

1 *Dioszegia antarctica* and *D. cryoxerica* spp. nov., two novel psychrophilic basidiomycetous
2 yeasts from polar desert soils in Antarctica

3

4 Laurie B. Connell¹, Regina Redman^{2,3}, Russel Rodriguez^{4,5}, Anne Barrett¹, Melissa Iszard¹,
5 and Álvaro Fonseca^{6*}

6

7 1 School of Marine Sciences, University of Maine, 5735 Hitchner Hall, Orono, ME 04469, USA

8 2 College of Forest Resources, University of Washington, Seattle, WA 98195, USA

9 3 Adaptive Symbiotic Technologies, Seattle WA 98125, USA

10 4 U.S. Geological Survey, Western Fisheries Research Center, Seattle, WA, 98115, USA

11 5 Department of Biology, University of Washington, Seattle, WA 98195

12 6 Centro de Recursos Microbiológicos (CREM), Departamento de Ciências da Vida, Faculdade de
13 Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

14

15 * Corresponding author:

16 Email: amrf@fct.unl.pt; tel.: +351 212948500; fax: +351 212948530

17

18 Running title: Novel *Dioszegia* species from Antarctica

19

20 Contents Category: New taxa (Eukaryotic Micro-organisms)

21

22

23 The GenBank accession number for the LSU rRNA gene (D1/D2 domains) of *Dioszegia*
24 *antarctica* (CBS 10920^T) is FJ640575, and those for the LSU rRNA gene (D1/D2 domains)
25 and ITS sequences of *D. cryoxerica* (CBS 10919^T) are FJ640562 and FJ640565, respectively.

26

27 **Summary**

28 During a survey of the culturable soil fungal population in samples collected in Taylor Valley,
29 South Victoria Land, Antarctica, thirteen basidiomycetous yeast strains with orange-coloured
30 colonies were isolated. Phylogenetic analyses of ITS and partial LSU rRNA gene sequences
31 showed that the strains belong to the *Dioszegia* clade of the Tremellales (Tremellomycetes,
32 Agaricomycotina), but did not correspond to any of the hitherto recognised species. Two
33 novel species, *Dioszegia antarctica* (CBS 10920^T = PYCC 5970^T) and *D. cryoxerica* (CBS
34 10919^T = PYCC 5967^T) are described to accommodate ten and three of those strains,
35 respectively. Analysis of ITS sequences demonstrated intra-strain sequence heterogeneity in
36 *D. cryoxerica*. The latter species is also notable for producing true hyphae with clamp
37 connections and haustoria. However, no sexual structures were observed. The two novel
38 species can be considered obligate psychrophiles since they failed to grow above 20°C and
39 grew best between 10 and 15°C.

40

41 **Introduction**

42 South Victoria Land (Antarctica) habitats exhibit some of the most extreme conditions on
43 Earth, with very dry and cold locations, as well as some highly saline sites, with soils
44 characterised by low moisture and very low organic content (e.g., Connell *et al.*, 2008). Yet a
45 variety of fungi, including yeasts, were cultured from (Connell *et al.*, 2006, 2008; Vishniac,
46 2006a) or detected in (Fell *et al.*, 2006) these soils. During a sampling campaign in the 2003–
47 2004 austral summer season (November 2003–January 2004) several strains of two putative
48 novel species of *Dioszegia* (named *Dioszegia* sp. 1 and *Dioszegia* sp. 2) were isolated from
49 soil samples in Taylor Valley (Connell *et al.*, 2008). The two taxa had been previously
50 detected by Connell *et al.* (2006) in the same region during the 2002-2003 field season. The
51 genus *Dioszegia* presently includes thirteen species, which give rise to conspicuously orange-

52 coloured colonies and may or may not produce ballistoconidia (Bai *et al.*, 2002, Inácio *et al.*,
53 2005, Takashima *et al.*, 2001, Wang *et al.*, 2003, 2008). All members of the genus form a
54 monophyletic clade (Dioszegia clade) in the Tremellales (Tremellomycetes,
55 Agaricomycotina) according to phylogenetic analyses of SSU and LSU (D1/D2) rRNA gene
56 sequences (e.g., Inácio *et al.*, 2005, Wang *et al.*, 2003, 2006). The majority of *Dioszegia*
57 species were isolated from plant leaves (Wang *et al.*, 2008), but some species may occur in
58 soils and roots (Renker *et al.*, 2004, Vishniac, 2006b) and one species, *D. statzelliae*, was
59 isolated from soil in Antarctica (Inácio *et al.*, 2005, Thomas-Hall *et al.*, 2002). Here we
60 present the descriptions of two novel species of *Dioszegia* from Antarctic soils, for which the
61 names *D. antarctica* (*Dioszegia* sp. 2) and *D. cryoxerica* (*Dioszegia* sp. 1) are proposed.

