

Phylogeny of rock-inhabiting fungi related to *Dothideomycetes*

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Abstract: The class *Dothideomycetes* (along with *Eurotiomycetes*) includes numerous rock-inhabiting fungi (RIF), a group of ascomycetes that tolerates surprisingly well harsh conditions prevailing on rock surfaces. Despite their convergent morphology and physiology, RIF are phylogenetically highly diverse in *Dothideomycetes*. However, the positions of main groups of RIF in this class remain unclear due to the lack of a strong phylogenetic framework. Moreover, connections between rock-dwelling habit and other lifestyles found in *Dothideomycetes* such as plant pathogens, saprobes and lichen-forming fungi are still unexplored. Based on multigene phylogenetic analyses, we report that RIF belong to *Capnodiales* (particularly to the family *Teratosphaeriaceae* s.l.), *Dothideales*, *Pleosporales*, and *Myriangiiales*, as well as some uncharacterised groups with affinities to *Dothideomycetes*. Moreover, one lineage consisting exclusively of RIF proved to be closely related to *Arthoniomycetes*, the sister class of *Dothideomycetes*. The broad phylogenetic amplitude of RIF in *Dothideomycetes* suggests that total species richness in this class remains underestimated. Composition of some RIF-rich lineages suggests that rock surfaces are reservoirs for plant-associated fungi or saprobes, although other data also agree with rocks as a primary substrate for ancient fungal lineages. According to the current sampling, long distance dispersal seems to be common for RIF. *Dothideomycetes* lineages comprising lichens also include RIF, suggesting a possible link between rock-dwelling habit and lichenisation.

Key words: *Arthoniomycetes*, *Capnodiales*, *Dothideomycetes*, evolution, extremotolerance, multigene phylogeny, rock-inhabiting fungi.

INTRODUCTION

The *Dothideomycetes* constitute the largest class of ascomycetes with approximately 19 000 species, which are currently classified in 11 orders and 90 families (Kirk *et al.* 2008). This class is ecologically diverse, with many pathogens or saprobes on plants, some coprophilous species, and a few lichen-forming fungi (Schoch *et al.* 2009b; this volume). Early studies have shown that a large part of the non-lichenised, slow-growing melanised fungi isolated from rock surfaces (here referred to as rock-inhabiting fungi) also belong to this class (Sterflinger *et al.* 1997, 1999). Subsequent sampling efforts revealed a higher diversity of species than expected for these rock-inhabiting fungi (Ruibal 2004, Ruibal *et al.* 2005, 2008, Selbmann *et al.* 2005, 2008).

Rock-inhabiting fungi (RIF) are peculiar organisms that apparently lack sexual reproductive structures and form compact, melanised colonies on bare rock surfaces (Fig. 1). Although very common, RIF have often been overlooked due to their small size, their slow growth and the lack of diagnostic features. First discovered in hot and cold deserts (Krumbein & Jens 1981, Friedmann 1982, Staley *et al.* 1982), RIF are now known to be ubiquitous on hard surfaces, in extreme as well as in temperate climates (Urzi *et al.* 1995, Sterflinger & Prillinger 2001, Gorbushina 2007, Gorbushina & Broughton 2009). RIF are well adapted to nutrient-poor and dry habitats where they are particularly successful colonisers due to restricted competition with other microbes (Gorbushina 2007) and their extremotolerance.

Extremotolerance comprises some specific universally present adaptations that enable these fungi to tolerate surprisingly wide ranges of temperatures, irradiation and osmotic stresses (Palmer *et al.* 1990, Sterflinger 1998, Gorbushina *et al.* 2003, Ruibal 2004, Onofri *et al.* 2007, Gorbushina *et al.* 2008). Melanisation protects cells against UV radiations (Dadachova & Casadevall 2008), whereas the typical isodiametrical (meristematic) growth form ensures an optimal volume : surface ratio and, therefore, allows them to survive extreme temperatures and desiccation (Wollenzien *et al.* 1995). These oligotrophic organisms are able to rely only on sparse, airborne nutrients available on rock surfaces. Their growth on these substrates is limited, and, for some of them, the production of internal asexual spores further allows to save energy. All adaptations contribute to the amazing survival capabilities of RIF in hostile habitats. The environmental tolerance of these fungi, and, in some cases, their capacity to penetrate minerals, make them an attractive subject for studies in microbial ecophysiology and applied research, such as biodeterioration of monuments and exobiology (Gorbushina *et al.* 1993, Diakumaku *et al.* 1995, Wollenzien *et al.* 1997, Gorbushina *et al.* 2002, Gorbushina 2003, Onofri *et al.* 2008).

