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Drought meets acid: three new genera in a dothideallean clade of extremotolerant fungi

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Abstract: Fungal strains isolated from rocks and lichens collected in the Antarctic ice-free area of the Victoria Land, one of the coldest and driest habitats on earth, were found in two phylogenetically isolated positions within the subclass Dothideomycetidae. They are here reported as new genera and species, Recurvomyces mirabilis gen. nov., sp. nov. and Elasticomyces elasticus gen. nov., sp. nov. The nearest neighbours within the clades were other rock-inhabiting fungi from dry environments, either cold or hot. Plant-associated Mycosphaerella-like species, known as invaders of leathery leaves in semi-arid climates, are also phylogenetically related with the new taxa. The clusters are also related to the halophilic species Hortaea werneckii, as well as to acidophilic fungi. One of the latter, able to grow at pH 0, is Scytalidium acidophilum, which is ascribed here to the newly validated genus Acidomyces. The ecological implications of this finding are discussed.

Key words: Acidophilic fungi, Antarctica, black fungi, extremotolerance, halophilic fungi, ITS, lichens, phylogeny, rock-inhabiting fungi, SSU, taxonomy.

Taxonomic novelties: Recurvomyces Selbmann & de Hoog, gen. nov.; Recurvomyces mirabilis Selbmann & de Hoog, sp. nov.; Elasticomyces Zucconi & Selbmann, gen. nov.; Elasticomyces elasticus Zucconi & Selbmann, sp. nov.; Acidomyces Selbmann, de Hoog & De Leo, gen. nov.; Acidomyces acidophilus (Sigler & J.W. Carmich.) Selbmann, de Hoog & De Leo, comb. nov.

INTRODUCTION

Contrary to expectations, bare rocks in arid and semi-arid climates may harbour a bewildering biodiversity of black fungi. Many species have been reported from the Mediterranean basin (Sterflinger et al. 1997, Wollenzien et al. 1997, Bogomolova & Minter 2003, De Leo et al. 1999, 2003, Bills et al. 2004, Ruibal et al. 2005, Ruibal et al. 2008). These extremotolerant fungi live and even thrive on surfaces that are too harsh to support growth of competing microorganisms; they shelter in small depressions in the marble surface, called micropits (Sterflinger 1998). Similar extremotolerant fungi were discovered in the extremely cold and ice-free McMurdo Dry Valleys, a desert area in the Antarctic (Nienow & Friedmann 1993), where temperatures are only occasionally above zero, dropping to about –50 °C in winter. Onofri et al. (1999) and Selbmann et al. (2005) even reported on the existence of possibly endemic genera, Friedmanniomycetes Onofri and Cryomyces Selbmann, de Hoog, Mazzaglia, Friedmann & Onofri in these habitats, which apparently show active evolution under conditions of near-permanent frost and extreme dryness (Friedmann et al. 1987). These fungi may escape prohibitive environmental conditions by colonising air spaces in rocks, living in association with lichens and algae in cryptoendolithic communities (Friedmann & Ocampo 1976, Friedmann 1982).

In the present paper we describe three new fungal genera and species; their novelty is supported by molecular phylogeny, taking a clearly separate position within the Dothideomycetidae. One genus includes two strains isolated from rocks in the Antarctic desert, one strain from rocks collected in Monte Rosa in the Alps, Italy, and an unidentified rock fungus from Puebla de la Sierra, Spain; the other genus includes three strains isolated from different thalli of Antarctic lichens, one from cryptoendolithic Antarctic communities and one from rocks collected in Aconcagua in the Argentinian Andes. In contrast to most rock-inhabiting black fungi, which are generally scarcely differentiated, they show peculiar and distinguished morphological traits.

Fungi may also be encountered in extremely acidic environments. Some are able to grow at pH values down to pH 0 (Starkey & Waksman 1943, Harrison et al. 1966, Gould et al. 1974, Ivarson & Morita 1982, Gimmler et al. 2001). Sigler & Carmichael (1974) compared four strains from an acidic soil (pH 1.4–3.5) with the ones previously isolated by Starkey & Waksman (1943) and Ivarson & Morita (1982), referring them to the genus Scytalidium Pesante on the basis of scarcely differentiated brown arthroconidia. Our SSU and ITS comparison proved these fungi also to be members of a clade within the Dothideomycetidae, amidst rock-inhabiting fungi from cold and semi-arid climates.

MATERIALS AND METHODS

Strains

Black fungi were isolated from rock samples harbouring a cryptoendolithic lichen-dominated community and from epilithic lichens collected in different locations of Northern and Southern Victoria Land, Antarctica, in the framework of the Italian expedition
Table 1. List of strains studied.