62

63 **Methods**

64 Details on strain isolation were given by Connell *et al.* (2008). The origin of the strains
65 studied is given in Table 1. Collection site data is given in Supplementary Table S1.
66 Phenotypic characterization was carried out according to Yarrow (1998) and included the
67 determination of growth in liquid YPD medium during three weeks at the following
68 temperatures: 4, 6, 8.5, 10, 12, 15, 18, 20, 25, 30, and 37°C. Physiological tests were
69 incubated at 14-16°C. Production of ballistoconidia and filamentous structures were tested on
70 Corn Meal Agar (CMA) and Malt extract 0.7%, Soytone 0.25%, Yeast extract 0,05% (MYP)
71 agar plates. Determination of siderophore production was carried out on solid agar plates
72 based on the O-CAS assay and associated color change as described by Pérez-Miranda *et al.*
73 (2007). Isolation of genomic DNA used MasterPure DNA extraction kit (Epicenter Inc.). A
74 ribosomal DNA fragment containing the Internal Transcribed Spacer (ITS) region, which
75 includes the ITS1 and ITS2 spacers and the 5.8S rRNA gene was PCR amplified and
76 sequenced using primers ITS5 (5' GGA AGT AAA AGT CGT AAC AAG G 3') and ITS4 (5'

77 TCC TCC GCT TAT TGA TAT GC 3') (White *et al.*, 1990) as previously described (Connell
78 *et al.*, 2006). The D1/D2 domains of the LSU rRNA gene were PCR amplified and sequenced
79 using primers FG1 (5' TGT TTG GGA ATG CAG CTC 3') and R635 (5' GGT CCG TGT
80 TTC AAG ACG G 3') (Fell *et al.*, 2000). Due to the finding of a few ambiguous positions in
81 ITS sequences of some strains, rDNA amplicons from those strains were cloned using the
82 TOPO® (Invitrogen) cloning kit as previously described (Connell *et al.*, 2006) and a few
83 random clones were selected for sequencing. With the sequences obtained BLAST searches in
84 GenBank were performed to find the closest matching sequences. Selected sequences from
85 each region were then aligned with the Clustal algorithm of MegAlign (DNASTAR Inc.,
86 Madison, WI, USA). Phylogenetic trees were computed with PAUP version 4.0b8 (Sinauer
87 Associates Inc., Sunderland, MA, USA) using the neighbour-joining method and the Kimura
88 two-parameter model for calculating distances. Gaps were treated as missing data. Newly
89 determined nucleotide sequences were deposited in GenBank under the accession numbers
90 FJ640562 to FJ640575 and FJ643481. Additional sequences were retrieved from GenBank
91 (accession numbers are indicated on the phylogenetic trees).

92

93 **Results and Discussion**

94 Phylogenetic analysis of partial LSU rRNA gene sequences (Supplementary Fig. 1)
95 demonstrated that the thirteen Antarctic isolates formed two groups in the Dioszegia clade of
96 the Tremellales, each containing strains with identical sequences: one group comprised
97 isolates ANT-03-012, 013, 015, 031, 037, 100, 111, 112, 114 and 116 (representing *Dioszegia*
98 *sp.*2, Connell *et al.* 2008), and the other contained the remaining three isolates ANT-03-071,
99 096 and 101 (*Dioszegia sp.*1) (Table 1). LSU sequences of *Dioszegia sp.*2 were identical to
100 that of the type strain of *D. fristingensis* (PYCC 5861) and had a few differences to other
101 isolates ascribed to the latter species (AS 2.2519, AS 2.2631, CRUB 1150, CRUB 1152).

102 *Dioszegia* sp.1 strains differed in LSU sequences from the type strain of *D. changbaiensis*
103 (AS 2.2309) at four nucleotide positions (three substitutions and one insertion). To further
104 ascertain the taxonomic status of the putative novel species of *Dioszegia*, the sequences of the
105 ITS region were subjected to phylogenetic analysis (Fig. 1). The ten strains of *Dioszegia* sp.2
106 formed a sister clade to *D. fristingensis* from which they differed at 16-17 nucleotide
107 positions and thus appear to represent a separate species. The ITS sequences of strains CBS
108 10624, 10637 and 10650 differed from the other seven strains by two nucleotide substitutions.
109 However, these differences may represent intraspecific variation since no other physiological
110 or ecological characteristics correlate with those differences. The ten strains of *Dioszegia* sp.2
111 can be thus accommodated in a novel species, for which the name *D. antarctica* is proposed.
112 The three strains of *Dioszegia* sp.1 once again formed a sister clade to *D. changbaiensis* and
113 although there were a few variable sites among the former (see below) they differed from the
114 latter species in at least 13 nucleotide positions, which confirms separation at the species
115 level. The three strains of *Dioszegia* sp.1 can be thus accommodated in a novel species, for
116 which the name *D. cryoxerica* is proposed.

117 Analysis of the ITS sequencing chromatograms of *D. cryoxerica* strains revealed up to
118 six positions for which the corresponding nucleotide could not be attributed unambiguously
119 due to the presence of overlapping peaks (data not shown). ITS amplicons from the three *D.*
120 *cryoxerica* strains were cloned prior to sequencing and at least six clones were selected for
121 sequencing. The resulting chromatograms had no ambiguities, but sequences differed at the
122 previously mentioned positions between clones from the same strain (data not shown). This
123 finding strongly suggests the existence of intra-strain ITS heterogeneity in the rDNA repeats.
124 This situation is apparently uncommon among fungi, but a few examples of intra-strain ITS
125 heterogeneity have been reported by other authors in the chytrid *Phytium helicoides*
126 (Kageyama *et al.*, 2007), the polypore genus *Ganoderma* (Wang & Yao, 2005) and the

127 basidiomycetous yeast *Phaffia rhodozyma* (Fell *et al.*, 2007). The present finding appears to
128 be restricted to *D. cryoxerica* and to the ITS region and was not detected in *D. antarctica*.

