliforme* and *A.*
23 *dimorphosporum*, are described. The former is related to *Emericellopsis* and is
24 characterized by cylindrical conidia, acicular phialides and abundant moniliform
25 hyphae. *Acremonium dimorphosporum* resembles *Acremonium borodinense*. It
26 produces cylindrical, smooth-walled and ellipsoidal, rough-walled conidia. The
27 new genus *Brunneomyces* is proposed based on three species, including *B.*
28 *brunnescens* (formerly *A. brunnescens*), *B. europaeus* and *B. hominis*. All of
29 them are characterized by brown hyphae, sympodial conidiophores and chains
30 of ovoidal to ellipsoidal conidia. Also, the proposed new species *Chordomyces*
31 *albus* is characterized by its light-coloured colonies, simple or branched
32 conidiophores, phialides with percurrent proliferations and cylindrical collarettes,
33 and ellipsoidal to cylindrical conidia. The combined analysis of the LSU, ITS,

34 *RPB2* and *TEF1- α* loci supports the inclusion of *B. brunnescens*, *B. europaeus*,
35 *B. hominis* and *C. albus* in Plectosphaerellaceae.

36

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

39

40 **Introduction**

41 *Acremonium* accommodates saprobic species that can colonize diverse
42 substrates (Gams 1971, 1975; Domsch et al. 2007), important plant pathogens
43 (Alfaro-García et al. 1996; Lin et al. 2004) or agents of opportunistic infections
44 in humans (Summerbell 2003; Guarro 2012; de Hoog et al. 2015). Species-level
45 identifications in *Acremonium* is difficult on the basis of morphological
46 characters because their asexual structures are poorly differentiated. Molecular
47 phylogenetic analyses have demonstrated that the genus is polyphyletic.
48 Recent phylogenetic studies have shown that *Acremonium* species cluster in
49 different lineages throughout the Ascomycota, mainly in the Sordariomycetes
50 (Glenn et al. 1996; Zare et al. 2007; Schoch et al. 2009; Gräfenhan et al. 2011;
51 Perdomo et al. 2011; Summerbell et al. 2011; Giraldo et al. 2012, 2015).
52 Epitypification of the type species of the genus, *A. alternatum*, linked
53 *Acremonium sensu stricto* to the Bionectriaceae (Hypocreales) (Summerbell et
54 al. 2011), which also accommodates several other, partly teleomorphically
55 typified genera with an *acremonium*-like anamorph, such as *Emericellopsis*,
56 *Hapsidospora*, *Nigrosabulum*, *Bulbithecium* and *Mycoarachis* (Gams 1971;
57 Summerbell et al. 2011). Other species of *Acremonium* are distantly related to
58 the type species of the genus and belong to the Glomerellales or other families
59 of the Hypocreales (Zare et al. 2007; Gräfenhan et al. 2011; Carlucci et al.
60 2012; Giraldo et al. 2012; Grum-Grzhimaylo 2013a; Maharachchikumbura et al.
61 2015, 2016; Lombard et al. 2015).

62 In a previous study on *Acremonium* species from clinical samples in USA
63 (Perdomo et al. 2011), some of the isolates distributed in different groups within
64 the Hypocreales (informally named groups J and N) and Plectosphaerellaceae
65 (groups Q and R), could not be identified. The taxonomy of those isolates and
66 of other *Acremonium* species in Plectosphaerellaceae was resolved in the

67 present study by using multilocus DNA sequence analyses and phenotypic
68 methods.

69

70 **Materials and methods**

71

72 **Fungal isolates and sequences**

73 The fungi included in this study are shown in Table 1. Six clinical isolates
74 provided by the Fungus Testing Laboratory at the University of Texas Health
75 Science Center (UTHSC) were tentatively identified as *A. hyalinulum* and
76 previously linked to Hypocreales or Plectosphaerellaceae (Perdomo et al.
77 2011). In addition, one *Acremonium* isolate (FMR 11785) obtained from soil with
78 the procedure described in Giraldo et al. (2012), and five ex-type or reference
79 strains provided by the CBS-KNAW Fungal Biodiversity Centre (CBS) were also
80 included in our study. Numerous DNA sequences of *Acremonium* species and
81 related genera reported in different studies (Sigler et al. 2004; Zuccaro et al.
82 2004; Zare et al. 2007; Summerbell et al. 2011; Carlucci et al. 2012; Grum-
83 Grzhimaylo et al. 2013a,b, 2016; Giraldo et al. 2014) were retrieved from public
84 databases (Table 2) and included in the phylogenetic analyses.