| Species                                      | Strain no. | Source                                      | Geography                  | ITS         | Reference               |
|----------------------------------------------|------------|---------------------------------------------|----------------------------|-------------|-------------------------|
| Acidomyces acidophilum                      | CBS 335.97 | Acidophilic algae, Dunaliella acidophilica pH 1.0 | Germany                    | AJ244237    | Gimmler et al. 2001    |
| Acidomyces acidophilum (deposited as Scytalidium acidophilum) | CBS 270.74 T (ATCC 26772; UAMH 3460; IMI 193918) | Soil near acidic elemental sulphur pile pH 1.1 | Canada                    | –           | Sigler & Camichael 1974 |
| Acidomyces acidophilum (deposited as Boltnomyces caespitosus) | CBS 899.87 | Pyrite ore acidic drainage pH 2.0           | Germany                    | –           | –                       |
|                                               | dh 13081 = det 106/2003 | 2N Sulphuric acid pH 1                     | Denmark (supplied by GC Frisvad) | –           | Starkey & Waksman 1943   |
|                                               | dh 11526 = det 237-1999 | Volcanic soil                              | Iceland (supplied by S Gross, Berlin) | –           | –                       |
|                                               | dh 12881 = det 142-AF1 | –                                           | –                          | –           | –                       |
| Acidomyces sp.                               | dh 13119   | Acidic industrial process water pH 1.5      | Emmen, The Netherlands     | –           | –                       |
| Batchelromyces proteae                       | CBS 110996; CPC 1518 | Protea cynaroides                         | South Africa               | –           | Crous et al. 2007       |
| Capnobotryella renispora                    | CBS 214.90 T (CBS 176.88; IAM 13014; JCM 6332) | Capnobotrys neessii                     | Japan                      | –           | –                       |
| Catenulostroma abietis                       | CBS 290.90 | Man, skin lesion                            | The Netherlands            | –           | –                       |
|                                               | CBS 145.97 (dh 15369) | sandstone of cathedral                     | Zeitz, Germany             | –           | –                       |
|                                               | CBS 300.81 | Juniperus communis (Cupressaceae), needle   | Graubünden, Grüss, Switzerland | –           | –                       |
|                                               | CBS 279.86 | Kiel, Germany                               | –                          | –           | –                       |
|                                               | dh 12687 = det 396/2001 | Painted wall                              | –                          | –           | –                       |
|                                               | TRN 128    | Limestone                                   | Mallorca                   | AY559363    | –                       |
|                                               | dh 12697 = det 373/2001 RMF N113 | Desert soil                              | Namibia                    | –           | –                       |
|                                               | dh 13593   | See snail                                   | Italy                      | –           | –                       |
|                                               | CBS 618.84 | Ilex sp., leaf                              | Germany                    | AY128696    | –                       |
|                                               | CBS 118756 (TRN 127; dh 14531) | Limestone                                | Cala San Vincenc, Mallorca | AY559362    | –                       |
| Catenulostroma elginense                     | CBS 11030 (CPC 1958) | Protea grandiceps                          | South Africa               | AY206093    | Crous et al. 2007       |
| Catenulostroma germanicum                    | CBS 530.88 | Stone                                       | Germany, former West-Germany | EU019253    | Crous et al. 2007       |
| Catenulostroma macowanii                    | CBS 110756 (CPC 1872) | Protea nitida                              | South Africa               | –           | Crous et al. 2007       |
|                                               | CPC 1488   | –                                           | –                          | –           | –                       |
| Elasticomyces elasticus                      | CBS 122538 (CCFEE 5313) | Lecanora fuscbrunnea                      | Kay Island, Northern Victoria Land, Antarctica | FJ415474    | –                       |
|                                               | CBS 122539 (CCFEE 5319) | Lecanora sp.                               | Inexpressible Island, Northern Victoria Land, Antarctica | FJ415475    | –                       |
|                                               | CBS 122540 (CCFEE 5320) | Usnea antarctica                          | Edmondson Point, Northern Victoria Land, Antarctica | FJ415476    | –                       |
|                                               | Da-004-06  | Rock                                        | Mount Aconcagua, Andes, Argentina | –           | –                       |
|                                               | CCFEE 5474 (D007-06) | Sandstone                                  | Tarn Flat, Northern Victoria Land, Antarctica | –           | –                       |
| Friedmanniomyces endolithicus                | CBS 119423 (CCFEE 5208) | Sandstone                                  | Northern Victoria Land, Antarctica | –           | Seibmann et al. 2005    |
|                                               | CBS 119424 (CCFEE 5195) | Rock                                       | Northern Victoria Land, Antarctica | –           | Seibmann et al. 2005    |
|                                               | CBS 119429 (CCFEE 5193) | Sandstone                                  | Timber Peak, Northern Victoria Land, Antarctica | –           | Seibmann et al. 2005    |
| Species                                      | Strain no.            | Source              | Geography                                                                 | ITS                | Reference                |
|----------------------------------------------|-----------------------|---------------------|---------------------------------------------------------------------------|--------------------|--------------------------|
| Friedmanniomyces endolithicus                | CBS 119428 (CCFEE 5001) | Sandstone           | Timber Peak, Northern Victoria Land, Antarctica                           | –                  | Selbmann et al. 2005    |
| Friedmanniomyces simplex                     | CBS 116775 T (CCFEE 5184) | Sandstone           | Battleship Promontory, Southern Victoria Land, Antarctica                 | DQ028271           | Selbmann et al. 2005    |
| Hobsonia santessonii                         | CBS 113389 (dh 111932) | Pellicera scabrosa  | Sweden                                                                    | –                  | Sikaroodi et al. 2001   |
| Hortaea acidophila                           | CBS 10352 (dh 12843 = VPCI 176) | Lignite pH 1.0     | Germany                                                                   | –                  | Höcker et al. 2004      |
| Hortaea werneckii (preserved as Pseudotaeniolina globosa) |                       |                     |                                                                           |                    |                          |
| dH 12322; Poonwan 13-44-06648                | dH 12322; Poonwan 13-44-06648 | Beach soil         | La Palma, Spain                                                           | AJ228474           | –                        |
| CBS 37392 (dh 15913)                         | CBS 37392 (dh 15913)   | Beach soil         | La Palma, Spain                                                           | AJ228474           | –                        |
| CBS 35966 (dh 15003)                         | CBS 35966 (dh 15003)   | Can, tinea nigra palmaris | Suriname, Paramaribo                                                   | AJ244249           | –                        |
| CBS 12232 (dh 15340)                         | CBS 12232 (dh 15340)   | Can, tinea nigra palmaris | –                                                                          | AJ228473           | –                        |
| CBS 117.90 (UAMH 4978; dh 15327)             | CBS 117.90 (UAMH 4978; dh 15327) | Salted fish, Osteoglossum bicirrhosum | Brazil                                                                    | AJ228472           | Mok et al. 1981         |
| CBS 116.90 (UAMH 52681; UAMH 5369; dh 15311) | CBS 116.90 (UAMH 52681; UAMH 5369; dh 15311) | Cantharus cantharus, eye infection, black sea bream in aquarium | –                                                                          | AJ228471           | Todaro et al. 1983      |
| CBS 115.90 (UAMH 4985; dh 15303)             | CBS 115.90 (UAMH 4985; dh 15303) | Buo granulosus, kidney | Brazil                                                                    | AJ228470           | –                        |
| CBS 111.31 (dh 15294)                        | CBS 111.31 (dh 15294)   | Man, keratomyosisis nigricans palmaris | Brazil                                                                    | AJ228679           | –                        |
| CBS 100455 (MZKI B-675)                      | CBS 100455 (MZKI B-675) | Coral, sea water   | Croatia                                                                   | AY128704           | Zalar et al. 1999       |
| CBS 107.67 (dh 15206)                        | CBS 107.67 (dh 15206)   | Man, tinea nigra   | Lisbon, Portugal                                                          | AJ228468           | McSinnis 1979           |
| MZKI B-987                                  | MZKI B-987              | Ipersaline water   | Spain                                                                     | –                  | –                        |
| CBS 113416                                  | CBS 113416              | Angelfish          | –                                                                         | –                  | –                        |
| CBS 117931 (TRN 122, dh 14528)               | CBS 117931 (TRN 122, dh 14528) | Limestone         | Cala San Vincenzo, Mallorca                                              | AY559357           | –                        |
| BMU000057                                   | BMU000057               | Patient’s foot     | China                                                                     | –                  | –                        |
| Mycocolelum victoriae                       | CBS 108663              | Soil, garden museum | Messina, Italy                                                            | AJ312123           | –                        |
| Mycosphaerella tasmaniens                   | CBS 114566 (CMW 14663; STE-U 1556) | E. ntese           | Australia                                                                 | DG267592           | –                        |
| CBS 111687 (CMW 14790; STE-U 1555)           | CBS 111687 (CMW 14790; STE-U 1555) | E. ntese           | Australia                                                                 | AY667578           | –                        |
| Pseudotaeniolina globosa                    | CBS 110353 (dh 12840; det M1082002) | 58-yr-old man, aorta, at autopsy | Würzburg, Germany                                                       | –                  | Kurzai et al. 2003      |
| CBS 109869 T (MC 769)                       | CBS 109869 T (MC 769)   | Church roof        | Sicily, Italy                                                             | AY128700           | De Leo et al. 2003      |
| CBS 113249 (dh 13060)                        | CBS 113249 (dh 13060)   | –                  | Sicily, Italy                                                             | –                  | –                        |
| CBS 303.64                                  | CBS 303.64              | Wood               | Germany                                                                    | AJ224426           | de Hoog et al. 1999     |
| CBS 119293 (dh 16805)                        | CBS 119293 (dh 16805)   | Window             | Japan                                                                      | –                  | –                        |
| Recurvoryces mirabilis                      | CBS 119434 (CCFEE 5264; dh 14759) | Sandstone           | Battleship Promontory, Southern Victoria Land, Antarctica                | FJ415477           | –                        |
| Recurvoryces mirabilis                      | CCFEE 5480 (D016-02)    | Sandstone           | Battleship Promontory, Southern Victoria Land, Antarctica                | –                  | –                        |
| Recurvoryces sp.                            | CCFEE 5391              | Rock               | Mount Rosa, P.ta Indren, Alps, Italy                                      | –                  | –                        |
| Teratosphaeria cryptica                      | CBS 117957 (TRN 491; dh 14566) | Quartzite          | Puebla de la Sierra, Madrid, Spain                                      | AY1843175          | –                        |
| Teratosphaeria cryptica                      | TC 0.56                 | E. globulus        | Australia                                                                  | DG665661           | –                        |
| Species                  | Strain no.                        | Source       | Geography                        | ITS     | Reference         |
|-------------------------|----------------------------------|--------------|----------------------------------|---------|-------------------|
| *Teratosphaeria mexicana* | CBS 110502 (CMW 14461)           | *E. globulus*| Manjimup, Darling View, Plantation, Western Australia | AY725588 | Crous et al. 2007 |
| *Teratosphaeria microspora* | CBS 110380 (CPC 1382)            | *Protea leaf*| South Africa                     | AY260197 | Taylor et al. 2003 |
| *Teratosphaeria molleriana* | CBS 111164 (CMW 4940; STE-U 1214) | *E. globulus*| Abrantes, Portugal                | AF309620 | Crous et al. 2007 |
|                          | CPC 11842                         | *Eucalyptus sp.* | Portugal                         | DQ302989 | Crous et al. 2006 |
|                          | CPC 11845                         | *Eucalyptus sp.* | Portugal                         | DQ302990 | Crous et al. 2006 |
|                          | CBS 116370 (CPC 10397)           | *E. globulus*| Spain                            | DQ302991 | Crous et al. 2006 |
|                          | CPC 12056                         |              |                                  |         |                   |
| *Teratosphaeria rubiobsa* | CBS 116005 E (CMW 3282; CPC 937) | *E. globulus*| Austria                          | AY725572 | Crous et al. 2007 |
|                          | CPC 4661                          | *E. globulus*| Spain                            | AY725570 | Crous et al. 2004 |
|                          | CBS 111445 (CPC 4660)            | *E. globulus*| Spain                            | AY725569 | Crous et al. 2004 |
|                          | CPC 3722                          | *E. globulus*| Spain                            | AY725568 | Crous et al. 2004 |
|                          | CPC 1099                          | *E. globulus*| Tanzania                         | AY725567 | Crous et al. 2004 |
|                          | CBS 111969 (CPC 1078)            | *E. globulus*| Kenya                            | AY725563 | Crous et al. 2004 |
|                          | CPC 4663                          | *E. globulus*| Spain                            | AY725571 | Crous et al. 2004 |
|                          | CPC 11882                         | *E. globulus*| Portugal                         | DQ302999 | Crous et al. 2006 |
|                          | CPC 11723                         |              |                                  |         |                   |
|                          | CPC 11487                         | *E. globulus*| Spain                            | DQ302994 | Crous et al. 2006 |
|                          | CPC 11249                         | *E. globulus*| Spain                            | DQ302993 | Crous et al. 2006 |
|                          | CPC 11246                         | *E. globulus*| Spain                            | DQ302992 | Crous et al. 2006 |
|                          | CBS 114419 (CPC 10497)           | *Eucalyptus globulus* (*Myrtaceae*) | New Zealand                       | AY725574 | Crous et al. 2007 |
|                          | TC 0.42                           | *E. globulus*| Australia                        | DQ665659 |                   |
|                          | TC 0.40                           | *E. globulus*| Australia                        | DQ665657 |                   |
|                          | TC 0.47                           | *E. globulus*| Australia                        | DQ665658 |                   |
|                          | CBS 116283 (CPC 10495)           | *E. globulus*| New Zealand                      | AY725573 | Crous et al. 2004 |
| *Teratosphaeria rubiobsa* | CPC 11885                         | *E. globulus*| Portugal                         | DQ303000 | Crous et al. 2006 |
| *Teratosphaeria ohnowa*  | CPC 1005                          |              |                                  | AY725575 | Crous et al. 2007 |
|                          | CMW 9103                          |              |                                  |         |                   |
|                          | CBS 110949 (STE-U 1008)          | *E. grandis* (*Myrtaceae*), leaves | Hazyview, South Africa            | AY725576 |                   |
| *Teratosphaeria toledana* | CBS 113313 H (CMW 14457)         | *Eucalyptus sp., leaves* | Toledo, Spain                   | AY725580 |                   |
|                          | OPC 10840                         | *Eucalyptus sp.* | Spain                            | AY725581 | Crous et al. 2004 |