Sterflinger *et al.* (1997) provided the first molecular evidence of RIF phylogenetic affiliations, and they are known to belong to two groups of ascomycetes, namely *Dothideomycetes* and *Eurotiomycetes* (de Hoog *et al.* 1999, Sterflinger *et al.* 1999, Ruibal 2004, Ruibal *et al.* 2005, 2008, Sert *et al.* 2007a). In *Eurotiomycetes*, multigene phylogenetic analyses have shown that



Fig 1. Rock-inhabiting fungi related to *Dothideomycetes*. A–C: sampling localities (photos C. Ruibal and L. Selbmann). A. Metamorphic black slate from Atazar, Central Mountain System, Spain. B. Limestone from Cala Sant Vicenç, Serra de Tramuntana, Mallorca, Spain. C. Sandstone from Alatna Valley, McMurdo Dry Valleys, Antarctica. D–G: *Coniosporium apollinis*, a rock-inhabiting species from the Mediterranean region (CBS 100213, photos C. Gueidan). D. Colony on MEA. E. Melanised torulose hyphae. F. Hypha disarticulating into bi- to multi-cellular clumps; G. Meristematic growth. H–J: Antarctic rock-inhabiting fungi (photos L. Selbmann). H. RIF growing on a crystal of sandstone. I. Melanised hypha of *Friedmanniomyces endolithicus*. J. Meristematic growth of *Cryomyces antarcticus*. K–L: *Cystocoleus ebeneus*, a lichenised species assigned to *Capnodiales* (photos L. Muggia). K. Microfilamentous thallus. L. Melanised hyphae of the mycobiont forming a furrow around the filamentous algae. Scale bars: D = 2 mm, E–G and I–J = 10 μ m, H = 0.5 mm, K–L = 20 μ m.

RIF cluster in early diverging lineages of *Chaetothyriales*, whereas two species seem to be more closely related to the lichenised order *Verrucariales*, the sister group of *Chaetothyriales* (Gueidan *et al.* 2008). Gueidan *et al.* (2008) also demonstrated that the most recent common ancestor of both lichenised *Verrucariales* and pathogen-rich *Chaetothyriales* was probably a rock-inhabiting fungus. It was hypothesised that adaptations to life in extreme conditions might have been a prerequisite for the evolution of human pathogenicity (de Hoog 1993, Haase *et al.* 1999, Gueidan *et al.* 2008) and lichenisation in this class (Gueidan *et al.* 2008). In contrast, despite the high diversity of RIF within *Dothideomycetes*, only very few human pathogens are known from this class of *Ascomycota* (de Hoog *et al.* 2000). Alternatively, associations with plants and in particular plant pathogenicity are very common (Schoch *et al.* 2006, Arzanlou *et al.* 2007, Crous *et al.* 2007a–c, 2009; this volume). Additionally, lichenised species also appeared to be nested within *Dothideomycetes* (Lutzoni *et al.* 2004, James *et al.* 2006, Del Prado *et al.* 2006, Muggia *et al.* 2008, Nelsen *et al.* 2009). Presently no strong phylogenetic hypothesis is available to assess the placement of RIF within *Dothideomycetes*. Moreover, no studies have investigated phylogenetic relationships among RIF, lichen-forming fungi and plant-associated fungi within *Dothideomycetes*. Our main goal was to infer phylogenetic relationships of RIF within *Dothideomyceta*, a lineage including *Dothideomycetes* and *Arthoniomycetes*, to explore more specifically their diversity, origins and evolution.

MATERIAL AND METHODS

Taxon and gene sampling

Representative taxa of most of the main orders and families of *Dothideomyceta* (*Dothideomycetes* and *Arthoniomycetes*) were sampled. Two separate sets of data matrices were assembled. The first set (three-gene analysis; Table 1 - see online Supplementary Information) is composed of 182 taxa (including 102 rock-inhabiting strains) for which DNA sequences of three ribosomal genes have been obtained: the large and small subunits of the nuclear ribosomal RNA gene (nucLSU and nucSSU, respectively) and the small subunit of the mitochondrial ribosomal RNA gene (mtSSU). Because this first set of data matrices included only ribosomal genes, low phylogenetic confidence was expected for deep relationships within *Dothideomyceta*. To overcome this problem, a second set of data matrices was assembled (five-gene analysis; Table 1 in Supplementary Information) consisting of DNA

sequences of five loci from 113 taxa (including 40 rock-inhabiting strains): the largest and second largest subunits of the RNA polymerase II (*RPB1* and *RPB2*, respectively), nucLSU, nucSSU and mtSSU. The outgroup for the three-gene analysis included *Hypozygma lignicola*, *Symbiotaphrina buchneri* and *S. kochii*, whereas only the latter two species were selected as outgroup for the five-gene analysis. These species were chosen because they constituted a sister group to *Dothideomyceta* in a previous study (Schoch *et al.* 2009a).

DNA isolation and sequencing

Different laboratories contributed data using various protocols, but most DNA sequence information was produced as follows: genomic DNA was isolated from cultures grown on MEA. Fungal biomass was transferred to a tube with 500 μ L of TES buffer and ground with a micro-pestle for 1–2 min, with or without silica-mix (2/3 silica-gel, 1/3 Celite® 545). A volume of 140 μ L of 5 M NaCl was then added, followed by 65 μ L of 10 % (w/v) CTAB (cetyltrimethylammoniumbromid). After an incubation of 30 min at 65 °C, 700 μ L of (24:1) chloroform/isoamylalcohol was added, the tubes were mixed carefully by hand, stored on icy water for 30 min, and centrifuged for 10 min at 4 °C (10 000 \times g). The supernatant was recovered and the genomic DNA precipitated using isopropanol. After washing the pellets with 70 % ethanol, they were dried in a vacuum centrifuge and re-suspended in 60 μ L of TE buffer (protocol modified from Möller *et al.* 1992).