85

86 **Phenotypic studies**

87 Morphological features were examined on potato dextrose agar (PDA;
88 Pronadisa, Madrid, Spain) and oatmeal agar (OA; filtered oat flakes after 1 h of
89 simmering, 30 g; agar, 20 g; distilled water to final volume of 1 000 mL).
90 Cultures were incubated at 25 °C in the dark for 4 wk. Colony diameters were
91 measured after 14 days of incubation and the colony colour rated after
92 Kornerup and Wanscher (1978). Microscopic features were examined and
93 measured from cultures grown on OA under an Olympus CH-2 light microscope
94 (Olympus Corporation, Tokyo, Japan) from direct wet mounts with either 85 %
95 lactic acid or Shear's solution, or from slide cultures. At least 30 randomly
96 selected elements were measured for each structure using an ocular
97 micrometer. Photomicrographs were obtained with a Zeiss Axio-Imager M1 light
98 microscope (Zeiss, Oberkochen, Germany), using phase contrast and Nomarski
99 differential interference. The ability of the fungi to grow at 4, 12, 15, 20, 25, 30,
100 32, 35, 37 and 40 °C was determined on PDA in duplicate.

101

102 **DNA extraction, amplification and sequencing**

103 Total genomic DNA was extracted from fresh colonies using PrepMan Ultra
104 Sample Preparation Reagent (Applied Biosystems, Foster City, CA, USA),
105 following the manufacturer's protocol. The DNA was quantified using a
106 NanoDrop 3000 fluorospectrometer (Thermo Scientific, Asheville, NC, USA).
107 The internal transcribed spacer (ITS) regions and the 5' end of the 28S nrDNA
108 gene (LSU) were amplified and sequenced with the primer pairs ITS5/ITS4
109 (White et al. 1990) and LR0R/LR5 (Vilgalys and Hester 1990; Vilgalys and Sun
110 1994), respectively. Fragments of the translation elongation factor 1-alpha
111 (*TEF1- α*), RNA polymerase II second largest subunit (*RPB2*) and β -tubulin
112 (*BT2*) genes were amplified with the following primer sets: EF 983F/EF 2218R
113 (Rehner and Buckley 2005) for *TEF1- α* , RPB2-5F/RPB2-7R (Liu et al. 1999) for
114 *RPB2* and Bt1a/Bt1b (Glass and Donaldson 1995) for *BT2* using PCR protocols
115 described elsewhere (Zuccaro et al. 2004; Grum-Grhimaylo et al. 2013a). PCR
116 products were purified and sequenced at Macrogen Europe (Amsterdam, The
117 Netherlands) with the same primers used for amplification. The program
118 SeqMan v. 7.0.0 (DNASTAR, Madison, WI, USA) was used to obtain consensus
119 sequences of each isolate.

120

121 **Phylogenetic analysis**

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

135 and Nearest-Neighbor-Interchange (NNI). The internal branch support was
136 assessed by a search of 1000 bootstrapped data sets. A bootstrap support (BS)
137 ≥ 70 was considered as statistically significant. Phylogenetic distance values
138 among isolates were estimated with Kimura 2-parameter as nucleotide
139 substitution model under the same software. A second phylogenetic
140 reconstruction via Bayesian inference (BI) was done using MrBayes v. 3.2.1
141 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012). Markov chain Monte
142 Carlo (MCMC) sampling was performed with two simultaneous runs for 3 million
143 generations, with samples taken every 100 generations. Bayesian posterior
144 probabilities (PP) were obtained from the 50 % majority-rule consensus tree
145 after removing the first 25 % of the collected trees. A PP value ≥ 0.95 was
146 considered statistically significant. The best nucleotide substitution model for
147 each gene in the Bayesian analysis (GTR+G+I) was determined using
148 MrModelTest v. 2.3 (Nylander 2004). Congruency of the sequence datasets for
149 the separate loci were determined using tree topologies of 70 % reciprocal
150 Neighbour-Joining (NJ) bootstrap trees with Maximum Likelihood distances for
151 identifying topology conflict visually (Gueidan et al. 2007). Because no
152 incongruence was observed, the different matrices were combined in the final
153 phylogenetic analyses. All novel DNA sequences were deposited in GenBank
154 (Table 1), the alignments and the resulting trees in TreeBASE
155 (<http://www.treebase.org>), and taxonomic novelties in MycoBank
156 (<http://www.MycoBank.org>; Crous et al. 2004).