Abbreviations used: ATCC – American Type Culture Collection, Manassas, VA, U.S.A.; CBS – Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; dH – GS de Hoog private collection, CBS, Utrecht, The Netherlands; CFEE – Culture Collection of Fungi From Extreme Environments, Università degli Studi della Tuscia, Viterbo, Italy; CMW – Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CPC – Culture collection of P Crous, housed at the CBS; IMI – International Mycological Institute, U.K.; M – Collection of Istituto di Microbiologia di Messina, Italy; MZKI – Microbiological Culture Collection, National Institute of Chemistry, Ljubljana, Slovenia; STE-U – University of Stellenbosch fungal culture collection, Stellenbosch, South Africa; TRN – T Ruibal private collection; UAMH – The University of Alberta Microfungus Collection and Herbarium, Edmonton, AB, Canada.

*Ex-type cultures.*
2003/2004, and from rock samples collected in Mount Aconcagua, Andes, Argentina, and Monte Rosa in the Alps, Italy, as reported in Table 1. The samples were collected using a sterile chisel, sterilised in the field before each sampling, and preserved in sterile bags at -20 °C. To remove potential contaminants, rocks collected in other environments than Antarctica were washed 15 min in sterile physiological solution added with 0.1 % Tween 20 (Sigma - Aldrich, Munich, Germany) and rinsed 4 times with physiological solution to remove any trace of the detergent. Isolations from rocks were performed by powdering the samples and seeding fragments in triplicate Petri dishes filled with 2 % malt extract agar (MEA, AppliChem, GmbH) and dichloran-rose bengal agar (DRBC, Oxoid Ltd., Basingstoke, Hampshire, U.K.). Media were supplemented with chloramphenicol 100 ppm to prevent bacterial growth. Fungi from lichens were isolated as follows: small fragments of thalli were sterilised by washing with H2O2 (8 %) for 5 min, washed with sterile deionised water to remove any trace of H2O2 and finally seeded on MEA and DRBC. Plates were incubated at 5 and 15 °C and inspected every 15 d until no new colonies appeared. Colonies were transferred to MEA slants and incubated at 15 °C. Strains analysed for comparison are listed in Table 1; they were taken from reference collections of the Culture Collection of Fungi from Extreme Environments (CCFEE, Viterbo, Italy) and the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands), including a large set of strains sampled by P.W. Crous from plants with leathery leaves in a semi-arid climate. 

Morphology

Hyphal maturation and conidiogenesis were studied using both light and scanning electron microscope (SEM). Slide cultures were seeded onto MEA, incubated for 10 wk and mounted in lactic acid. Samples for SEM observations were prepared according to methods described by Onofri et al. (1980).

DNA extraction and sequencing

DNA was extracted from mycelial fragments taken from 6-mos-old MEA slants grown at 10 °C, using NucleoSpin Plant kit (Macherey-Nagel, Düren, Germany) following the protocol optimised for fungi. PCR reactions were performed using BioMix (BioLine GmbH, Luckenwalde, Germany). In each 25 µL reaction tube 5 pmol of each primer and 40 ng on template DNA were added. The amplification was carried out using MiniCycler™ (MJ Research, Waltham, Massachusetts, U.S.A.) equipped with a heated lid. The first denaturation step at 95 °C for 3 min was followed by: denaturation at 95 °C for 2 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s. The last three steps were repeated 35 times, with a last extension 72 °C for 5 min. The products were purified using NucleoSpin Extract kit (Macherey-Nagel, Düren, Germany). Primers NS1, NS2, NS3, NS4, NS5, NS8, ITS1, ITS4 (White et al. 1990), SR10R (Bruns et al. 1992), ITS5, and ITS4a (Larena et al. 1999) were employed to amplify SSU and ITS rDNA portions. Sequencing reactions were performed according to the dideoxynucleotide method (Sanger et al. 1977) using the TF Big Dye Terminator 1.1 RR kit (Applied Biosystems). Fragments were analysed using an ABI 310 Genetic Analyser (Applied Biosystems). Sequence assembly was done using the software Chromas (v. 1.45 1996–1998, Conor McCarthy School of Health Science, Griffith University, Southport, Queensland, Australia).

