Dioszegia antarctica sp. nov. and *Dioszegia cryoxerica* sp. nov., psychrophilic basidiomycetous yeasts from polar desert soils in Antarctica

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During a survey of the culturable soil fungal population in samples collected in Taylor Valley, South Victoria Land, Antarctica, 13 basidiomycetous yeast strains with orange-coloured colonies were isolated. Phylogenetic analyses of internal transcribed spacer (ITS) and partial LSU rRNA gene sequences showed that the strains belong to the *Dioszegia* clade of the Tremellales (Tremellomycetes, Agaricomycotina), but did not correspond to any of the hitherto recognized species. Two novel species, *Dioszegia antarctica* sp. nov. (type strain ANT-03-116^T = CBS 10920^T = PYCC 5970^T) and *Dioszegia cryoxerica* sp. nov. (type strain ANT-03-071^T = CBS 10919^T = PYCC 5967^T), are described to accommodate ten and three of these strains, respectively. Analysis of ITS sequences demonstrated intrastrain sequence heterogeneity in *D. cryoxerica*. The latter species is also notable for producing true hyphae with clamp connections and haustoria. However, no sexual structures were observed. The two novel species can be considered obligate psychrophiles, since they failed to grow above 20 °C and grew best between 10 and 15 °C.

South Victoria Land (Antarctica) exhibits some of the most extreme conditions on Earth, with very dry and cold locations, as well as some highly saline sites, with soils characterized by low moisture and very low organic content (e.g. Connell *et al.*, 2008). Yet a variety of fungi, including yeasts, have been cultured from (Connell *et al.*, 2006, 2008; Vishniac, 2006a) or detected in (Fell *et al.*, 2006) these soils. During a sampling campaign in the 2003–2004 austral summer (November 2003–January 2004), several strains of two putative novel species of *Dioszegia*

(named *Dioszegia* sp. 1 and *Dioszegia* sp. 2) were isolated from soil samples in Taylor Valley (Connell *et al.*, 2008). The two taxa had been detected previously by Connell *et al.* (2006) in the same region during the 2002–2003 field season. The genus *Dioszegia* currently includes 13 species, which give rise to conspicuously orange-coloured colonies and may or may not produce ballistoconidia (Bai *et al.*, 2002; Inácio *et al.*, 2005; Takashima *et al.*, 2001; Wang *et al.*, 2003, 2008). All members of the genus form a monophyletic group (the *Dioszegia* clade) in the Tremellales (Tremellomycetes, Agaricomycotina) according to phylogenetic analyses of SSU and LSU (D1/D2) rRNA gene sequences (e.g. Inácio *et al.*, 2005; Wang *et al.*, 2003, 2008). The majority of *Dioszegia* species were isolated from plant leaves (Wang *et al.*, 2008), but some species may occur in soils and roots (Renker *et al.*, 2004; Vishniac, 2006b) and one species, *Dioszegia statzelliae*, was isolated from soil in Antarctica (Inácio *et al.*, 2005; Thomas-Hall *et al.*, 2002). Here, we present the descriptions of two novel species of *Dioszegia* from Antarctic soils.

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the LSU rRNA gene D1/D2 domain sequences of strains ANT-03-116^T and ANT-03-071^T are respectively FJ640575 and FJ640562 and those for the ITS sequences of ANT-03-116^T and ANT-03-071^T are DQ402529 and FJ640565.

Details of sampling sites and a neighbour-joining tree based on D1/D2 sequences are available as supplementary material with the online version of this paper.

Details on strain isolation were given by Connell *et al.* (2008). The origin of the strains studied is given in Table 1. Collection site data are given in Supplementary Table S1, available in IJSEM Online. Phenotypic characterization was carried out according to Yarrow (1998) and included the determination of growth in liquid YPD medium up to 3 weeks at 4, 6, 8.5, 10, 12, 15, 18, 20, 25, 30 and 37 °C. Physiological tests were incubated at 14–16 °C. Production of ballistoconidia and filamentous structures were tested on cornmeal agar (CMA) and MYP (0.7 % malt extract, 0.25 % soytone, 0.05 % yeast extract) agar plates. Determination of siderophore production was carried out on solid agar plates based on the O-CAS assay and associated colour change as described by Pérez-Miranda *et al.* (2007). Isolation of genomic DNA used a MasterPure DNA extraction kit (Epicenter Inc.). An rRNA gene fragment containing the internal transcribed spacer (ITS) region, which includes the ITS1 and ITS2 spacers and the 5.8S rRNA gene, was amplified by PCR and sequenced using primers ITS5 (5'-GGAAGTAAAAGTCGTAACA-AGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990) as described previously (Connell *et al.*, 2006). The D1/D2 domains of the LSU rRNA gene were amplified by PCR and sequenced using primers FG1 (5'-TGTTTGGGAATGCAGCTC-3') and R635 (5'-GGTC-CGTGTTTCAAGACGG-3') (Fell *et al.*, 2000). Because a few ambiguous positions were found in the ITS sequences of some strains, rRNA gene amplicons from those strains were cloned using the TOPO cloning kit (Invitrogen) as described previously (Connell *et al.*, 2006) and a few random clones were selected for sequencing. With the sequences obtained, BLAST searches in GenBank were performed to find the closest matching sequences. Selected sequences from each region were then aligned