157

158 **Results**

159

160 The phylogenetic analysis based on LSU sequences of the isolates UTHSC 08-
161 2284 (group N), UTHSC 08-3639 (group J) and FMR 11785 together with
162 hypocrealean *Acremonium* species and related genera reported by Summerbell
163 et al. (2011) is shown in the Figure 1. The final tree is based on 75 aligned
164 sequences, 847 characters including gaps, of which 580 were conserved, 267
165 were variable and 195 were phylogenetically informative. Tamura-Nei with
166 gamma distribution (TN+G) and the general time reversible with gamma
167 distribution and a portion of invariable sites (GTR+G+I) were found as the best-

168 fit nucleotide substitution models for ML and BI, respectively. The phylogenetic
169 tree revealed that the isolates clustered in the “*Emericellopsis*” and “*fusidioides*”
170 clades (Bionectriaceae) as defined by Summerbell et al. (2011). The isolates
171 UTHSC 08-2284 and FMR 11785 fell into the *Emericellopsis* clade (BS = 98 %,
172 PP = 1.00) together with the type species of *Emericellopsis*, *E. terricola* (CBS
173 120.40), *A. exuviarum* (UAMH 9995), *A. fuci* (CBS 113889) and *A. salmoneum*
174 (CBS 721.71). The two mentioned unidentified isolates showed identical
175 sequences and were grouped in a highly supported subclade (*Acremonium* sp.
176 I). The isolate UTHSC 08-3639 (*Acremonium* sp. II) was represented by a
177 single branch, phylogenetically related (BS = 83 %, PP = 1.0) to *A. fusidioides*,
178 *A. hennebertii* and the recently described species *A. citrinum*, *A. parvum* and *A.*
179 *pilosum* (Giraldo et al. 2014), all belonging to the *fusidioides* clade (Fig. 1).

180 To better resolve the phylogenetic relationships obtained from the LSU
181 analyses of *Acremonium* sp. I and *Acremonium* sp. II, a multilocus study was
182 performed for each of the unidentified species and their respective closely
183 related species. The first one (Fig. 2) was based on ITS, *BT2*, *RPB2* and *TEF1-*
184 *α* sequences and targeted the two isolates of *Acremonium* sp. I, members of the
185 *Emericellopsis* clade, and additional species previously reported to be related to
186 *Emericellopsis* (Sigler et al. 2004; Zuccaro et al. 2004, Grum-Grzhimaylo et al.
187 2013b) such as *Stanjemonium grisellum*, *S. ochroroseum* and *Acremonium*
188 *potronii*. The data set included 48 sequences of different strains and 2807
189 characters (2004 conserved, 803 variables and 604 phylogenetically
190 informative). *Verrucostoma freycinetiae* and *Selinia pulchra* were used as
191 outgroup. Tamura 3-parameter with gamma distribution (T92+G) and GTR+G+I
192 were found to be the best nucleotide substitution models for ML and BI,
193 respectively. The trees generated by using ML and BI had a similar topology.
194 The phylogenetic tree was consistent with previously reported phylogenies
195 (Sigler et al. 2004; Zuccaro et al. 2004; Grum-Grzhimaylo et al. 2013b). The two
196 isolates of *Acremonium* sp. I formed a highly supported basal clade (BS = 84 %,
197 PP = 1.00), distant from the species of *Acremonium*, *Emericellopsis* and
198 *Stanjemonium*. *Acremonium* sp. I is described below as a new species, named
199 *Acremonium moniliforme*.

200 The other analysis (Fig. 3) included a combination of the ITS and LSU
201 sequences of *Acremonium* sp. II and the ex-type and reference strains of the