Alignment and tree reconstruction

SSU sequences were aligned with ARB beta-package (v. 22-08-2003, Ludwig et al. 2004; www.mikro.biologie.tu-muenchen.de/pub/ARB). The SSU alignment spanned positions 141–2512, which corresponds to 1515 bp with reference to Saccharomyces cerevisiae. Trees based on SSU sequences were reconstructed with neighbour-joining in ARB.

ITS sequences were aligned iteratively with Ward’s averaging (Van Ooijen 2002) in a research data base of black yeasts present at CBS using the BioNumerics package (Applied Maths, Kortrijk, Belgium). Due to gaps necessary for alignment, the ITS1 domain spanned 187 positions (real lengths 147–154 bp), the 5.8S gene 156 positions and the ITS2 domain 184 positions (real lengths 143–155 bp). The alignments were based on the positions 28–481, the initial and the final parts were cut off to compare fragments with the same length. Alignments were exported and the best-fit substitution model was determined using Modelfest MrAIC.pl 1.4.3 (Nylander 2004, program distributed by the author) estimated using PHYML (Guindon & Gascuel 2003) through hierarchical likelihood ratio tests. MrAIC calculates the Akaike Information Criterion (AIC), corrected Akaike Information Criterion (AICc) and Bayesian Information Criterion (BIC); Akaike weights for nucleotide substitution model and model uncertainty. All 56 models implemented in MrDistel were evaluated. Phylogenetic trees were reconstructed by Maximum Likelihood, using TREEFINDER (Jobb et al. 2004) and the resulting tree was displayed using TREEVIEW v. 1.6.6 (Page 1996). The robustness of the phylogenetic inference was estimated using the bootstrap method (Felsenstein 1985) with 100 pseudoreplicates generated and analysed with TREEFINDER.

As alignment over the entire complex was highly ambiguous, an algorithm for tree reconstruction without alignment, the DNA-walk Divergence method (DNAWD, Licinio & Caligiore 2004; Caligiore et al. 2005) was used, involving the entire spacer region. DNA-walks are defined by incrementing walk steps for each nucleotide in the sequence (for example a positive step for purines, and negative for pyrimidines). It makes simultaneous comparisons of the three-dimensional walks (representing three composition skews): AG, TC, AC-TG, and AT-CG for each pair of sequences. One sequence slides against the other until the minimum squared walk difference is found, corresponding to a global alignment. This is then taken as a measure of their distance since statistically independent mutations and indels increase the mean square walk differences linearly. The resulting distance matrices are then fed into the Kitsch program of the Phylip package (v. 3.572c, Felsenstein 1996).

Cultural preferences

Cultural characteristics and growth rates were recorded on Potato-Dextrose Agar (PDA), MEA, Czapek Dox Agar (CzA) and Oatmeal Agar (OA). Strain CBS 119434 was incubated at 10 °C, strains CBS 122538, 122539 and 122540 at 20 °C and strains CBS 899.87, CBS 335.97, dH 12881, dH 11526 and dH 13081 at 25 °C. The diameter of the colonies was recorded monthly. Tests were performed in triplicate.

Temperature preferences

Temperature preferences for the strains CBS 119434, 122538, 122539 and 122540 were tested by incubating them on MEA,
### Table 2. Physiological profiles of Antarctic strains.

| Species                     | Strain no. | Cultural preferences | Thermal preferences (° C) |
|-----------------------------|------------|----------------------|---------------------------|
|                             |            | PDA | MEA | CzA | OA | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 |
| Recurvomycetaceae mirabilis | CBS 119434 | 1.5±0.14 | 1.8±0.14 | 0.5±0.14 | 1.65±0.07 | 0.45±0.07 | 0.8±0.2 | 1.5±0.26 | 1.9±0.26 | 0.7±0.2 | – | – | – |
| Elasticomyces elasticus     | CBS 122538 | 1.7±0.2 | 1.5±0.14 | 0.5±0.14 | 1.5±0.14 | 0.82±0.064 | 0.64±0.079 | 1.2±0.2 | 1.6±0.26 | 1.54±0.09 | 0.95±0.15 | – | – |
|                             | CBS 122539 | 1.5±0.1 | 1.43±0.15 | 0.3±0.02 | 1.23±0.2 | 0.77±0.1 | 0.6±0.2 | 1.1±0 | 1.5±0.14 | 1.2±0.2 | 0.6±0.2 | – | – |
|                             | CBS 122540 | 1.0±0.2 | 1.1±0.1 | 0.2±0.02 | 1.0±0.2 | 0.72±0.1 | 0.5±0.14 | 1.13±0.2 | 1.4±0.17 | 1.2±0.2 | 0.8±0.08 | – | – |

Cultural and thermal preferences reported as diameter of the colonies (cm), after two mos of incubation. The values represent the average of three different tests. Plates for cultural preferences were incubated at 10 °C for strain CBS 119434 and at 20 °C for strains CBS 122538, 122539 and 122540; plates for temperature preferences were seeded on MEA; – = no growth.

### Table 3. Cultural preferences and salt tolerance of acidophilic strains.

| Species                        | Strain no. | Cultural preferences | NaCl % |
|--------------------------------|------------|----------------------|--------|
|                                |            | PDA | MEA | CzA | OA | 1.2 | 1.5 | 3 | 5 | 7 | 10 | 12 |
| Acidomyces acidophilum         | CBS 899.87 | 1.17±0.1 | 1.3±0.15 | 0.3±0.02 | 1.17±0.1 | 1.3±0.02 | 0.7±0.02 | 0.6±0.03 | 0.6±0 | 0.5±0 | 0.2±0.02 | – |
|                                | CBS 335.97 | 1.08±0.1 | 1.9±0.14 | 0.6±0.2 | 1.0±0.2 | 1.5±0.02 | 0.5±0.02 | 0.4±0.04 | 0.3±0.02 | 0.2±0.02 | – | – |
|                                | dh 12881   | 2.0±0.14 | 1.5±0.14 | 1.2±0.2 | 1.5±0.1 | 1.5±0.02 | 1.5±0.02 | 0.7±0.03 | 0.5±0.04 | – | – |
|                                | dh 11526   | 2.5±0.2 | 3.0±0.2 | 1.5±0.14 | 1.2±0.2 | 2.5±0.02 | 2.0±0.04 | 2±0.04 | 0.8±0.03 | 0.6±0.008 | – | – |
|                                | dh 13081   | 1.8±0.14 | 2.2±0.2 | 1.1±0.1 | 1.0±0.2 | 1.0±0.04 | 0.8±0.02 | 0.8±0.02 | 0.6±0.02 | 0.3±0.02 | – | – |

Growth on different media and salt concentration expressed as diameters of the colonies (cm); – = no growth.

### Table 4. Thermal and pH preferences of acidophilic strains.

| Species                        | Strain no. | Thermal preferences (° C) | pH |
|--------------------------------|------------|---------------------------|----|
|                                |            | 4 | 10 | 18 | 25 | 30 | 37 | 1 | 3 | 5 | 7 | 9 |
| Acidomyces acidophilum         | CBS 899.87 | – | – | + | ++ | + | – | + | + | ++ | + | ± |
|                                | CBS 335.97 | + | + | ++ | + | + | – | + | ++ | + | – |
|                                | dh 12881   | + | + | ++ | ++ | + | – | + | ++ | + | ± | – |
|                                | dh 11526   | + | + | + | ++ | + | – | + | ++ | ++ | + | – |
|                                | dh 13081   | + | + | + | ++ | + | – | ± | ++ | ++ | + | – |

++ = maximum growth recorded; + = growth; ± = weak growth; – = no growth.
incubating in shaken culture at 70 r.p.m. After 30 d of incubation at 122540 grew in a wider range of temperatures between 0 and CzA. CBS 119434 was able to grow in the range 0–20 °C, with a marked reduction of ultimate colony diameter when seeded on visible growth on MEA as well as on PDA and OA, whilst there was dH12881, dH11526 and dH13081 are reported in Tables 3 and 4. Physiological data for the strains CBS 899.87, CBS 335.97, 119434, 122538, 122539 and 122540 are shown in Table 2.