Six regions covering five genes were amplified: nucLSU, nucSSU, mtSSU, *RPB1* region A–D, *RPB2* region 5–7, and *RPB2* region 7–11 (see Table 2 for primers used). Genomic DNA (1 μ L of a 1/10 or 1/100 dilution) was added to a PCR mix comprising 2.5 μ L of PCR buffer (buffer IV with 15 mM MgCl₂, Abgene, Epsom, U.K.), 2.5 μ L of dNTPs (2 mM), 2.5 μ L of BSA (10 mg/mL), 2.0 μ L of primers (10 μ M), 0.15 μ L *Taq* polymerase (5 U/ μ L, Denville, Metuchen NJ, U.S.A.), and water for a total volume of 25 μ L. Amplification cycles for nucLSU, nucSSU and *RPB1* (same conditions applied for *RPB2*) are described in Gueidan *et al.* (2007), and in Zoller *et al.* (1999) for mtSSU. The PCR products were purified using Microcon PCR cleaning kits (Millipore, Billerica MA, U.S.A.). Sequencing was carried out using Big Dye Terminator Cycle sequencing Kits (ABI PRISM version 3.1, Perkin-Elmer, Applied Biosystems) on ABI 3730xl DNA Analyzers (Applied Biosystems, Foster City CA, U.S.A.) from the Duke Center for Evolutionary Genomics (Durham NC, U.S.A.) and the Hubrecht Institute (Utrecht, Netherlands).

Table 2. List of primers for the five genes used in this study (*RPB2* was amplified in two regions).

Gene regions	PCR primers	Additional primers used for sequencing
nucLSU	LR0R ^a , LR7 ^a	LR3, LR3R, LR5, LR5R, LR6, LR6R ^a
nucSSU	nssu131 ^c , NS24 ^d	nssu1088, nssu1088R, nssu897R, nssu634 ^c , SR11R ^e , NS23, NS22 ^d , SR7R, SR7, SR10R ^f
mtSSU	mtSSU1, mtSSU3R ^g	mtSSU2, mtSSU2R ^g
<i>RPB1</i> region A–D	<i>RPB1</i> -AF ^h , <i>RPB1</i> -6R1asc ⁱ	–
<i>RPB2</i> region 5–7	<i>RPB2</i> -5F, <i>RPB2</i> -7cR ⁱ	–
<i>RPB2</i> region 7–11	<i>RPB2</i> -7cF, <i>RPB2</i> -11aR ⁱ	–

^aRehner & Samuels (1994), ^bVilgalys & Hester (1990), ^cKauff & Lutzoni (2002), ^dGargas & Taylor (1992), ^eSpatafora *et al.* (1995), ^fVilgalys (unpubl.; www.biology.duke.edu/fungi/mycolab/primers.htm), ^gZoller *et al.* (1999), ^hHall (unpubl.; <http://faculty.washington.edu/benhall/>), ⁱHofstetter *et al.* (2007), ^jLiu *et al.* (1999).

Alignments and phylogenetic analyses

Sequences were assembled and edited using Sequencher (Gene Codes Corporation, Ann Arbor MI, U.S.A.). Manual alignments were performed using MacClade v. 4.08 (Maddison & Maddison 2003). Ambiguous regions (*sensu* Lutzoni *et al.* 2000) and introns were delimited manually and excluded from the alignments. Congruence was tested using a 70 % reciprocal bootstrap criterion (Mason-Gamer & Kellogg 1996, Reeb *et al.* 2004). For the three-gene dataset, the test was performed using Compat (Kauff & Lutzoni 2002) on all possible gene pairs (mtSSU vs. nucSSU, mtSSU vs. nuLSU, and nuLSU vs. nucSSU) and based on bootstrap consensus trees. Bootstrap trees were obtained using Neighbor-Joining bootstrap analyses with Maximum Likelihood distances in PAUP v. 4.0b10 (Swofford 2003). Models of molecular evolution were estimated using the Akaike Information Criterion implemented in Modeltest v. 3.7 (Posada & Crandall 1998). For the five-gene dataset, congruence was also tested using a 70 % reciprocal bootstrap criterion, but the comparison was done manually based on trees obtained with 500 bootstrap replicates using RAxML VI-HPC (Stamatakis *et al.* 2005, 2008) on the Cipres Web Portal (www.phylo.org/sub_sections/portal/). Taxa or sequences responsible for incongruence were removed from the dataset, and the markers were combined. Final phylogenetic analyses of the three-gene and five-gene datasets were performed using RAxML on the Cipres Web Portal. The ML search followed a GTRMIX model of molecular evolution applied to the following nine partitions: *RPB1* first, second and third codon positions, *RPB2* first, second and third codon positions, nuLSU, nucSSU and mtSSU. Support values were obtained with bootstrap analyses of 1 000 pseudoreplicates using RAxML.