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

212 The phylogenetic reconstruction using the LSU, ITS, *TEF1- α* and *RPB2* loci
213 from the clinical isolates of the groups Q (UTHSC 06-415 and UTHSC R-3853)
214 and R (UTHSC 06-874 and UTHSC 08-3693), and other representative species
215 of the Plectosphaerellaceae (Fig. 4) is based on a combined dataset consisting
216 of 3271 characters, including 805 phylogenetically informative positions (135
217 LSU, 151 ITS, 158 *TEF1- α* and 361 *RPB2*), and 39 strains or taxa including the
218 outgroup *Colletotrichum orbiculare* and *C. lagerarium*. The best-fit nucleotide
219 substitution model for ML and BI analysis was K2+G. The phylogenetic tree
220 showed that the isolates UTHSC 06-874 and UTHSC 08-3693 clustered in
221 *Chordomyces* (Grum-Grzhimaylo et al. 2016). While UTHSC 08-3693 was
222 grouped with the ex-type strain of *C. antarcticus* (BS = 85 %; PP = 0.98),
223 UTHSC 06-874 clustered with strain CBS 987.87 in a separated subclade (BS =
224 86 %; PP = 0.98). Because the last two isolates were phylogenetically distant
225 from the clade of *C. antarcticum*, the new species *Chordomyces albus* is
226 proposed. In the same analysis, a monophyletic group (BS = 94 %; PP = 1.00)
227 was formed by all isolates of the group R, two reference strains of *A. hyalinulum*
228 formed a well-supported clade (BS = 98 %; PP = 1.00), two clinical isolates
229 (UTHSC R-3853 and UTHSC 06-415) clustered together with high support (BS
230 = 97 %; PP = 1.00), while the ex-type strain of *A. brunnescens* formed a single
231 branch. This novel lineage of acremonium-like fungi within the
232 Plectosphaerellaceae is proposed as a new genus, *Brunneomyces* based on *A.*
233 *brunnescens*, and the two new species, *B. hominis* and *B. europaeus*.

234

235 **Taxonomy**

236

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

238 [MB 811461] Fig. 5

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

241 *Colonies* at 25 °C after 14 days on OA reaching 10–11 mm diam, white (1A1)
242 to yellowish white (4A2), flat, with scarce aerial mycelium, reverse colourless; at
243 25 °C after 14 days on PDA reaching 14–15 mm diam, pinkish white (7A2), flat,
244 cottony, reverse orange (6A6). *Mycelium* consisting of hyaline, smooth- and
245 thin-walled, 1.5–2 µm wide hyphae. *Conidiophores* erect, usually reduced to
246 single phialides emerging from vegetative hyphae, occasionally basitonously
247 branched and then bearing 2–4 phialides, straight, up to 60 µm long, hyaline,
248 smooth, with cell walls usually thicker than those of the vegetative hyphae.
249 *Phialides* subulate, 17–30(45) µm long, 1–1.5 µm wide at the base, hyaline,
250 thick- and smooth-walled, often borne on short cylindrical subtending cells;
251 percurrently proliferating phialides are occasionally present. *Conidia* arranged in
252 slimy heads, 1-celled, hyaline, of two types: i) cylindrical with more or less
253 rounded ends, 3–7 × 1–1.5 µm, thin- and smooth-walled; ii) ellipsoidal, 3–4 × 2–
254 3 µm, thick- and rough-walled. *Chlamydospores* and *sexual morph* not
255 observed.

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

258 *Specimen examined*. **USA**, Texas, from bronchoalveolar lavage fluid, 2008,
259 D.A. Sutton (holotype CBS H-22021, dried culture on OA; cultures ex-type CBS
260 139050 = FMR 10548 = UTHSC 08-3639).

261 *Notes*: Although *A. dimorphosporum* is phylogenetically distant to *A.*
262 *borodinense*, it is morphologically similar to that species in producing both
263 ellipsoidal rough-walled and cylindrical smooth-walled conidia (Ito et al. 2000).
264 However, *A. borodinense* differs from *A. dimorphosporum* by its faster growth at
265 25 °C (27–29 mm diam. after 10 d) and by its ability to grow at 37 °C.
266 Furthermore, its cylindrical conidia are slightly curved and smaller (4.5–5.5 µm
267 long), and its ellipsoidal and rough-walled conidia are larger (4.2–5.5 × 3–4 µm)
268 than those of *A. dimorphosporum*.

269

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

271 [MB 811462] Fig. 6

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

273 Colonies at 25 °C after 14 days on OA reaching 45–60 mm diam, yellowish
274 white (4A2), flat, glabrous; reverse colourless; at 25 °C after 14 days on PDA
275 reaching 36–50 mm diam, pinkish white (7A2), radially folded, zonate towards
276 the periphery, felty, greyish red (7B4); reverse salmon (6A4). *Mycelium*
277 consisting of branched, septate, hyaline, smooth- and thick-walled hyphae,
278 initially 2–2.5 µm wide, often swelling at maturity, becoming barrel-shaped, up
279 to 7 µm wide. *Conidiophores* reduced to conidiogenous cells, emerging laterally
280 from hyphae. *Phialides* acicular with a slightly flexuose apex, 30–50 µm long,
281 1.5–2 µm wide at the base, with a distinct periclinal thickening, thick- and
282 smooth-walled, hyaline. *Conidia* arranged in slimy heads, 1-celled, cylindrical
283 with rounded ends, 3–5(6) × 1–2 µm, hyaline, thick- and smooth-walled.
284 *Chlamydospores* and *sexual morph* not observed.