129 Another unique characteristic of *D. cryoxerica* was the production of abundant true
130 hyphae with clamp connections and haustoria by all three strains. However, no sexual
131 structures were ever observed on the hyphae, even after prolonged incubation periods on
132 CMA and MYP agar media at low temperature and upon transfer to water-agar. Elongated
133 conidiogenous cells that gave rise to large cylindrical conidia were frequently found, but no
134 structures resembling basidia. The clamped mycelium arose from single cells, apparently
135 without prior cell-cell conjugation. Mixing of the three strains in pairs yielded similar
136 structures. The presence of haustoria suggests a possible mycoparasitic habit for this taxon. A
137 similar situation has been reported in *Cryptococcus mycelialis*, another anamorphic
138 tremellaceous yeast, which is a member of the Holtemannia clade (Golubev & Golubev,
139 2003).

140 The two novel species share an obligately psychrophilic phenotype with *D. statzelliae*,
141 the only other *Dioszegia* species isolated in Antarctica, but from a different region (Davis
142 Base, Princess Elizabeth Land; Thomas-Hall *et al.*, 2002). The three taxa are unable to grow
143 at 25°C and optimal growth was observed between 10 and 15°C for the two novel species
144 (data not shown) and between 15 and 18°C for *D. statzelliae* (Thomas-Hall *et al.*, 2002). *D.*
145 *aurantiaca* and *D. fristingensis* are also unable to grow at 25°C (e.g., Inácio *et al.*, 2005), but
146 they are apparently not restricted to regions with cold climates since they were recently found
147 in subtropical regions of China by Wang *et al.* (2008).

148 The two novel species can be distinguished phenotypically by the ability of *D.*
149 *cryoxerica* to assimilate citric acid, saccharic acid and urea (Table 2). The two species also
150 differ in siderophore production, which is strong in *D. cryoxerica* but only slightly positive in
151 *D. antarctica*. Discrimination from other *Dioszegia* species is possible based on a

152 combination of phenotypic characteristics, namely the ability of *D. antarctica* and *D.*
153 *cryoxerica* to assimilate inulin and to grow without added vitamins, and their inability to grow
154 at 25°C and to produce ballistoconidia (Table 3). *D. antarctica* differs from the closest
155 relative, *D. fristingensis*, in the assimilation of inulin and in the growth without added
156 vitamins (Table 3). *D. cryoxerica* differs from the closest relative, *D. changbaiensis*, in the
157 assimilation of inulin and inositol, and the growth at 25°C (Table 3).

158

159 **Latin diagnoses and standard descriptions**

160 Latin diagnosis of *Dioszegia antarctica* L. Connell, R. Redman, R. Rodriguez et Á. Fonseca,
161 sp. nov.

162 Status teleomorphosis incognitus. In medio liquido “YM” post 4 dies ad 14°C, cellulae
163 ellipsoidae, 6.2-8.2 x 3.7-4.5 µm. Cultura in agar “MYP” post dies 7 ad 16°C aurantiaca,
164 glabra, nitida, butyracea, margine integro. Ballistoconidia nullae. Mycelium et
165 pseudomycelium non formatur. In tabula “Table 2” characteres biochemices physiologicesque
166 declarates sunt. Characteres moleculares (culturae typi): sequentiae acidi nucleici ‘rDNA 26S
167 (D1/D2)’, FJ640575, et ‘rDNA ITS’, DQ402529, in collectione sequentiarum acidi nucleici
168 “NCBI (GenBank)” depositae sunt. Typus: ANT-03-116^T isolatus ex terra, prope Taylor Valley
169 in Antarctica, praeservatus in collectione zymotica “Centraalbureau voor Schimmelcultures”
170 (CBS), Hollandia (CBS 10920^T) et in collectione zymotica lusitanica (PYCC 5970^T).

171

172 Description of *Dioszegia antarctica* L. Connell, R. Redman, R. Rodriguez et Á. Fonseca, sp.
173 nov.

174 *Dioszegia antarctica* (L. fem. adj., *antarctica*, of the south polar region; referring to the origin
175 of the isolates)

176 Teleomorph: unknown. In YM broth, after 4 days at 14°C, cells are mainly ellipsoidal, 6.2-8.2
177 x 3.7-4.5 µm (Fig. 2). On MYP agar, after 7 days at 16°C, colonies are dark orange, glossy,
178 smooth, butyrous, with entire margins. Ballistoconidia are not produced. Hyphae or
179 pseudohyphae are not formed, but ramified chains of slightly elongated cells may be present.
180 Physiological and biochemical characteristics are listed in Table 2. Molecular characteristics
181 (type strain): nucleotide sequences of the D1/D2 domains of the LSU rRNA gene (FJ640575),
182 and of the ITS region (DQ402529) were deposited in NCBI (GenBank). Deposits: strain ANT-
183 03-116^T, isolated from soil in Taylor Valley, Antarctica, and designated as type culture, was
184 deposited in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS
185 10920^T) and in the Portuguese Yeast Culture Collection, Caparica, Portugal (PYCC 5970^T);
186 nine additional isolates from the same origin also belong to this species (Table 1).