RESULTS

DNA sequence alignments

Not all markers were recovered or available for all taxa. For the three-gene dataset, 20 nuLSU, 11 nucSSU and 54 mtSSU sequences were missing. Among the 182 taxa, 119 had sequences for three genes, 61 for two genes, and 12 for one gene (Table 1 in Supplementary Information). After exclusion of ambiguous regions and introns, the combined dataset included 3 274 characters (1 106 for nuLSU, 1 616 for nucSSU and 552 for mtSSU). Among these, 2 063 were constant while 931 were parsimony-informative. For the five-gene dataset, missing data comprised 5 nuLSU, 8 nucSSU, 30 mtSSU, 48 *RPB1* and 30 *RPB2* sequences. Among the 113 taxa, 32 had sequences for five genes, 46 for four genes, 30 for 3 genes, and 5 for 2 genes (Table 1 in Supplementary Information). After exclusion of ambiguous regions and introns, the combined dataset included 6 045 characters (1 133 for nuLSU, 1 607 for nucSSU, 593 for mtSSU, 1 011 for *RPB1* and 1 701 for *RPB2*). Among these, 2 912 were constant while 2 693 were parsimony-informative.

Phylogenetic inference

For the three-gene analysis (Figs 2–3), results show that, within the two classes *Dothideomycetes* and *Arthoniomycetes*, rock-inhabiting fungi belong to 13 groups, either well-known orders or families, or lineages that have not previously been characterised. Among the rock-inhabiting fungi clustering with well-known groups of *Dothideomycetes*, two strains are found in the order *Dothideales*, four in the order *Pleosporales*, one in *Myriangiales*, 12 forming a monophyletic group sister to the remaining members of *Davidiellaceae*, and one in the family *Capnodiaceae*. The family *Teratosphaeriaceae* is not monophyletic in this analysis (also see Crous *et al.* 2009; this volume). In a first group including the generic type *Teratosphaeria fibrillosa* (*Teratosphaeriaceae* 1, Fig. 3), many rock-inhabiting strains are present, including taxa from the three genera *Friedmanniomyces*, *Elasticomyces* and *Recurvomyces*. The second group (*Teratosphaeriaceae* 2, Fig. 3), including the three leaf-colonising species *Devriesia strelitziae*, *Mycosphaerella eurypotami* and *Triposphermum myrti*, an unknown species of *Capnodiales*, the lichen species *Cystocoleus ebeneus* as well as 20 undescribed rock inhabiting strains, is supported as sister to the family *Mycosphaerellaceae* (91 % bootstrap). The two rock-inhabiting species *Coniosporium uncinatum* and *C. apollinis* are well supported (100 % bootstrap), but their sister relationship is not. Neither these two species of *Coniosporium* nor the Antarctic genus *Cryomyces* can be assigned to any known family or order sampled here. Amongst the unknown lineages, one does not seem to be part of *Dothideomycetes* (lineage 1, Fig. 2), and appears as sister to *Arthoniomycetes* (98 % bootstrap). Due to the lack of support for many deep internodes, it is not possible to determine if lineages 2 and 3 can be accommodated by the expansion of known groups of *Dothideomycetes*, or if the recognition of new taxonomical entities are needed. Finally, the rock isolates A6, AN13, TRN 437 and CCFFEE 5413 do not significantly cluster with any other taxa.

With the five-gene analysis (Fig. 4), the inferred deep branching pattern within *Dothideomycetes* is still poorly supported, but additional well-supported nodes are recovered (e.g., *Capnodiaceae* as sister to the lineage including *Mycosphaerellaceae* and *Teratosphaeriaceae*, and the monophyly of *Teratosphaeriaceae* 1). As in the three-gene analysis, the sister relationship between lineage 1 and *Arthoniomycetes* obtains high support (100 % bootstrap), even though the two rock-inhabiting strains included do not seem to form a monophyletic group. The placement of the lichen family *Trypetheliaceae* as sister to *Arthoniomycetes* (70 % bootstrap) might be an artifact, as this relationship was not recovered in any other studies (Del Prado *et al.* 2006, Spatafora *et al.* 2006, Nelsen *et al.* 2009). Within *Dothideomycetes*, the orders *Dothideales* and *Myriangiales* form a sister group (100 % bootstrap), and are sister to the well-supported *Capnodiales* (100 % bootstrap), which includes most of the rock-inhabiting strains. Within *Capnodiales*, the second group of *Teratosphaeriaceae* (*Teratosphaeriaceae* 2; Fig. 4) is still supported as sister to *Mycosphaerellaceae* (89 % bootstrap). Other lineages comprising exclusively RIF (*Cryomyces*, *Coniosporium uncinatum*, and *C. apollinis*) do not significantly cluster with any known group of *Dothideomycetes*.