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

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

292 *Notes*: *Acremonium moniliforme* is phylogenetically distant from the species
293 of the *Emericellopsis* clade. It can be morphologically differentiated from other
294 *Acremonium* species by the production of abundant moniliform hyphae.
295 *Acremonium fuci* occasionally produces small rounded hyphal swellings, similar
296 to the moniliform hyphae of *A. moniliforme*. However, both species can be
297 distinguished by the conidial shape, which is obovoid or broadly ellipsoidal in
298 the former, and cylindrical in the latter. Additionally, the maximum temperature
299 for growth in *A. fuci* is 33 °C (Zuccaro et al. 2004), while in *A. moniliforme* it is
300 37 °C.

301

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

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

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

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

317

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

320 Basionym. *Acremonium brunnescens* W. Gams, Trans. Br. Mycol. Soc., 64:
321 398. 1975.

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

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

332 A detailed description of *B. brunnescens* was given in Gams (1975). Most
333 relevant features of the ex-type strain of this species (CBS 559.73) studied here
334 were: slow growing colonies (6–8 mm and 21–22 mm diam. after 14 d on PDA
335 and OA, respectively) with a mushroom-like odour, pigmented verrucose
336 hyphae and dark brown conidia appearing after 21 days, phialides with short

337 cylindrical and slightly pigmented collarettes, adelophialides of 6–10 × 1.5–2.5
338 µm, and conidial chains often collapsing soon in slimy heads. In addition, this
339 fungus was unable to grow above 32 °C.

340

341 ***Brunneomyces hominis*** Giraldo, Deanna A. Sutton & Gené, **sp. nov.**
342 [MB811473] Fig. 8

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

345 *Colonies* at 25 °C after 14 days on OA reaching 26–28 mm diam, orange
346 white (6A2), flat, dusty; reverse colourless; at 25 °C after 14 days on PDA
347 reaching 17–18 mm diam, grey (5F1) at the centre, yellowish white (4A2) at the
348 periphery, crateriform and radially folded, felty; reverse grey (5F1). Strong
349 mushroom-like (moist soil) odour. *Mycelium* consisting of septate, hyaline,
350 smooth- and thin-walled hyphae, 1.5–2 µm wide at the beginning, becoming
351 dark brown, verrucose and thick-walled, up to 3 µm wide with age.
352 *Conidiophores* erect, mostly unbranched, occasionally with a few branches and
353 proliferating sympodially, straight or slightly bent, up to 35 µm long, hyaline,
354 smooth-walled. *Phialides* subulate, 12–20(30) µm long, 1.5–2 µm wide at the
355 base, hyaline at first, dark brown in old cultures, thick- and smooth-walled, with
356 conspicuous periclinal thickening and cylindrical collarettes; adelophialides
357 sometimes present, up to 10 µm long; polyphialides with up to two
358 conidiogenous loci commonly present. *Conidia* arranged in long dry chains, 1-
359 celled, pyriform or ellipsoidal, 4–5(6) × 2–2.5 µm, with truncate base, subhyaline,
360 thin- and smooth-walled. *Chlamydospores* and *sexual morph* not observed.

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

363 *Specimens examined*. **USA**, Minnesota, from human sputum, 2006, D.A.
364 Sutton (holotype CBS H-22023, dried culture on OA; cultures ex-type CBS
365 139053 = FMR 10429 = UTHSC 06-415). California, from human sputum, D.A.
366 Sutton (CBS 139054 = FMR 10437 = UTHSC R-3853).

367 *Notes*: The colourless colony reverse on OA distinguishes *B. hominis* from
368 the dark grey reverse in *B. brunnescens*. *Brunneomyces hominis* produces
369 long, dry conidial chains, while in *B. europaeus* and *B. brunnescens* chains tend

370 to collapse in slimy heads. It is the only *Brunneomyces* species able to grow at
371 35 °C.