Growth at different salt concentrations

The ability to grow at different salinities was tested in duplicate on plates of MEA amended with 1.2, 1.5, 3, 5, 7, 10 or 12 % NaCl. Strains were inoculated in three spots on each plate and incubated at 25 °C for one mo, when the colony diameter was recorded. Colonies with a diameter >2 mm were considered positive (Kane & Summerbell 1987).

Growth at different pH

The ability to grow at different pH values for the strains CBS 899.87, CBS 335.97, dH12881, dH11526 and dH13081 was tested in duplicate using MEB2 % medium at pH 1, 3, 5, 7 and 9. Values of pH 5 were obtained by the addition of 1N HCl; remaining pH values were obtained according to Küster & Thiel (1990) as follows: McIlvaine solution for pH 2–7, Clark & Lubs solution for pH 8–9; buffer HCl/KCl for pH 1. Strains were incubated at 25 °C in shaken culture at 70 r.p.m. for one mo, cultures were filtered and the biomass dry-weighed.

RESULTS

Physiology

Temperature relations and cultural features of the strains CBS 119434, 122538, 122539 and 122540 are shown in Table 2. Physiological data for the strains CBS 899.87, CBS 335.97, dH12881, dH11526 and dH13081 are reported in Tables 3 and 4. All fungi tested were able to grow on natural media showing clearly visible growth on MEA as well as on PDA and OA, whilst there was a marked reduction of ultimate colony diameter when seeded on CzA. CBS 119434 was able to grow in the range 0–20 °C, with an optimum at 15 °C, whereas strains CBS 122538, 122539 and 122540 grew in a wider range of temperatures between 0 and 25 °C, with an optimum at 15–20 °C. Strains CBS 899.87, CBS 335.97, dH12881, dH11526 and dH13081 grew best in the range of 18–30 °C, with the optimum temperature at 18 °C for the strains CBS 335.97 and dH 12881, 25 °C for the strains CBS 899.87 and dH 13081, and 30 °C for the strain dH 11526. Since almost all strains (except CBS 899.87) were still able to reproduce at 4 °C, they can be referred as mesophilic-psycrotolerant (Zucconi et al. 1996). None of them grew at 37 °C. Furthermore, they were able to grow in very acidic conditions (pH 1) and most of them grew best at pH 5 or below. In particular the optimum was pH 5 for the strain CBS 899.87, around pH 3–5 for the strains CBS 335.97, dH11526 and dH 13081 and pH 3 for the strain dH 12881. All the strains studied showed a decreasing growth at pH 7 and stopped to grow at pH 9, with the exception of strain CBS 899.87 which was able to grow in a very wide range of pH values showing a weak growth even at pH 9. The strains demonstrated also to be moderately halophilic being able to grow in up to 7 % NaCl (strains CBS 335.97, dH11526, dH 13081, dH 12881) and in up to 10 % for the strain CBS 899.87.

Phylogeny

SSU sequences were analysed for 71 strains of ascomycetous black yeasts and relatives belonging to the orders Capnodiales, Dothideales, Myriangiales as well as the recently proposed new order Botryosphaeriales (Schoch et al. 2006). The recently described taxon Baudonia componiacensis (Richon) J.A. Scott & Unter. (Scott et al. 2007) with an SSU similarity around 97 % with some capnodialean/dothidealean strains, was not included in this comparison. Fig. 1 shows a Neighbour Joining tree based on the SSU comparison where the outgroup is represented by Aliquandostipite khaoaiensis (Jahnuales).

Three Antarctic rock strains (CBS 122538, 122539 and 122540), with nearly identical sequences, were included in a group paraphyletic to Friedmanniomycetes endolithicus Onofri. The strains shared a remarkable morphology, with straight fertile hyphae of which fragments detached, sometimes by basiaxially expanding connectives.

Strain CBS 119434 clustered in a clade composed of mainly meristematic species, which included the Antarctic rock-inhabiting genus Friedmanniomycetes. This strain also had characteristic morphology, showing a unique pattern of recurved hyphal branching at conidiation. Another strain with nearly identical sequence, CBS 117957 from Mediterranean rocks, showed a simple, undiagnostic meristematic micromorphology. The lichenicolous fungus Habsonia santessonii Lowen & D. Hawksw. belonged to the same group, together with the black fungus Myccocalicum victoriae (C. Knight ex F. Wilson) Tibell.

The acidophilic species Hortaea acidophila Höller et al. and Acidomyces acidophilum as “Acidomyces richmondensis” B.J. Baker et al. composed a sister clade to the Friedmanniomycetes complex. The halophilic species Hortaea werneckii (Horta) Nishimura & Miyaji was found at a larger distance, in a heterogeneous clade with Pseudotaeniolina globosa De Leo et al., Catalanostroma abietis (Butin & Pehl) Crous & Braun and Coccodinium bartschii A. Massal. As the backbone of the tree shows low bootstrap values for most clades, the exact phylogenetic positions of the newly added Antarctic and acidophilic species are difficult to determine.

The ITS tree shown in Fig. 2 was generated on the basis of the manually optimised alignment containing 95 sequences of halophilic, acidophilic, rock and plant-pathogenic fungi; it is based on a length of 452 characters, including alignment gaps. The AlCc selected HKY+G (Hasegawa et al. 1985) as the best model. The base frequencies was as follows: T = 0.2293, C = 0.3120, A = 0.2009, G = 0.2574, TC = 0.5413, AG = 0.4583. The entire ITS region showed too many polymorphisms to allow alignment with a sufficient degree of confidence. For this reason, DNAWD was applied, which is insensitive to alignment. Topology of this tree was identical (data not shown).

In general the ITS trees showed excellent resolution of entities. Inter-group variability was constant and invariably significantly larger than intra-group variability. The tree contained anamorph taxa from extreme environments, as well as a number of plant-associated species of Teratosphaeria Syd. & P. Syd. Three main clades were discernible: an upper clade around Teratosphaeria microspora J.E. Taylor & Crous, a clade with T. nubilosa (Cook) Crous & U. Braun as central species, and an outgroup with strains isolated at very low pH (Fig. 2).