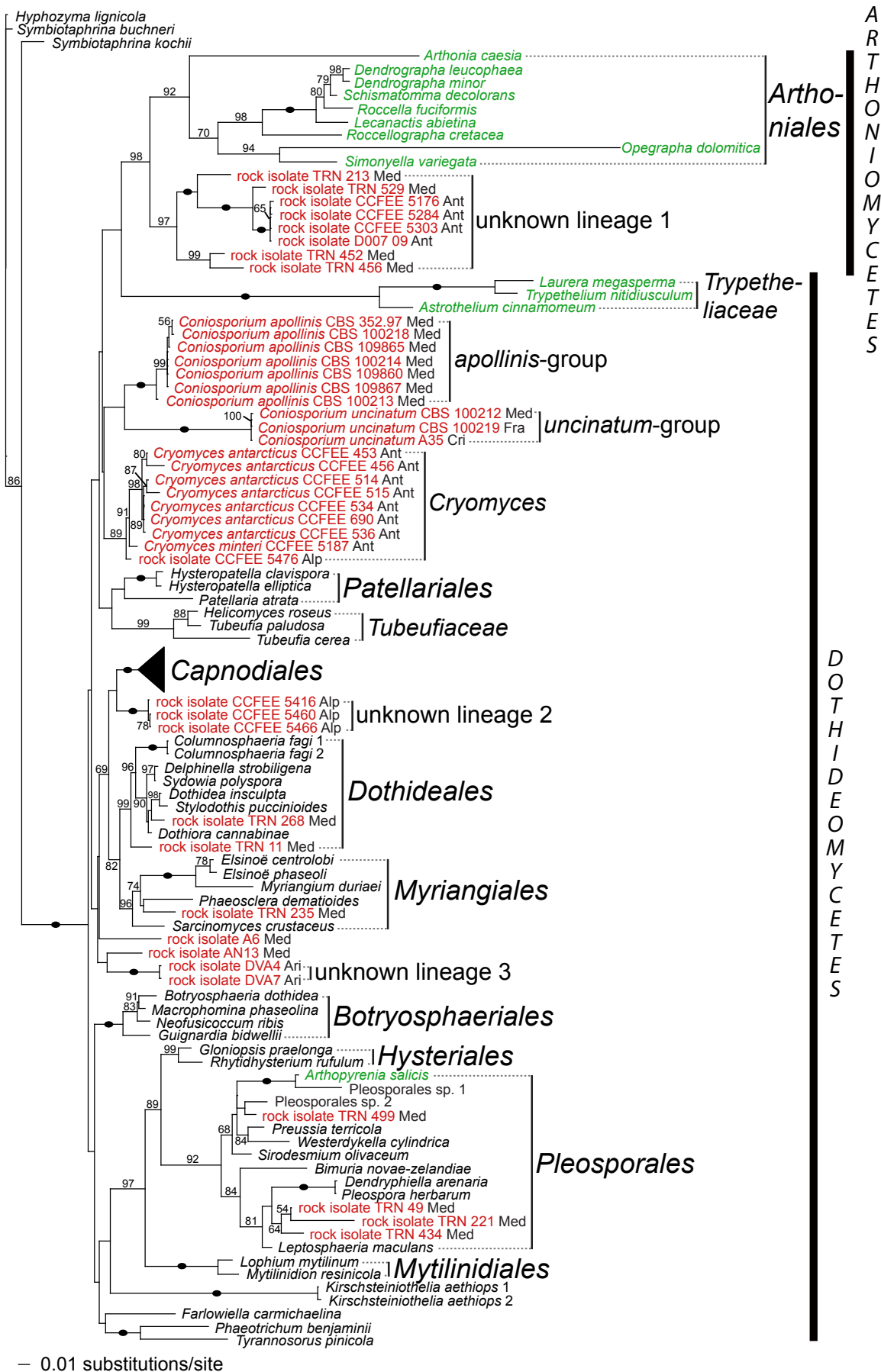


Fig. 2. Phylogenetic placement of 102 rock-inhabiting strains within *Dothideomyceta* (*Dothideomycetes* and *Arthoniomycetes*). The tree is based on a Maximum Likelihood analysis of the combined nuLSU, nucSSU and mtSSU (three-gene analysis). A black oval on a branch indicates a bootstrap support value of 100 %. Other bootstrap values ≥ 50 % are shown below or above branches. RIF are highlighted in red and lichens in green. Geographical origins are also labeled for RIF (Alp = Alps, And = Andes, Ant = Antarctica, Ari = Arizona desert, Cri = Crimea, Fra = France, Med = Mediterranean region, including Greece, Israel, Italy, Slovenia, Spain and Turkey). Phylogenetic relationships within *Capnodiales* are detailed in Fig. 3.