372

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

374 Fig. 9

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

376 *Colonies* at 25 °C after 14 days on OA reaching 31–50 mm diam, yellowish
377 white (4A2), flat, dusty; reverse colourless; at 25 °C after 14 days on PDA
378 reaching 25–36 mm diam, greyish brown (6E2) at the centre, white (1A1) to
379 orange-white (6A2) towards the periphery, radially folded, felty; reverse brown
380 (6E2). Slight mushroom-like (moist soil) odour. *Mycelium* consisting of septate,
381 hyaline, smooth- and thin-walled hyphae, 2–2.5 µm wide, becoming brownish,
382 verrucose and thick-walled with age. *Conidiophores* erect, usually unbranched,
383 some proliferating sympodially, up to 45 µm long, straight or slightly bent,
384 hyaline to subhyaline, smooth-walled. *Phialides* subulate or somewhat
385 cylindrical, 15–35(40) µm long, 2–3 µm wide at the base, hyaline, thick- and
386 smooth-walled, with a distinct periclinal thickening at the conidiogenous locus
387 and short cylindrical collarettes; adelophialides sometimes present, up to 15 µm
388 long; polyphialides with up to three conidiogenous loci commonly present.
389 *Conidia* forming chains that soon collapse in slimy heads, 1-celled, ovoidal to
390 ellipsoidal, 5–6(7) × 2–3 µm, with a distinctly truncate base, subhyaline, thin-
391 and smooth-walled. *Chlamydospores* and *sexual morph* not observed.

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

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

398 *Notes*: The two isolates of *B. europaeus* were previously identified wrongly
399 as *A. hyalinulum* because they form brownish pigmented and verrucose
400 hyphae, sympodially proliferating conidiophores and intercalary phialides that all
401 are not mentioned in the protologue of *A. hyalinulum* (Gams 1971). The latter
402 was described with hyaline smooth-walled hyphae, and lacking adelophialides.
403 However, there is no ex-type strain of *A. hyalinulum* for a reliable comparison

404 and, according to different studies, it seems to be a polyphyletic species
405 (Perdomo et al. 2011; Summerbell et al. 2011).

406

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

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

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

421

422 ***Chordomyces albus*** Giraldo, Deanna A. Sutton & Guarro, **sp. nov.** [MB
423 811476] Fig. 10

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

425 *Colonies* at 25 °C after 14 days on OA reaching 40–41 mm diam, yellowish
426 white (4A2), flat, dusty; reverse colourless; at 25 °C after 14 days on PDA
427 reaching 10–11 mm diam, pale yellow (4A3), raised, cerebriform; reverse
428 colourless. *Mycelium* consisting of septate, hyaline, smooth- and thin-walled
429 hyphae, 1.5–2 µm wide. *Conidiophores* erect, unbranched, consisting of one or
430 two cells, or with branches from near the middle bearing 3–4 phialides, up to 30
431 µm long, straight or slightly curved, hyaline to subhyaline, smooth-walled.
432 *Synnemata* absent. *Phialides* cylindrical or subulate, 12–22 µm long, 2–2.5 µm
433 wide at the base, with a distinct periclinal thickening at the conidiogenous locus
434 and cylindrical collarettes, occasionally with a percurrent proliferation, hyaline,
435 thick- and smooth-walled, sometimes with a second conidiogenous locus
436 emerging laterally as an up to 5 µm long cylindrical projection near the basal
437 septum. *Conidia* in slimy heads, 1-celled, ellipsoidal to near cylindrical, 3–4 × 2–

438 2.5 µm, subhyaline, thick- and smooth-walled. *Chlamydoconidia* and *sexual*
439 *morph* not observed.

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

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

446 *Notes*: The genus *Chordomyces* was recently proposed by Grum-Grzhimaylo
447 et al. (2016) to accommodate *C. antarcticus*. Most isolates of *C. antarcticus* but
448 CBS 987.87 derived from soda soil and were alkalitolerant. Strain CBS 987.87
449 was previously wrongly identified as *A. antarcticum*; however, it differs from the
450 protologue of that species in the presence of schizophialides with conspicuous
451 cylindrical collarettes and percurrent conidiophores (Spegazzini 1910;
452 Hawksworth 1979). Furthermore, its DNA sequences differ significantly from *C.*
453 *antarcticus* strains in Grum-Grzhimaylo et al. (2016). In the present study, CBS
454 987.87 and the clinical isolate UTHSC 06-874 have been found to be
455 morphologically and genetically similar and, since they formed a novel lineage
456 into the *Chordomyces* clade, they are proposed as a second species in the
457 genus. *Chordomyces albus* morphologically differs from *C. antarcticus* in the
458 absence of synnemata in culture, in having a faster growth on OA at 25 °C (40–
459 41 mm vs. 22–28 mm in 14 days), shorter phialides (12–22 µm long vs. 28–30
460 µm long) and conidia without a protuberant hilum (Grum-Grzhimaylo et al.
461 2016).