with the CLUSTAL algorithm of MEGALIGN (DNASTAR Inc.). Phylogenetic trees were computed with PAUP version 4.0b8 (Sinauer Associates) using the neighbour-joining method and Kimura's two-parameter model for calculating distances. Gaps were treated as missing data. Additional sequences were retrieved from GenBank (accession numbers are indicated on the phylogenetic trees).

Phylogenetic analysis of partial LSU rRNA gene sequences (Supplementary Fig. S1) demonstrated that the 13 Antarctic isolates formed two groups in the *Dioszegia* clade of the Tremellales, each containing strains with identical sequences: one group comprised isolates ANT-03-012, -013, -015, -031, -037, -100, -111, -112, -114 and -116 (representing *Dioszegia* sp. 2; Connell *et al.*, 2008) and the other contained the remaining three isolates, ANT-03-071, -096 and -101 (*Dioszegia* sp. 1) (Table 1). LSU rRNA gene sequences of *Dioszegia* sp. 2 were identical to that of the type strain of *Dioszegia fristingensis* (PYCC 5861^T) and had a few differences from those of other isolates ascribed to the latter species (AS 2.2519, AS 2.2631, CRUB 1150 and CRUB 1152). *Dioszegia* sp. 1 strains differed in LSU sequences from the type strain of *Dioszegia changbaiensis* (AS 2.2309^T) at four nucleotide positions (three substitutions and one insertion). To further ascertain the taxonomic status of the putative novel species of *Dioszegia*, the sequences of the ITS region were subjected to phylogenetic analysis (Fig. 1). The ten strains of *Dioszegia* sp. 2 formed a sister clade to *D. fristingensis* from which they differed at 16–17 nucleotide positions and thus they appear to represent a separate species. The ITS sequences of strains ANT-03-013, -037 and -100 differed from the other seven strains by two nucleotide substitutions. However, these differences may represent intraspe-

Table 1. Cultures used in this study

Isolate names correspond to the original strain names from Connell *et al.* (2008). Strains have been deposited in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS), and in some cases in the Portuguese Yeast Culture Collection, Caparica, Portugal (PYCC). Data on collection sites are provided in Supplementary Table S1 and by Connell *et al.* (2006, 2008).

Isolate	Other strain names	Origin
<i>Dioszegia antarctica</i> sp. nov.		
ANT-03-116 ^T	CBS 10920 ^T , PYCC 5970 ^T	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 ^T	CBS 10919 ^T , PYCC 5967 ^T	Moss, Taylor Valley (03 CW moss)
ANT-03-096	CBS 10623, PYCC 5968	Soil, Taylor Valley (03 CW2)
ANT-03-101	CBS 10921, PYCC 5969	Soil, Taylor Valley (03 YB1)

Analysis of the ITS sequencing chromatograms of *D. cryoxerica* strains revealed up to six positions for which the corresponding nucleotide could not be attributed unambiguously because of the presence of overlapping peaks (not shown). ITS amplicons from the three *D. cryoxerica* strains were cloned prior to sequencing and at least six clones were selected for sequencing. The resulting chromatograms had no ambiguities, but sequences differed at the previously mentioned positions between clones from the same strain (not shown). This finding strongly suggests the existence of intrastrain ITS heterogeneity in the rRNA gene repeats. This situation is apparently uncommon among fungi, but a few examples of intrastrain ITS heterogeneity have been reported by other authors in the chytrid *Pythium helicoides* (Kageyama *et al.*, 2007), the polypore genus *Ganoderma* (Wang & Yao, 2005) and the basidiomycetous yeast *Phaffia rhodozyma* (Fell *et al.*, 2007). The present finding appears to be restricted to *D. cryoxerica* and to the ITS region, and was not detected in *D. antarctica*.

and haustoria by all three strains. However, no sexual structures were ever observed on the hyphae, even after prolonged incubation periods on CMA and MYP agar media at low temperature and upon transfer to water agar. Elongated conidiogenous cells that gave rise to large cylindrical conidia were frequently found, but no structures resembling basidia. The clamped mycelium arose from single cells, apparently without prior cell–cell conjugation. Mixing of the three strains in pairs yielded similar structures. The presence of haustoria suggests a possible mycoparasitic habit for this taxon. A similar situation has been reported in *Cryptococcus mycelialis*, another anamorphic tremellaceous yeast, which is a member of the *Holtemannia* clade (Golubev & Golubev, 2003).