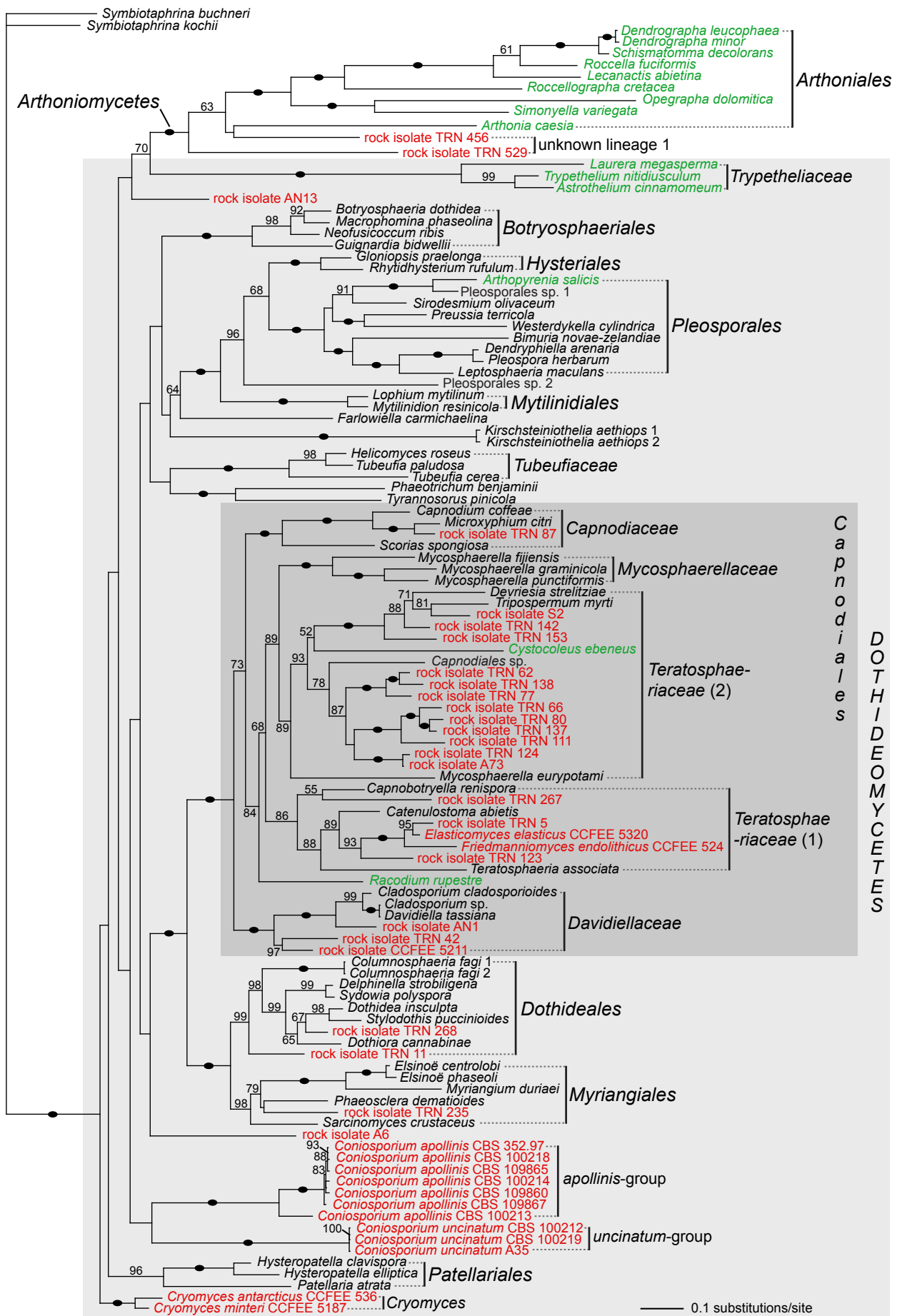


Fig. 4. Phylogenetic relationships of rock-inhabiting lineages with known groups of Dothideomyceta based on a Maximum Likelihood analysis of the combined nuclSU, nucSSU, mtSSU, *RPB1* and *RPB2* (five-gene analysis). A black dot on a branch indicates a bootstrap support value of 100 %. Other bootstrap values ≥ 50 % are shown below or above the branches. RIF are highlighted in red and lichens in green.

DISCUSSION

Species diversity in *Dothideomycetes*

The *Dothideomycetes* are very diverse in term of species, some of which are well known for their pathogenicity on crops (e.g., *Mycosphaerella fijiensis*, the agent of the leaf spot disease of banana, or *Leptosphaeria maculans*, the agent of the blackleg disease of cabbage). Whilst many species are associated with plants (either as pathogens or as epiphytes), saprobic, coprophilous, lichen-forming and rock-inhabiting fungi are also present in this class. The importance of RIF in term of species richness is still under-investigated. A thorough sampling of dothideomycetous RIF from few localities in Mallorca and Central Spain formed the basis of the analyses described here (Ruibal 2004, Ruibal *et al.* 2005, 2008). RIF from Antarctica, the Alps and the Andes (Selbmann *et al.* 2005, 2008), as well as the Arizona and Negev deserts (Staley *et al.* 1982, A.A. Gorbushina, unpubl. data) extended the geographical range of the sampled taxa. Finally, isolates from monuments in the Mediterranean area supplemented the sampling (Gorbushina *et al.* 1996, Sterflinger *et al.* 1997, Volkmann & Gorbushina 2006). In comparison to known RIF habitats (Gorbushina 2007), our sampling was very restricted and does not permit a realistic overview of fungal diversity on rock surfaces. Nevertheless, an impressive number of rock-inhabiting species is already evident. Our data show that rock-inhabiting fungi are not only present in well-known orders, such as *Capnodiales* or *Pleosporales*, but also in novel lineages (e.g., lineage 1, Fig. 2). Moreover, very few species with overlapping distribution were recovered from neighbouring geographical localities in Mallorca and Central Spain (Ruibal *et al.* 2005, 2008). Therefore, we can hypothesise that species richness within *Dothideomycetes* remains woefully underestimated, and that many more species will need to be described within this class in the future, especially for fungi colonising rocky substrates.