462

463 **Discussion**

464

465 Two bionectriaceous species are newly described. *Acremonium dimorphosporum*
466 is phylogenetically related to species of the *fusidioides* clade (Summerbell et al.
467 2011; Giraldo et al. 2014). Conidial dimorphism seen also in other species of
468 the *fusidioides* clade supports this phylogenetic inference, although no conidial
469 chains but slimy masses were observed in *A. dimorphosporum* (Gams 1971; Ito
470 et al. 2000; Giraldo et al. 2012, 2014). *Acremonium moniliforme* is
471 phylogenetically closely related to species of the *Emericellopsis* clade, a well-

472 defined monophyletic group within the Bionectriaceae accomodating also the
473 type species of the synnematos genus *Stilbella*, *S. fimetaria* (Seifert 1985), the
474 type species of *Stanjemonium*, *S. grisellum* (Gams et al. 1998), and
475 *Acremonium* species, such as *A. tubakii*, *A. fuci*, *A. exuviarum* and *A.*
476 *salmonium* (Sigler et al. 2004; Zuccaro et al. 2004; Summerbell et al. 2011;
477 Grum-Grzhimaylo et al. 2013b). Members of this clade are commonly isolated
478 from soil, dung, marine water, and occasionally from animal lesions (Sigler et al.
479 2004; de Hoog et al. 2015; Grum-Grzhimaylo et al. 2013b). The origin of the two
480 strains of *A. moniliforme*, i.e. human nail from USA and soil from Spain,
481 suggests that it is a widespread species as other species of this group.

482 Phylogenetic analyses of concatenated sequences from four loci support the
483 monophyly of *Brunneomyces* and places the genus in the
484 Plectosphaerellaceae. This family was introduced by Gams, Summerbell and
485 Zare (Zare et al. 2007) and recently assigned to the Glomerellales
486 (Maharachchikumbura et al. 2016). Currently, it comprises nine genera, i.e.
487 *Acrostalagmus*, *Gibellulopsis*, *Lectera*, *Musicillium*, *Plectosphaerella*,
488 *Stachylidium*, *Verticillium sensu stricto* and the recently described *Chordomyces*
489 and *Sodiomyces* (Zare et al. 2007; Inderbitzin et al. 2011; Réblová et al. 2011;
490 Cannon et al. 2012; Grum-Grzhimaylo et al. 2013a, 2016). In addition,
491 *Gliocladium cibotii* and some *Acremonium* species, including *A. collariferum*, *A.*
492 *furcatum*, *A. nepalense*, *A. restrictum* and *A. stromaticum*, belong to this family
493 (Zare et al. 2007; Weisenborn et al. 2010; Carlucci et al. 2012). Morphologically,
494 species of *Acrostalagmus*, *Gibellulopsis*, *Musicillium*, *Stachylidium* and
495 *Verticillium* are mainly characterized by hyaline or light brown verticillate
496 conidiophores (Hughes 1951; Zare et al. 2007; Inderbitzin et al. 2011; Réblová
497 et al. 2011); *Lectera* produces brightly coloured sporodochia and brown setae
498 (Cannon et al. 2012); the asexual morphs of *Sodiomyces* and *Plectosphaerella*
499 have verticillate or penicillate conidiophores and septate conidia (Carlucci et al.
500 2012; Grum-Grzhimaylo et al. 2013a, 2016); *Chordomyces*, *G. cibotii* and
501 above-mentioned *Acremonium* species form mostly cylindrical or ellipsoidal
502 conidia arranged in slimy heads (Gams 1971, 1975; Zare et al. 2007;
503 Weisenborn et al. 2010; Grum-Grzhimaylo et al. 2016). By contrast, the species
504 of *Brunneomyces* are characterized by the production of sympodially
505 proliferating conidiophores, ovoidal or ellipsoidal conidia arranged in chains and

506 dark verruculose hyphae. Subglobose to oval conidia, conspicuous funnel-
507 shaped collarete and olive-brown chlamydospores distinguishes *A. collariferum*
508 from *Brunneomyces* although it also forms verruculose hyphae (Weisenborn et
509 al. 2010). In addition, *A. collariferum* did not produce conidial chains in any of
510 the culture media tested here.