187

188 Latin diagnosis of *Dioszegia cryoxerica* L. Connell, R. Redman, R. Rodriguez et Á. Fonseca,
189 sp. nov.

190 Status teleomorphosis incognitus. In medio liquido “YM” post 4 dies ad 14 °C, cellulae
191 cylindratae, 11.2-13.9 x 3.3-4.2 µm. Cultura in agaro “MYP” post dies 7 ad 16°C aurantiaca,
192 glabra, nitida, butyracea, margine integro. Ballistoconidia nullae. Mycelium formatum, septis
193 fibulatis. In tabula (Table 2) characteres biochemici physiologici declarati sunt.

194 Characteres moleculares (culturae typi): sequentiae acidi nucleici ‘rDNA 26S (D1/D2)’,
195 FJ640562, et ‘rDNA ITS’, FJ640565, in collectione sequentiarum acidi nucleici NCBI
196 (GenBank) depositae sunt. Typus: ANT-03-071^T isolatus ex musco, prope Taylor Valley in
197 Antarctica, praeservatus in collectione zymotica “Centraalbureau voor Schimmelcultures”
198 (CBS), Hollandia (CBS 10919^T) et in collectione zymotica lusitana (PYCC 5967^T).

199

200 Description of *Dioszegia cryoxerica* L. Connell, R. Redman, R. Rodriguez et Á. Fonseca, sp.
201 nov.
202 *Dioszegia cryoxerica* (Gr. masc. n., *cryos*, cold; Gr. adj., *xeros*, dry; N.L. fem. adj.,
203 *cryoxerica*, referring to the cold and dry environment of the sampling site where the species
204 was isolated)
205 Teleomorph: unknown. In YM broth, after 4 days at 14°C, cells are mainly cylindrical, 11.2-
206 13.9 x 3.3-4.2 µm (Fig. 3a). On MYP agar, after 7 days at 16°C, colonies are orange, glossy,
207 smooth, butyrous, with entire margins. Ballistoconidia are not produced. Septate hyphae with
208 clamp connections are formed from single cells (Fig. 3b). Haustoria and conidiogenous cells
209 may develop from clamp connections (Fig. 3c,d). Physiological and biochemical
210 characteristics are listed in Table 2. Molecular characteristics (type strain): nucleotide
211 sequences of the D1/D2 domains of the LSU rRNA gene (FJ640562), and of the ITS region
212 (FJ640565) were deposited in NCBI (GenBank). Deposits: strain ANT-03-116^T, isolated from
213 moss in Taylor Valley, Antarctica, and designated as type culture, was deposited in the
214 Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS 10920^T) and in the
215 Portuguese Yeast Culture Collection, Caparica, Portugal (PYCC 5967^T); two additional
216 isolates from soil in the same region also belong to this species (Table 1).

217

218 **Acknowledgements**

219 B. Schulz and A. Stoyles (TEA participant) for field work; Raytheon Polar Support Service,
220 UNAVCO, and PHI for logistical and laboratory support while in Antarctica. Funding was
221 provided for this project by NSF Office of Polar Programs to LC and RR (OPP-0125611) and
222 by USGS (RR).). The use of trade, firm, or corporation names in this publication is for the
223 information and convenience of the reader. Such use does not constitute an official

224 endorsement or approval by the U.S. Department of Interior or the U.S. Geological Survey of
225 any product or service to the exclusion of others that may be suitable.

226

227 **References**

228 - **Bai, F.Y., Takashima, M., Jia, J.H. & Nakase, T.** (2002). *Dioszegia zsolttii* sp. nov., a new
229 ballistoconidium-forming yeast species with two varieties. *J Gen Appl Microbiol* **48**, 17–23.

230 - **Connell, L.B., Redman, R., Craig, S.D. & Rodriguez, R.** (2006). Distribution and
231 abundance of fungi in the soils of Taylor Valley, Antarctica. *Soil Biol Biochem* **38**, 3083–
232 3094.

233 - **Connell, L.B., Redman, R., Craig, S.D., Scorzetti, G., Iszard, M. & Rodriguez, R.**
234 (2008). Diversity of Soil Yeasts Isolated from South Victoria Land, Antarctica. *Microb Ecol*
235 **56**, 448–459.

236 - **Fell, J.W., Boekhout, T., Fonseca, Á., Scorzetti, G. & Stätzell-Tallman, A.** (2000).
237 Biodiversity and systematics of basidiomycetous yeasts as determined by large subunit rDNA
238 D1/D2 domain sequence analysis. *Int J Syst Evol Microbiol* **50**, 1351–1371.

239 - **Fell, J.W., Scorzetti, G., Connell, L. & Craig, S.** (2006). Biodiversity of micro-eukaryotes
240 in Antarctic Dry Valley soils with < 5% soil moisture. *Soil Biol Biochem* **38**, 3107–3119.

241 - **Fell, J.W., Scorzetti, G., Stätzell-Tallman, A. & Boundy-Mills, K.** (2007). Molecular
242 diversity and intragenomic variability in the yeast genus *Xanthophyllomyces*: the origin of
243 *Phaffia rhodozyma*. *FEMS Yeast Res* **7**, 1399–1408.