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

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

+, Positive; D, delayed positive; w, weak; –, negative; v, variable. Utilization of salicin and xylitol as sole carbon sources was not determined.

Test	<i>D. antarctica</i>	<i>D. cryoxerica</i>
Utilization of carbon sources		
D-Glucose, D-galactose, D-ribose, D-xylose, L-arabinose, sucrose, maltose, α,α -trehalose, methyl α -D-glucoside, cellobiose, lactose, raffinose, melezitose, inulin	+	+
L-Sorbose, L-rhamnose	v	+
D-Glucosamine	v	v
D-Arabinose	+, D	+
Melibiose	+, w, D	+
Soluble starch	–	v
Glycerol	+, D	+
Erythritol	v	v
Ribitol, myo-inositol	v	+
D-Glucitol, D-mannitol, galactitol	+	+
D-Gluconic acid, D-glucuronic acid	+	+
DL-Lactic acid, succinic acid	v	+
Citric acid, saccharic acid	–	+
Methanol	–	–
Ethanol, n-hexadecane	v	+
Utilization of nitrogen sources		
Nitrate	–	–
Nitrite	+	+
Ethylamine	v	–
L-Lysine	–	–
Urea	–	+
Other tests		
Growth in vitamin-free medium	+	+
Growth in 10 % NaCl plus 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	–	–

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

Latin diagnosis of *Dioszegia antarctica* Connell, Redman, Rodriguez et Fonseca sp. nov.

Status teleomorphosis incognitus. In medio liquido YM post 4 dies ad 14 °C, cellulae ellipsoideae, 6.2–8.2 × 3.7–4.5 µm. Cultura in agar MYP post dies 7 ad 16 °C aurantiaca, glabra, nitida, butyracea, margine integro. Ballistoconidia nullae. Mycelium et pseudomycelium non formatur. In Table

2 characteres biochemices physiologicesque declarates sunt. Characteres moleculares (culturae typi): sequentiae acidi nucleici rRNA gene 26S (D1/D2), FJ640575, et rRNA gene ITS, DQ402529, in collectione sequentiarum acidi nucleici NCBI (GenBank) depositae sunt. Typus: ANT-03-116^T isolatus ex terra, prope Taylor Valley in Antarctica, praeservatus in collectione zymotica Centraalbureau voor Schimmelcultures (CBS), Hollandia (CBS 10920^T) et in collectione zymotica lusitanica (PYCC 5970^T).

Description of *Dioszegia antarctica* Connell, Redman, Rodriguez et Fonseca sp. nov.

Dioszegia antarctica (an.tarc'ti.ca. L. fem. adj. *antarctica* southern, referring to the south polar region, the origin of the first isolates).

Teleomorph: unknown. In YM broth, after 4 days at 14 °C, cells are mainly ellipsoidal, 6.2–8.2 × 3.7–4.5 µm (Fig. 2).

Table 3. Discriminating phenotypic characteristics of *Dioszegia* species

Species: 1, *D. antarctica* sp. nov.; 2, *D. cryoxerica* sp. nov.; 3, *D. athyri*; 4, *D. aurantiaca*; 5, *D. buhagiarii*; 6, *D. butyracea*; 7, *D. catarinonii*; 8, *D. changbaiensis*; 9, *D. crocea*; 10, *D. fristingensis*; 11, *D. hungarica*; 12, *D. statzelliae*; 13, *D. takashimae*; 14, *D. xingshanensis*; 15, *D. zsolitii*. Data were taken from Inácio *et al.* (2005) and Wang *et al.* (2003, 2008) or obtained in the present study for the newly proposed species; test results are scored as outlined in Table 2.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Utilization of:															
Melibiose	+	+	+	+	–	+	+	+	+	+	–	w	+	+	+
Inulin	+	+	w/D	–	–	+	–	–	–	–	–	–	–	–	–
Ribitol	v	+	–	–	D	–	–	–	+	D	–	w	–	–	–
Glucitol	+	+	w/D	+	D	–	–	D	+	+	+	+	–	–	–
Inositol	v	+	–	–	–	–	–/D	–	–	–	+/D	w	–/D	w	–/D
Erythritol	v	v	–	–	–	–	–	–	–	–	–/D	–	–	–	–
Growth in/at:															
Vitamin-free medium	+	+	–	–	+	–	–	+	–	–	–	–	–	w/D	–
25 °C	–	–	+	–	+	+	+	+	+	–	+	–	+	+	+
Formation of:															
Ballistoconidia	–	–	+	+	–	+	v	–	+	+	v	–	+	+	+
Mycelium	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–