Classification of rock fungi related to *Dothideomycetes*

Although very diverse within *Dothideomycetes*, RIF have not been included in recent phylogenetic studies of this class (Lumbsch *et al.* 2001, Schoch *et al.* 2006). Only very few of these rock-inhabiting species have been taxonomically described (Sterflinger *et al.* 1997, Bills *et al.* 2005, Sert *et al.* 2007b), and the molecular marker available for most of these species (ITS) does not allow their inclusion in large-scale phylogenetic analyses. The few attempts to produce phylogenies involving RIF have shown that they belong to two diverse classes of *Ascomycota*, namely *Eurotiomycetes* (particularly the order *Chaetothyriales*) and *Dothideomycetes* (preponderantly the orders *Capnodiales*, *Dothideales* and *Pleosporales*) (Sterflinger *et al.* 1999, Ruibal 2004, Ruibal *et al.* 2005, 2008).

Our results confirm the placement of RIF in the same orders of *Dothideomycetes*, although some lineages are shown to belong to additional groups. Based on our results, many RIF should be classified within *Dothideales*, *Pleosporales* and *Capnodiales*, the latter order holding the largest number in rock-colonising species. The genera *Elasticomyces* and *Recurvomyces*, as well as the Antarctic genus *Friedmanniomyces*, were previously attributed to *Capnodiales* based on nucSSU data (Selbmann *et al.* 2008). Our multigene analyses confirm this placement, and show that these three genera belong to *Teratosphaeriaceae* s. str., the family currently showing the highest diversity in RIF (Fig. 3). We

also showed that one RIF (TRN 235) previously thought to be related to the *Dothideales* (Ruibal *et al.* 2008) actually belongs to *Myriangiiales*, along with *Sarcinomyces crustaceus*, a species similarly melanised and meristematic, but isolated from plant material (Sigler *et al.* 1981).

Several well-supported groups of RIF could not be attributed to any known families and orders according to our data. As a consequence, *Cryomyces* should still be considered as *Dothideomycetes incertae sedis*, as no close relationship was recovered for this enigmatic Antarctic genus (Selbmann *et al.* 2005). The positions of RIF-rich genera *Coniosporium* and *Sarcinomyces* are also problematic. Previous studies placed them either in *Dothideales* or *Chaetothyriales* based on ITS or nucSSU data (de Leo *et al.* 1999, Sterflinger *et al.* 1999, Sert *et al.* 2007a). Yet, the limited taxon and gene sampling on which these analyses were based was probably insufficient to demonstrate clear phylogenetic relationships. Our results show that *Coniosporium apollinis* (including the type strain CBS 352.97), *C. uncinatum* (including the type strain CBS 100219) and *Sarcinomyces crustaceus* belong to *Dothideomycetes* (Fig. 4). However, a previous multigene analysis showed that two other species, *Coniosporium perforans* and *Sarcinomyces petricola*, belong to *Chaetothyriales* (Gueidan *et al.* 2008). These anamorphic genera are therefore not monophyletic, and additional research is required to clarify their status.

Among lineages lacking known reference taxa, two groups seem to belong to *Dothideomycetes* (unknown group 2, a lineage comprising RIF from the Alps, and unknown group 3, a lineage including strains isolated in Arizona; Fig. 2). Another unknown group (lineage 1) clusters outside *Dothideomycetes*, sister to the *Arthoniales* (Figs 2, 4). A previous study had noted the problematic placement of this latter group (Ruibal *et al.* 2008). Many lineages including RIF still need to be named. In the past, several melanised meristematic species and genera have been described such as *Lichenothelia* (Hawksworth 1981; see also Henssen 1987), which could potentially correspond to some of these RIF lineages. However, little is known about these formerly named taxa, and no molecular data or cultures are available for many of them. Naming RIF will therefore require an extensive study of both rock-inhabiting species and formerly described melanised meristematic species, whether they grow on rock or not.

Rock surfaces: “terroirs” for ancient lineages or reservoirs for plant-associated fungi?