A single cluster is composed of strains listed as Teratosphaeria
Fig. 1. Molecular phylogeny based on SSU sequences indicating the positions of the clades in Dothideomycetidae; the described new genera were highlighted with coloured rectangles. The tree has been built with neighbour-joining algorithm in ARB package with 100 replications. Branches of the clades supported by a bootstrap value above 95% are in bold.
Fig. 2. Molecular phylogeny of the analysed ITS rDNAs showing the relationship of the new genera described, highlighted with coloured rectangles, in the Capnodiales. The neighbour-joining tree, based on 95 sequences and 452 nucleotide positions, has been generated using HKY+G model; the model was calculated using ML in MrAIC software. Bootstrap values from 100 resampled data sets are shown.
microspora, Catenulostroma abietis (Butin & Pehl) Crous & U. Braun and C. germanicum Crous & U. Braun. The strain TRN 128 from Mediterranean rock is found in a paraphyletic position. Pseudotaeniolina globosa is a member of the same clade, while the endemic Antarctic Friedmanniomyces species are members of a neighbouring clade which includes Myccocalicium victoriae CBS 109663 and the lichenicolous fungus Hobsonia santessonii, without any known teleomorph. Five further undescribed Antarctic strains, among which was CBS 122539, constituted a sister clade (Fig. 2), and phenetically all showed conidial dehiscence with basauxically expanding connectives; we here describe a new genus, Elasticomyces Zucconi & Selbmann for this group. Another, separate cluster of four strains from Antarctic and Mediterranean rocks was found around CBS 119434, strains microscopically either showing recurved conidial branches or a reduced, meristematic morphology; for which we describe a new genus, Recurvomyces Selbmann & de Hoog. The halo- respectively acidophilic Hortaea species, H. werneckii and H. acidophila, were located at clearly separate positions. The same clade included also the plant-pathogenic teleomorph species Teratosphaeria ohnowa (Crous & M.J. Wingf.) Crous & U. Braun occurring on leaves of Myrtaceae. A basal cluster comprised some strains, among which Scytalidium acidophilum CBS 270.74 was selected as outgroup. The clade contained the plant-pathogenic Mycosphaerella nubilosa (Cooke) Hansf. and, as sister clade, a group of acidophilic fungi which included the ex-type strain of Scytalidium acidophilum Sigler & J.W. Carmich. CBS 270.74, isolated from acidic soil, was found to be identical to that species. No teleomorph relationships are known for this group. S. acidophilum was found to be identical to strains of the the invalidly described genus “Acidomyces” B.J. Baker et al. That genus is validated below as Acidomyces B.J. Baker & al. ex Selbmann et al.

**DESCRIPTIONS**

**Recurvomyces** Selbmann & de Hoog, gen. nov. – MycoBank MB511293.

Ad fungos anamorphos, hyphomycetes pertinens. Coloniae in agaro maltoso lente crescentes, compactae, velutinae, nigrae vel olivaceo-nigrae. Mycelium ex hyphis longis, levibus, pallide brunneis et crassitunicatis compositum. Hyphae torulosae interdum productae, brunneae, crassitunicatae, enteroblastice proliferantes. Conidiophora macronematosa vel semi-macronematosa, saepe angulo recto dispositis deorumque inflexis. Conidia enteroblastica, schizolytice secedentia. Ramoconidia interdum producta. Teleomorphosis ignota.

Species typica: Recurvomyces mirabilis Selbmann & de Hoog, sp. nov.

Fig. 3. Recurvomyces mirabilis. A. 1-celled conidia and ramoconidia. B–D. Septate and branched conidiophores; branches forming right angle and bent down. E. Conidiophore with recurved hyphal branching. F. Conidiogenous cells producing conidia by enteroblastic proliferation. G. Schizolytic conidial secession. H. Swelling cells. Scale bar = 10 µm.
Anamorphic fungi, hyphomycetes. Colonies very slowly growing, compact, heaped, black or olive-black. Mycelium composed of long, smooth, yellowish brown and thick-walled hyphae. Torulose hyphae sometimes present, brown, thick-walled, smooth to verrucose, enteroblastically proliferating. Conidiophores macronematous or semi-macronematous, often branched with lateral branches mostly at roughly right angle and bent down. Conidia produced by enteroblastic proliferation released by schizolytic secession. Ramoconidia sometimes present.

Teleomorph: Unknown; phylogenetic affinity to the ascomycete order Capnodiales.

Type species: Recurvomyces mirabilis Selbmann & de Hoog, sp. nov.

Recurvomyces mirabilis Selbmann & de Hoog, sp. nov. – MycoBank MB511294, Figs 3–5.

Colonies in agar maltose acetaev, compactae, marígne regulari atque plano, velutinae, nigrae vel olivaceae-nigrae, revertex nigrum, lenite crescentes, post tres menses usque ad 18 mm diam. Mycelium ex hyphis longis, levibus, pallide brunneis et crassitunicatis, apicum versus gradatim pallidius et subtilius tunicatis, subhyalinis, interdum anastomosis fenestritis, septatis, ramosis compositum. Torulose hyphae interdum productae, brunneae, crassitunicatae, leves vel verrucose, paribus inflationis 4.5–7.5 μm crassis, proliferatione apicali enteroblastica. Conidiophora ex mycelio superficiali compása, macronomatosa vel semi-macronomatosa, mononematosa, erecta, brevia, septata, levia, crassitunicata, brunnea, apicum versus gradatim pallidius et ruritunicata, ex proliferatione enteroblastica percurrentia obversum, saepe ramosa, cum ramis lateraliis saepae angulo recto dispositiis, deorsum inflexiis. Cellulae conidigeneae polyblasticae, integratae, terminales vel intercalares, tenenitunicatae, brunneae, enteroblasticae, post schizophilicum secessionem conidiorum terui cicalihe praedite. Conidia enteroblastica, sica, solitaria, 0–1-septata, subhyalin elm, dulce brunnea, tenenitunicata, levia, elipsoidea vel obovoida, raro modice ad septum constricta, ad apicem rotundata, plano hilo basilari incospicuus praedita post schizophilicum secessionem, 7.2–11.2 × 2.5–4.7 μm. Propagula liberata e partibus terminalibus conidiophororum vel cellulis conidigenis orientia; ramoconidia etiam producta, saepe 1-septata, brunnea, crassitunicata, forma irregular, quae a late enteroblastica conidia igniere possunt. Teleomorphia ignota.

Holotypus: CBS H-20178, cultura ex-CBS 119434 = CCFEE 5264, in Promontorio Pugiae Navaliss, Terra Victoriae Meridionalis, Antarctica, isolated from sandstone. Leg. L. Zucconi, 24 Jan. 2004.

Strains examined: CBS 119434; CCFEE 5480; CCFEE 5391; CBS 117957.

Elasticomyces Zucconi & Selbmann, gen. nov. – MycoBank MB511296.

Ad fungos anamorphos, hyphomycetes pertinens. Coloniae compactae, coherentae, cumulatae, nigrae, lenite crescentes. Mycelium ex hyphis longis, levibus, ramosis, tenenitunicatis, hyalinis vel pallide pigmentatis compositum. Hyphae fertiles obscuriores et magis crassitunicatae, repetite ramosae, septatae, fissione ad septa in conidia disarticulatae. Arthroconidia catenatae, univel pluricellularia, cylindrica, utrime secessione schizolytica truncata. Teleomorphia ignota.

Species typica: Elasticomyces elaticus Zucconi & Selbmann, sp. nov.

Anamorphic fungi, hyphomycetes. Colonies compact, felted, clumped, black, slow growing. Mycelium composed of long, branched, septate, thin-walled, colourless or yellowish to pale brown hyphae. Fertile hyphae more pigmented and thicker-walled, repeatedly branched at roughly right angle, septate, forming conidia by fragmentation. Arthroconidia catenate, one or pluricellular, cylindrical, with truncated ends due to schizolytic secession.

Teleomorph: Unknown.

Phylogenetic affinity to the ascomycete order Capnodiales.

Type species: Elasticomyces elaticus Zucconi & Selbmann, sp. nov.

Elasticomyces elaticus Zucconi & Selbmann, sp. nov. – MycoBank MB511297, Figs 6, 7.

Coloniae in agar maltoso compactae, coerctae, partim immersae, margine pneumque irregulari, cumulatae, obversum reversumque nigra, lenite crescentes, post duos menses usque ad 15 mm diam. Mycelium ex hyphis longis, septatis, levibus, tenenitunicatis, luteolis vel pallide brunneis, ramosis, anastomosantibus, 3.5–5.3 μm latis compositum. Hyphae fertiles brunneae, crassitunicatae, ad angulo recto repetite ramosae, septatae, primum leves, demum crenulates, fissones ad septa in numerosa brevia segmenta, ex uno vel pluribus conidia composita, disarticulantes, interdum connectiva coniuncta. Arthroconidia catenata, pneumque bicellularia, rarius unicellularia, levia vel crenulata, cylindrica, truncata et magis crassitunicata ad apices secessione schizolytica, modice ad septum constricta, 12.5–16 × 3.5–5.3 μm. Cellulae intercalares clamydotosporium semel interdum orientes, parietibus crassis et brunneis. Teleomorphia ignota.