On MYP agar, after 7 days at 16 °C, colonies are dark orange, glossy, smooth and butyrous with entire margins. Ballistoconidia are not produced. Hyphae or pseudohyphae are not formed, but ramified chains of slightly elongated cells may be present. Physiological and biochemical characteristics are listed in Table 2. Nucleotide sequences of the D1/D2 domains of the LSU rRNA gene (FJ640575) and of the ITS region (DQ402529) of the type strain have been deposited in GenBank/EMBL/DDBJ.

The type strain, ANT-03-116^T (=CBS 10920^T =PYCC 5970^T), was isolated from soil in Taylor Valley, Antarctica. Nine additional isolates from the same origin also belong to this species (Table 1).

Fig. 2. Yeast cells of *Dioszegia antarctica* sp. nov. ANT-03-116^T in YM broth after 4 days at 14 °C. Bar, 10 µm.

Latin diagnosis of *Dioszegia cryoxerica* Connell, Redman, Rodriguez et Fonseca sp. nov.

Status teleomorphosis incognitus. In medio liquido YM post 4 dies ad 14 °C, cellulae cylindraceae, 11.2–13.9 × 3.3–4.2 µm. Cultura in agaro MYP post dies 7 ad 16 °C aurantiaca, glabra, nitida, butyracea, margine integro. Ballistoconidia nullae. Mycelium formatur, septis fibulatis. In Table 2 characteres biochemices physiologicesque declarates sunt. Characteres moleculares (culturae typi): sequentiae acidi nucleici rRNA gene 26S (D1/D2), FJ640562, et rRNA gene ITS, FJ640565, in collectione sequentiarum acidi nucleici NCBI (GenBank) depositae sunt. Typus: ANT-03-071^T isolatus ex muscus, prope Taylor Valley in Antarctica, praeservatus in collectione zymotica Centraalbureau voor Schimmelcultures (CBS), Hollandia (CBS 10919^T) et in collectione zymotica lusitanica (PYCC 5967^T).

Description of *Dioszegia cryoxerica* Connell, Redman, Rodriguez et Fonseca sp. nov.

Dioszegia cryoxerica (cry.o.xe'ri.ca. Gr. masc. n. *cryos* cold; Gr. adj. *xeros* dry; L. fem. suff. *-ica* suffix used with the sense of pertaining to; N.L. fem. adj. *cryoxerica* cold and dry, referring to the cold and dry environment of the sampling site where the first strains were isolated).

Teleomorph: unknown. In YM broth, after 4 days at 14 °C, cells are mainly cylindrical, 11.2–13.9 × 3.3–4.2 µm (Fig. 3a). On MYP agar, after 7 days at 16 °C, colonies are orange, glossy, smooth and butyrous with entire margins. Ballistoconidia are not produced. Septate hyphae with clamp connections are formed from single cells (Fig. 3b). Haustoria and conidiogenous cells may develop from clamp connections (Fig. 3c, d). Physiological and biochemical characteristics are listed in Table 2. Nucleotide

Fig. 3. Micrographs of *Dioszegia cryoxerica* sp. nov. ANT-03-071^T. (a) Yeast cells in YM broth after 4 days at 14 °C; (b) mycelium with clamp connections (arrows) on CMA after 1 week at 16 °C; (c) mycelium with clamp connections (arrows) and an haustorium (H) on CMA after 2 weeks at 16 °C; (d) conidiogenous cell (arrow) developing from a clamp connection on CMA after 2 weeks at 16 °C. Bars, 10 µm.

sequences of the D1/D2 domains of the LSU rRNA gene (FJ640562) and of the ITS region (FJ640565) of the type strain have been deposited in GenBank/EMBL/DDBJ.

The type strain, ANT-03-116^T (=CBS 10920^T =PYCC 5967^T), was isolated from moss in Taylor Valley, Antarctica; two additional isolates from soil in the same region also belong to this species (Table 1).

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