244 - **Golubev, W.I. & Golubev, N.W.** (2003). A new basidiomycetous yeast species,
245 *Cryptococcus mycelialis*, related to *Holtermannia* Saccardo et Traverso. *Microbiology*
246 (Moscow) **72**, 728–732.

247 - **Inácio, J., Portugal, L., Spencer-Martins, I. & Fonseca, Á.** (2005). Phylloplane yeasts
248 from Portugal: seven novel anamorphic species in the Tremellales lineage of the

249 Hymenomycetes (Basidiomycota) producing orange-coloured colonies. *FEMS Yeast Res* **5**,
250 1167–1183.

251 - **Kageyama, K., Senda, M., Asano, T., Suga, H. & Ishiguro, K.** (2007). Intra-isolate
252 heterogeneity of the ITS region of rDNA in *Pythium helicoides*. *Mycological Res* **111**, 416–
253 423.

254 - **Pérez-Miranda, S., Cabirol, N., George-Télez, R., Zamudio-Rivera, L.S. & Fernández,**
255 **F.J.** (2007). O-CAS, a fast and universal method for siderophore detection. *J Microbiological*
256 *Methods* **70**, 127-131.

257 - **Renker, C., Blanke, V., Börstler, B., Heinrichs, J. & Buscot, F.** (2004). Diversity of
258 *Cryptococcus* and *Dioszegia* yeasts (Basidiomycota) inhabiting arbuscular mycorrhizal roots
259 or spores. *FEMS Yeast Res* **4**, 597-603.

260 - **Takashima, M., Deak, T. & Nakase, T.** (2001). Emendation of *Dioszegia* with
261 redescription of *Dioszegia hungarica* and two new combinations, *Dioszegia aurantiaca* and
262 *Dioszegia crocea*. *J Gen Appl Microbiol* **47**, 75–84.

263 - **Thomas-Hall, S., Watson, K. & Scorzetti, G.** (2002). *Cryptococcus statzelliae* sp. nov. and
264 three novel strains of *Cryptococcus victoriae*, yeasts isolated from Antarctic soils. *Int J Syst*
265 *Evol Microbiol* **52**, 2303–2308.

266 - **Vishniac, H.S.** (2006a). Yeast biodiversity in the Antarctic. In: G. Peter & C.A. Rosa (Eds.),
267 *Biodiversity and Ecophysiology of Yeasts*. Springer-Verlag, Berlin, pp. 428-440.

268 - **Vishniac, H.S.** (2006b). A multivariate analysis of soil yeasts isolated from a latitudinal
269 gradient. *Microbial Ecol* **52**, 90–103.

270 - **Wang, D.M. & Yao, Y.J.** (2005). Intrastrain internal transcribed spacer heterogeneity in
271 *Ganoderma* species. *Can J Microbiol* **51**, 113–121.

272 - **Wang, Q.M., Bai, F.Y., Zhao, J.H. & Jia, J.H.** (2003). *Dioszegia changbaiensis* sp. nov., a
273 basidiomycetous yeast species isolated from northeast China. *J Gen Appl Microbiol* **49**, 295–
274 299.

275 - **Wang, Q.M., Jia, J.H. & Bai, F.Y.** (2008). Diversity of basidiomycetous phylloplane
276 yeasts belonging to the genus *Dioszegia* (Tremellales) and description of *Dioszegia athyri* sp.
277 nov., *Dioszegia butyracea* sp. nov. and *Dioszegia xingshanensis* sp. nov. *Antonie van*
278 *Leeuwenhoek* **93**, 391–399.

279 - **White, T.J., Bruns, T., Lee, S. & Taylor, J.W.** (1990). Amplification and direct
280 sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: A Guide to
281 Methods and Applications, M. Innis, et al., Eds, Academic Press: Orlando, FL. Pp. 315-322.

282 - **Yarrow, D.** (1998). Methods for the isolation, maintenance, and identification of yeasts. In:
283 *The Yeasts: A taxonomic study* (Fourth ed.), edited by Kurtzman CP and Fell JW. Amsterdam:
284 Elsevier, p. 77-100.

285

286

287 Table 1 – List of cultures used in this study
 288

Species	Isolate*	Strain†	Origin‡
<i>Dioszegia antarctica</i> sp. nov.	ANT-03-116	CBS 10920 ^T , PYCC 5970	Soil, Taylor Valley (03 YB3)
	ANT-03-012	CBS 10613	Soil, Taylor Valley (03 YB2)
	ANT-03-013	CBS 10750, PYCC 5966	Soil, Taylor Valley (03 T30)
	ANT-03-015	CBS 10615	Soil, Taylor Valley (03 YB3)
	ANT-03-031	CBS 10635	Soil, Taylor Valley (03 YB2)
	ANT-03-037	CBS 10637	Soil, Taylor Valley (03 CW2)
	ANT-03-100	CBS 10624	Soil, Taylor Valley (03 YB1)
	ANT-03-111	CBS 10626	Soil, Taylor Valley (03 YB3)
	ANT-03-112	CBS 10627	Soil, Taylor Valley (03 YB3)
	ANT-03-114	CBS 10767	Soil, Taylor Valley (03 YB3)
	<i>Dioszegia cryoxerica</i> sp. nov.	ANT-03-071	CBS 10919 ^T , PYCC 5967
ANT-03-096		CBS 10623, PYCC 5968	Soil, Taylor Valley (03 CW2)
ANT-03-101		CBS 10921, PYCC 5969	Soil, Taylor Valley (03 YB1)

289

290 * Original strain numbers of the isolates, Connell *et al.* (2008).