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

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

521 The habitats of the members of Plectosphaerellaceae are quite diverse.
522 *Verticillium*, *Musicillium*, *Plectosphaerella* and *Lectera* are well-known
523 pathogens of different kinds of plants, including legumes, banana, cucurbits,
524 potatoes, and others (Cannon et al. 2012; Carlucci et al. 2012; Masudi and
525 Bonjar 2012; Hyde et al. 2014). *Acrostalagmus luteoalbus* has been reported
526 also as a fungicolous species (Gams et al. 2004); *Gibellulopsis nigrescens* and
527 most of the plectosphaerellaceous *Acremonium* species are soil-borne
528 saprobes (Gams 1975; Domsch et al. 2007; Zare et al. 2007). *Gibellulopsis*
529 *piscis* and *Plectosphaerella oratosquillae* have occasionally been reported as
530 pathogens of fish and shrimp, respectively (Batista and da Silva 1959; Duc et al.
531 2009), and some species such as *Stachylidium bicolor*, *Acremonium*
532 *alcalophilum*, *Chordomyces* and *Sodiomyces* species possess alkaliphilic or
533 alkalitolerant abilities (Grum-Grzhimaylo et al. 2013a,b; 2016). The species of
534 *Brunneomyces* and *Chordomyces* studied here seem to be saprotrophic; they
535 are typically recovered from plant debris. Although, the isolates included in *B.*
536 *hominis*, and some of *C. albus* and *C. antarcticus* are from human specimens
537 (human nails and sputum), their human-related pathogenic role is unknown.

538

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543 P.
544

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806 **Figure legends**

807

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

816

817 **Fig. 2** Maximum composite likelihood tree based on sequences of ITS and
818 partial protein encoding genes (*BT2*, *RPB2* and *TEF1- α*) of selected
819 acremonium-like taxa of the Bionectriaceae. Bootstrap support values above 70
820 % / Bayesian posterior probability values above 0.95, are shown at the nodes. [†],
821 Type strain

822

823 **Fig. 3** Maximum composite likelihood tree based on sequences of ITS and
824 partial LSU genes from *A. dimorphosporum* and species of the *fusidioides*
825 clade. Bootstrap support values above 70 % / Bayesian posterior probability
826 values above 0.95, are shown at the nodes. [†], Type strain

827

828 **Fig. 4** Maximum composite likelihood tree based on analysis of ITS and partial
829 LSU, *RPB2* and *TEF1- α* sequences of genera of the Plectosphaerellaceae and
830 related genera. Bootstrap support values above 70 % / Bayesian posterior
831 probability values above 0.95, are shown at the nodes. [†], Type strain

832

833 **Fig. 5** *Acremonium dimorphosporum* UTHSC 08-3639. **a** Colonies on OA, after
834 21 days at 25 °C. **b, c** Simple conidiophores and conidia forming heads. **d, e**
835 Phialides with percurrent proliferation (arrow). **f–h** Smooth-walled, ellipsoidal (**f**)
836 and thick-walled, verrucose, ovoidal conidia (**h**). *Scale bars*: **b–f** = 10 μ m, **g, h** =
837 5 μ m

838

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

843

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

848

849 **Fig. 8** *Brunneomyces hominis* a–c, f, g UTHSC 06-415; d, e UTHSC R-3853. **a**
850 Colonies on OA after 14 days at 25 °C. **b, c** Colonies on PDA after 21 days at
851 25 °C obverse and reverse, respectively. **d** Unbranched conidiophores. **e**
852 Unbranched conidiophores with terminal polyphialides and a sympodial
853 conidiophore (arrow). **f** Pigmented verrucose hyphae and intercalary phialide
854 (arrow). **g** Conidia. *Scale bars* = 10 µm

855

856 **Fig. 9** *Brunneomyces europaeus* a–c, f–h, k, l CBS 560.86; d, e, i, j CBS
857 652.96. **a** Colonies on OA after 14 days at 25 °C. **b, c** Colonies on PDA after 14
858 days at 25 °C obverse and reverse, respectively. **d**. Phialide producing a long
859 conidial chain. **e** Pigmented verrucose hyphae. **f** Polyphialide. **g** Adelophialide.
860 **h** Sympodial conidiophore. **i, j** Phialides with short cylindrical collarettes. **k, l**
861 Conidia. *Scale bars* = 10 µm

862

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