Holotypus: CBS H-20177, cultura ex-CBS 122538 = CCFEE 5313, Insula Kay, Terra Victoriae Selentrenisalis, Antarctica, isolated ex hallo Usea Antarcticae. Leg. L. Zucconi, 30.I.2004.
Fig. 4. A. Battleship Promontory, Southern Victoria Land, Antarctica, view from the helicopter. B. Battleship Promontory, Southern Victoria Land, Antarctica, landscape. C. Sandstone sample from which Recurvomyces mirabilis CBS 119434 has been isolated. The surface is weathered by the activity of cryptoendolithic microorganisms. Scale bar = 5 cm. D. Magnification at the dissecting microscope of the black fungi endolithic colonization inside the sample of sandstone used for the isolation. Scale bar = 2 mm. E, F. Rock colonised by the fungus observed at the Scanning Electron Microscope.
Fig. 5. Recurvomyces mirabilis, CBS 119434 (= CCFEE 5264). A. Strain grown on different media after two mo of incubation at 15 °C. B–D. Hyphae with branched and unbranched conidiophores producing 0–1 septated conidia. E. Curved branched conidiophores schizolytically seceding (black arrow) showing enteroblastic elongation (white arrow) at the apex. F, G. High magnification of branched conidiophores producing 1-celled conidia. H, I. 1- and 2-celled conidia and ramoconidia. L. Terminal swelling cell. M, N. Swelling hyphae showing enteroblastic elongation. O, P. Unbranched conidiophores producing 1-celled conidia, scar is visible after schizolytic secession (P, arrow). Q. Branched conidiophore. R, S. Swelling cell at intercalary (R) and terminal position (S). B–N. Light microscopy; Scale bars = 10 µm. O–S. SEM.
Colonies compact, felted, partially immersed in the agar, margin rather irregular, obverse and reverse black, clumped on MEA2 % and PDA, almost completely flat on OA; slow growth: diameter after two mos 15 mm on MEA2 % and OA, 17 mm on PDA, and 5 mm on CzA. Mycelium composed of long, septate, branched, smooth, thin-walled, yellowish to pale brown hyphae, 3.5–5.3 µm wide, with anastomoses. Fertile hyphae more pigmented, thicker-walled, at roughly right angle repeatedly branched, septate, at first smooth, then crenulate, forming by fragmentation numerous short segments, composed of one or more conidia, sometimes joined by connectives. Arthroconidia catenate, mostly bicellular; rarely aseptate, smooth or crenulate, cylindrical, with thickened and truncated ends due to schizolytic secession, slightly constricted at the septum, 12.5–16 × 3.5–5.3 µm. Intercalary chlamydospore-like cells, with thickened and brown wall, sometimes present.

Teleomorph: Unknown.

Holotype: CBS H-20177, culture ex-type CBS 122538 = CCFEE 5313, Kay Island (75°04'13.7''S, 165°19'0.2.0''E), Northern Victoria Land, Antarctica, isolated from lichen thallus (Usnea antarctica Du Rietz). Leg. L. Zucconi, 30 Jan. 2004.

Strains examined: CBS 122538; CBS 122539; CBS 122540; Da-004-06; CCFEE 5474.

Notes: The conidium ontogeny is holoarthric, involving an irregular basipetal maturation of cells and fragmentation of fertile hyphae. Often short portions of fertile hyphae are released by fragmentation.
Fig. 7. *Elasticomyces elasticus*, CCFEE 5313. A. Strain grown on different media after 1.5 mo of incubation at 15 °C. B–D. Vegetative and fertile hyphae. E–H. High magnification of fertile hyphae; anastomoses in the vegetative hyphae (black arrows in E). I–O. Uncompleted disarticulation of artic conidia and hyphal fragments remaining joint by connectives (white arrows). P. High magnification of conidia remaining joint after disarticulation. Q, R. 1- and 2-celled conidia produced after schyzolithic secession (white arrow). S–U. Enteroblastic elongation at the apexes (white arrows). Scale bars = 20 µm.
Acidomyces acidophilus. A. Strain CBS 899.87 grown on different media after 1 mo of incubation at 25 °C. B–D. G. Toruloid unbranched hyphae with melanised and thick-walled cells. E. Meristematic development of the hyphae. F. Fungus grown at pH 1 in liquid culture. H. Chain of 1- 2- and 3- celled conidia. I. Strain CBS 335.97 grown on different media after 1 mo of incubation at 25 °C. L, M. Filamentous hyphae with intercalary and terminal swelling cells (black arrows). N. Fungus grown at pH 1 in liquid culture. Scale bars = 20 µm.

of longer hyphae, functioning as propagules. Apical growth of fertile hyphae occurs concomitantly with holoarthric development. A circumscissile scar remains at both ends, after schizolytic secession of adjacent conidia; complete disarticulation is retarded by the presence of thin strands of wall material at the central convexity of septa. Sometimes conidial secession is not completed; new wall material is laid down in the existing septum, and adjacent cells remain connected by narrow and pale connectives. Connectives can eventually elongate to form new pale hyphae, sometimes evolving in new fertile hyphae.

Acidomyces B.J. Baker, M.A. Lutz, S.C. Dawson, P.L. Bond & J.F. Banfield ex Selbmann, de Hoog & De Leo, gen. nov. – MycoBank MB511298.

Ad fungos anamorphos, hyphomycetes pertinens. Coloniae lente crescentes, celeriis in acido agar, compactae, nigrae. Mycelium ex septatis, interdum ramosis, brunneis crassitunicatisque hyphis compositum, demum meristematice increscens. Conidia arthrice secedentia. Teleomorphosis ignota.

Anamorphic fungi, hyphomycetes. Colonies growing slowly, faster in acidic medium, compact, dark. Mycelium composed of septate, scarcely branched, brown and thick-walled hyphae, eventually
 converting into a meristemmatic mycelium. Conidia produced by arthric disarticulation of hyphae.

**Teleomorph:** Unknown; phylogenetic affinity to the ascomycete order Capnodiiales.

**Type species:** Acidomyces acidophilus (Sigler & J.W. Carmich.) de Hoog & De Leo

**Acidomyces acidophilus** (Sigler & J.W. Carmich.) Selbmann, de Hoog & De Leo, *comb. nov.* – MycoBank MB511856, Fig. 8.

Basionym: Scytalidium acidophilum Sigler & J.W. Carmich., *Canad. J. Microbiol.* 20: 267, 1974.  
≡ Fungus D, Starkey & Waksman, *J. Bacteriol.* 45: 512, 1943 (without description, without type).  
≡ Acidomyces richmondensis B.J. Baker, M.A. Lutz, S.C. Dawson, P.L. Bond & J.F. Banfield, *Appl. Environm. Microbiol.* 70: 6270, 2004 (nom. inval., Arts 36.1, 37.1 ICBN).

**Type:** UAMH 3460, from field soil, adjacent to elementary sulphur stockpile from natural gas purification plant, Bowden, Alberta, Canada [*as Scytalidium acidophilum*].