291 † Strains deposited in culture collections: CBS, Centraalbureau voor Schimmelcultures (The
 292 Netherlands); PYCC, Portuguese Yeast Culture Collection (Portugal); T, type strain.

293 ‡ Collection site designation between parentheses; site data provided in Supplementary Table
 294 S1 and in Connell *et al.* (2006) and Connell *et al.* (2008).

295

296

Table 2 – Physiological/biochemical test responses of the newly proposed species

Test Responses *	<i>Dioszegia antarctica</i>	<i>Dioszegia cryoxerica</i>
C-sources:		
D-Glucose	+	+
D-Galactose	+	+
L-Sorbose	V	+
D-Glucosamine	V	V
D-Ribose	+	+
D-Xylose	+	+
L-Arabinose	+	+
D-Arabinose	+,D	+
L-Rhamnose	V	+
Sucrose	+	+
Maltose	+	+
α,α Trehalose	+	+
Methyl- α -D-glucoside	+	+
Cellobiose	+	+
Salicin	ND	ND
Melibiose	+,W,D	+
Lactose	+	+
Raffinose	+	+
Melezitose	+	+
Inulin	+	+
Soluble starch	-	V
Glycerol	+,D	+
Erythritol	V	V
Ribitol	V	+
Xylitol	ND	ND
D-Glucitol	+	+
D-Mannitol	+	+
Galactitol	+	+
<i>myo</i> -Inositol	V	+
D-Gluconic acid	+	+
D-Glucuronic acid	+	+
DL-Lactic acid	V	+
Succinic acid	V	+
Citric acid	-	+
Methanol	-	-
Ethanol	V	+
n-Hexadecane	V	+
Saccharic acid	-	+
N-sources:		
Nitrate	-	-
Nitrite	+	+
Ethylamine	V	-
L-lysine	-	-
Urea	-	+
Other tests:		
Growth in vitamin-free medium	+	+
Growth in 10%NaCl+5%Glucose	V	+
Growth on 50%Glucose	V	W
Formation of starch-like compounds	+	+
Hydrolysis of urea	-	+
Colour reaction with Diazonium Blue B	+	+
Growth at 20°C	D	D
Growth at 25°C	-	-

* Test results: +, positive; D, delayed positive; W, weak; -, negative; V, variable; ND, not determined

298
299
300
301

302 Table 3 – Discriminating phenotypic characteristics of *Dioszegia* spp.*
 303

Species	Melibiose	Inulin	Ribitol	Glucitol	Inositol	Erythritol	Vitamin-free medium	Growth at 25°C	Ballistocomidia	Mycelium
<i>D. antarctica</i>	+	+	V	+	V	V	+	-	-	-
<i>D. cryoxerica</i>	+	+	+	+	+	V	+	-	-	+
<i>D. aurantiaca</i>	+	-	-	+	-	-	-	-	+	-
<i>D. athyrii</i>	+	w/D	-	w/D	-	-	-	+	+	-
<i>D. buhagiarii</i>	-	-	D	D	-	-	+	+	-	-
<i>D. butyracea</i>	+	+	-	-	-	-	-	+	+	-
<i>D. catarinonii</i>	+	-	-	-	-/D	-	-	+	v	-
<i>D. changbaiensis</i>	+	-	-	D	-	-	+	+	-	-
<i>D. crocea</i>	+	-	+	+	-	-	-	+	+	-
<i>D. fristingensis</i>	+	-	D	+	-	-	-	-	+	-
<i>D. hungarica</i>	-	-	-	+	+/D	-/D	-	+	v	-
<i>D. statzelliae</i>	W	-	W	+	W	-	-	-	-	-
<i>D. takashimae</i>	+	-	-	-	-/D	-	-	+	+	-
<i>D. xingshanensis</i>	+	-	-	-	W	-	w/D	+	+	-
<i>D. zsoldii</i>	+	-	-	-	-/D	-	-	+	+	-

304
 305 * Data from: a. Inácio *et al.* (2005); Wang *et al.* (2008); Wang *et al.* (2003) or obtained in the
 306 present study for the newly proposed species (in bold); test results as in Table 2.
 307
 308

309 Figure legends

310

311 Fig. 1 – Phylogenetic tree of strains of *Dioszegia antarctica* and *D. cryoxerica* and closely
312 related species in the *Dioszegia* clade (Tremellales, Tremellomycetes, Agaricomycotina)
313 obtained by neighbour-joining analysis of ITS sequences using PAUP 4.0b8. The numbers
314 given on the branches are the frequencies (>50%) with which a given branch appeared in
315 1000 bootstrap replications. Members of the *D. catarinonii* subclade were used as outgroup.
316 Sequences determined by the authors of the present study are typed in boldface. Additional
317 sequences were retrieved from GenBank (species names followed by the corresponding strain
318 number and accession number between parentheses).

319

320 Fig. 2 – *Dioszegia antarctica* CBS 10920^T: yeast cells in YM broth, after 4 days at 14°C. Bar
321 = 10 µm.

322

323 Fig. 3 – *Dioszegia cryoxerica* CBS 10919^T: a. yeast cells in YM broth, after 4 days at 14°C; b.
324 mycelium with clamp connections (arrows) on CMA, after 1 week at 16°C; c. mycelium with
325 clamp connections (arrows) and an hastorium (H) on CMA, after 2 weeks at 16°C; d.
326 conidiogenous cell (arrow) developing from a clamp connection on CMA, after 2 weeks at
327 16°C. Bar = 10 µm.

328

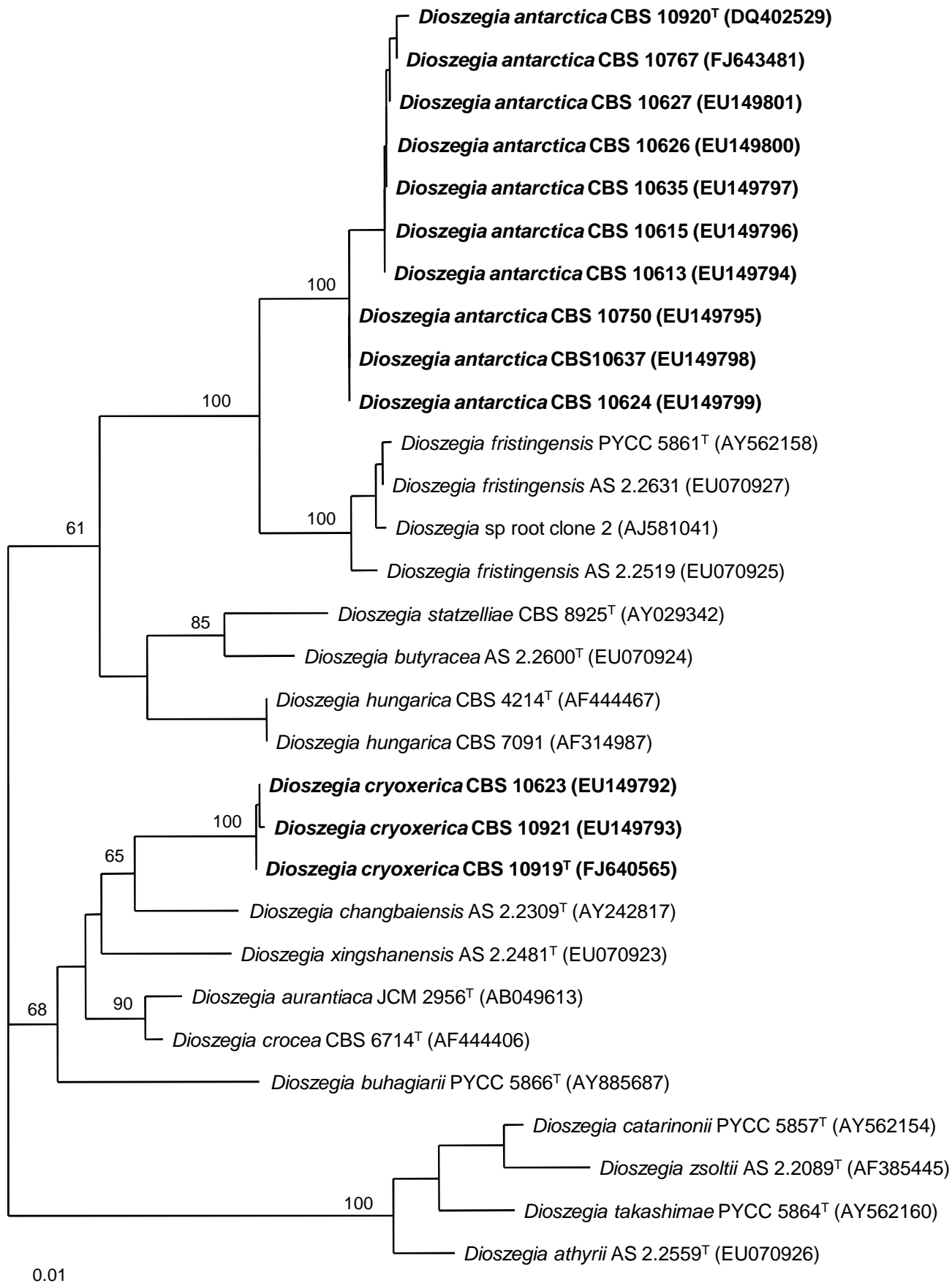
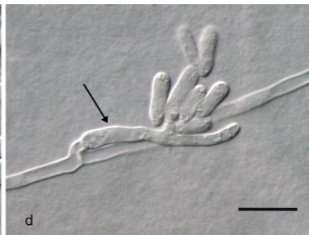
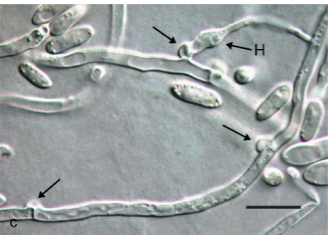