**Ex-type strain:** CBS 270.74 (= ATCC 26772 = IMI 183518 = UAMH 3460).

**Additional strains examined:** See Table 2.

**DISCUSSION**

Melanised rock-inhabiting fungi are phylogenetically quite heterogeneous and have been grouped at least in four different fungal orders: Capnodiiales, Dothideales, Chaetothyriales and Pleosporales (Sterflinger et al. 1997, Selbmann et al. 2005, Ruibal et al. 2008). Now that suitable isolation and identification techniques have been developed for these fungi (Wollenzenz et al. 1995, Ruibal et al. 2005), they appear to be very common in arid climates, from Polar Regions to the sub tropics. Stringent environmental conditions include high solar radiation (Urzi et al. 1993), repeated freezing and thawing cycles (Selbmann et al. 2002), low water activity (Sterflinger 1998, Zalar et al. 1999), acidity (Price 2000), and nutrient deficiency (Sterflinger et al. 1999).

The physiological studies indicated that all the strains studied were well adapted to the particular stressing conditions characterising their natural environments. For instance, the strains belonging to the newly validated genus *Acidomyces*, all isolated from very acid conditions (see Table 1), showed an acidophilic profile being able to grow very well at pH 1 and with optimum well below the neutral value (pH 3–5). This means that most probably they are scarcely competitive in other environments and remain trapped in the extremely acid ones where they have been isolated. Furthermore, they showed only a moderate tolerance to salinity. Similar data have been already reported for other acidophilic fungi phylogenetically distant from *Acidomyces* strains; for instance, *Hortaea acidophila*, was reported to be very sensitive to osmotic stresses being unable to grow at NaCl concentration above 2% (Hölker et al. 2004). These data suggest that the adaptation to acidic environments does not require a concomitant tolerance to osmotic stresses. The situation seems to be different for fungi colonising rocks; Sterflinger (1998) observed a certain degree of halotolerance for some fungal strains in a selected, phylogenetically heterogeneous, group of rock fungi. Halotolerance has been proven to be particularly pronounced in some fungi isolated from the exposed rocks of the Antarctic desert (Onofri et al. 2007), among the driest terrestrial ice-free areas on Earth; there, the high evaporation leads also to salt accumulation on rock surfaces and fungi adapted to that environment have to cope with both water deficiency and salinity (Rusi et al. 2007).

All Antarctic strains studied can also be referred to as psychrophilic according to criteria outlined by van Uden (1984) and Vishniac (1987), having an optimum at 15 °C and being unable to grow at temperatures above 20–25 °C. This result confirms what has been recently published for other strains isolated from Antarctic rocks (Selbmann et al. 2005). Remarkable is the finding that strains belonging to the genus *Elas ticomycetes* here described, isolated from Antarctic lichens, showed a wider temperature range for growth with respect to the Antarctic cryptoendolithic strain of *Recurrenomyces mirabilis* Selbmann & de Hoog. Their ability to grow at 0 as well as at 25 °C can be an adaptive strategy to withstand not only the very low temperatures characterising their natural environment but also the very wide thermal fluctuations, much more marked in the epilithic rather than in the endolithic environment.

Common features enhancing survival are high degrees of melanisation and thick cell walls (Figuera et al. 1996), slow, isodiametric growth, isodiametric expansion ensuring an optimal surface/volume ratio (Wollenzenz et al. 1995), ability to change cellular polarity (Yoshida et al. 1996), and hence little differentiation although with great polymorphism. These characters seem to favour a marked degree of convergent evolution.

In the SSU phylogeny (Fig. 1) a clade is recognisable, containing a large number of melanised fungi that can be isolated from bare rocks, among which there is *Pseudotanaeolina globosa* isolated from rock surfaces in Sicily (De Leo et al. 2003). The clade also contains some *Teratosphaeria* species inhabiting leathery plant leaves, mostly in semi-arid climates (Crou et al. 2004); this genus was re-established as separate from *Mycosphaerella* because of this ecology and because of its phylogenetic position (Crou et al. 2007). The phylogenetic relationship to numerous extremotolerant species is remarkable. Most plant-associated fungi are known with their teleomorph, while for the epilithic and endolithic species no teleomorphs are known. *Teratosphaeria microspora* can be found both on rock and plant leaves, suggesting that adaptation to a life at the extreme starts with dispersal under semi-arid conditions.

In general the ITS tree shows excellent resolution of species. Recognised entities were ecologically consistent (Fig. 2), such as the halophilic species *Hortaea werneckii* and the acidophilic *Acidomyces acidophilus*.

*Trimmatastoma* Corda, with the generic type species *T. salicis* Corda, was recently treated as a genus of *Leotiales*, while the capnodiaceous species were reclassified in *Catenulostroma* Crous & U. Braun (Crous et al. 2007). Teleomorph connections of *Catenulostroma* are in *Teratosphaeria*. Crou et al. (2007) mentioned differences in ecology and geography, as *Teratosphaeria microspora* would be an endemic species of Protea in South Africa, while other taxa within the *T. microspora* complex were observed on conifer needles and rocks in the Northern Hemisphere. Members of the complex are commonly observed on rocks and other relatively inert surfaces in temperate climates, but on conifer needles they produce well-defined acervuli (Bubn et al. 1996). This suggests that superficial rock-colonising strains of this group may have originated from leathery plant leaves; we did not find any match between observed ITS polymorphisms and geography or ecology (Table
reaching the stationary phase (Figueras 1976; Friedmann 1982), as well as from Andean rocks (4885 a.s.l.) rocks in the airspaces between crystals (Friedmann & Ocampo described as peculiar ability to produce connectives seems a distinctive feature of Antarctic lichens, but later on also from Antarctic lichen-dominated group of Recurvomyces mirabilis, respectively. From Spanish rocks (Ruibal 2004) and the Alps (unpublished data) sequence, CBS 117957 (TRN 491) and CCFEE 5391, were isolated a member of an Antarctic lichen-dominated cryptoendolithic community. Additional, sterile strains with a nearly identical ITS a further related species with a very prevalent mode of action is superficial biopitting (Sterflinger and physiological factors (Ruisi al. 2007). All factors discussed above of melanisation, meristematic morphology, growth at low water activity and at high/low temperature, and acid tolerance, are encountered in Dothideomycetidae as well as in Chaetothyriomycetidae, in varying combinations, but only in Chaetothyriales they play a role in infection. For example, meristematic morphology, particularly expressed at low pH (Mendoza et al. 1993), determines the invasive form in humans with the black yeast-specific skin disease chromoblastomycosis. The natural habitat of one of the agents of this disease, Cladophialophora carrionii (Trejo) de Hoog et al., was found to be in cactus debris in semi-arid climates (de Hoog et
al. 2007), where the same morphology is expressed as prevalent in human skin. This suggests that in C. carrionii the extremotolerant morphology directly enhances human invasion.Nevertheless, de Hoog et al. (2005) noticed that human pathogenicity is associated with a stress-factor like osmotolerance at the order level, but the two factors are nearly mutually exclusive at the species level. This means that extremotolerance may facilitate pathogenicevolution, but this has to be additive to other factors, such as, in the case of Chaetothyriales, oligotrophy with the ability to assimilate monoaromates (Prenafeta-Boldú et al. 2006). We therefore consider the characters listed above as primarily suited for growth on exposed surfaces under harsh climatic conditions, rather than for the capacity to evade immune cells.

In summary, we determined a group with pronounced extremotolerance among semi-arid plant-associated fungi. It is probable that all these fungi share elaborate complexes of factors, as an adaptive response to these extreme conditions (Plemenitaš & Gunde-Cimerman 2005). Having acquired a basic set of vitality factors, a shift to a different habitat with a comparable degree of stress seems to be allowed. Phylogeny thus is predictive for ecology in that overall tendencies within a single clade are similar; the shifts to other extreme conditions may be possible provided that they fit the same framework of extremotolerance. In this group of fungi, the winning strategy consists in escaping competitors by colonising selective niches.

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