Fig. 1





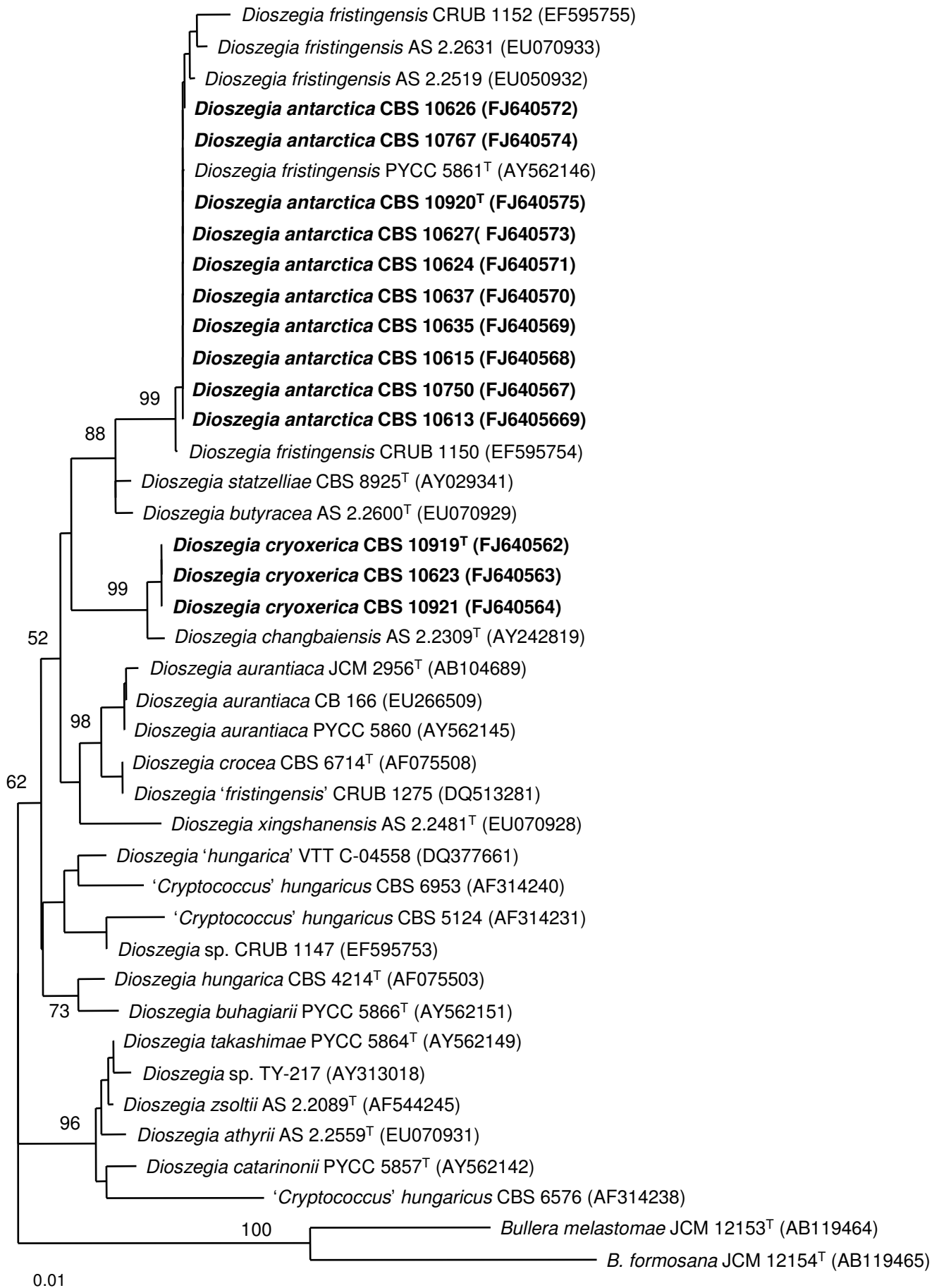


Fig. S1

1 Supplementary data:

2

3 Table S1 - Location and soil properties of sites in South Victoria Land. Isolates found at each
4 site are listed by number in Table 1.

5

Site	Longitude (east)	Latitude (south)	Elevation (m)	pH	Conductivity (mS.cm ⁻¹)	Salinity (ppt)	Moisture (%)
03 CW moss	163.37012	-77.57180	25	n/a	n/a	n/a	23.5
03 CW2	163.33315	-77.58864	26	9.4	503	0.3	5.3
03 T30	163.3677	-77.5600	130	10.1	414	0.2	3.8
03 YB1	163.35742	-77.62176	125	9.9	119	0.1	3.5
03 YB2	163.36777	-77.62318	140	9.6	105	0.1	3.6
03 YB3	163.37953	-77.62327	175	9.9	161	0.1	3.5

6

7

8

9 Fig. S1 - Phylogenetic tree of strains of *Dioszegia antarctica* and *D. cryoxerica* and related
10 species in the *Dioszegia* clade (Tremellales, Tremellomycetes, Agaricomycotina) obtained by
11 neighbour-joining analysis of LSU rRNA gene (D1/D2 domains) sequences using PAUP
12 4.0b8. The numbers given on the branches are the frequencies (>50%) with which a given
13 branch appeared in 1000 bootstrap replications. *Bullera formosana* and *B. melastomae* were
14 used as outgroup. Sequences determined by the authors of the present study are typed in
15 boldface. Additional sequences were retrieved from GenBank (species names followed by the
16 corresponding strain number and accession number between parentheses).

17