Despite the prevailing extreme conditions, rock surfaces host a large variety of specialised fungi. Fungal colonisation of subaerial rocks can be explained by two non-exclusive hypotheses. Firstly, atmosphere-exposed rock substrates could constitute “terroirs” for ancient fungal lineages. Rock surfaces were among the first terrestrial substrates available for living organisms on earth (Gorbushina & Broughton 2009). It is therefore likely that, early on, some species became adapted to colonise rock surfaces. RIF are persistent to different types of physical stress, but are poor competitors and surrender to more combative organisms (Gorbushina *et al.* 2008). Increasing competition with other rock-inhabiting organisms living under more permissive conditions may have restricted some of these ancient, morphologically reduced, slow-growing, fungal relicts to extreme habitats. The presence of lineages comprising exclusively RIF that diverged early in the evolution of *Dothideomycetes* (e.g., *Cryomyces* and lineage 1, Fig. 2) supports this hypothesis of rock surfaces as substrates for ancient fungal lineages.

Secondly, rock surfaces could form reservoirs for plant-associated or saprobic fungi. Through spore or propagule dispersal, some species of various unrelated groups of plant pathogens, epiphytes or saprobes can reach rock substrates. Their ability to survive in these environments will depend on some key features, namely oligotrophy, melanisation and pleiomorphism (or diversity of growth forms, amongst which meristematic growth). Under extreme conditions prevailing on rock surfaces, fungi possessing these key features can survive due to their slow, meristematic, clumpy growth and thick-walled, heavily melanised cells. These key features seem to have evolved several times in *Dothideomycetes*, allowing different lineages to colonise rock substrates. In *Dothideales*, phyllosphere fungi such as *Aureobasidium pullulans* and relatives, which have a filamentous or yeast-like growth under moist conditions, but convert to a meristematic form when colonising inert substrates, have also been isolated from rock surfaces (Ruibal *et al.* 2008). The family *Teratosphaeriaceae* s. l. is another example of a group in which some leaf-colonising species can also grow meristematically and form dark, thick-walled cells. According to our results, this family (as traditionally delimited; *i.e.*, including *Teratosphaeriaceae* 1 and 2) is also extremely diverse in RIF (Fig. 3). Rocks supporting growth of subaerial biofilms (Gorbushina & Broughton, 2009) may be viewed as a reservoir for all types of melanised meristematic fungi, from where other habitats can be re-colonised. Survival of new comers is probably additionally facilitated by the existing microbial community on rocks (Gorbushina & Broughton 2009) in a fashion known for immigrant bacteria on leaf surfaces (Monier & Lindow 2005).

Alternatively, rock-colonising lichens may supply buffered environments and refugia for RIF or organisms otherwise occupying other niches (Selbmann *et al.* 2010). Recent studies have shown that lichens harbour an amazing diversity of ascomycetous endophyte-like (endolichenic) fungi (Arnold *et al.* 2009), and phylogenetic relatedness was found between some endolichenic fungi isolated from saxicolous lichens and RIF (Harutyunyan *et al.* 2008). If in most cases, species from rock surfaces can still go back to their primary habitats, in some cases, these fungi keep specialising and get trapped in these extreme habitats. This may be the case for groups with no close relationships with plant-associated fungi, such as the genus *Friedmanniomyces* (Fig. 3).

Geographical distribution of rock-inhabiting fungi

The large majority of rock-inhabiting strains isolated thus far originated from rocks in the Mediterranean region or Antarctica (Sterflinger *et al.* 1999, Ruibal 2004, Ruibal *et al.* 2005, 2008 Selbmann *et al.* 2005, 2008). In Antarctica, RIF tend to grow within rocks, together with the cryptoendolithic lichen communities, finding shelter from extreme conditions prevailing on rock surfaces. In the Mediterranean area, RIF tend to grow on the rock surface or in cracks, causing damages to the substrate (*e.g.*, biopitting of marble). Despite differences in temperature, they share similar morphological and physiological adaptations, such as melanisation, meristematic growth and oligotrophism.

Similarly to previous studies (Selbmann *et al.* 2005, Ruibal *et al.* 2008), our results show that Antarctic RIF often share an evolutionary history with RIF from semi-arid areas. In our study, RIF sampled in geographically disjoint localities (Antarctica versus Mediterranean region) cluster together in *Davidiellaceae*, the two groups of *Teratosphaeriaceae*, and unknown lineage 1 (Figs 2–3). In some cases, Antarctic and Mediterranean strains are even phylogenetically very closely related, showing a recent

common evolutionary history (*e.g.*, in *Teratosphaeriaceae* 2, the Mediterranean rock isolates TRN 124 and A73 with the Antarctic strain CCFEE 5489). Likewise, some strains of *Recurvomyces mirabilis* and *Elasticomyces elasticus* have been recorded in the Antarctic as well as in high peaks of the Alps and Andes (Selbmann *et al.* 2008). Therefore, it seems that an efficient mechanism of dispersal, most probably wind-mediated (Gorbushina *et al.* 2007, Gorbushina & Broughton 2009), have led to a colonisation spanning different continents.

Rock-dwelling habit and evolution of lichenisation

Most of the diversity in lichen-forming fungi is found in *Lecanoromycetes*, a large and diverse class of ascomycetes including approximately 14 000 species (Miadlikowska *et al.* 2006, Kirk *et al.* 2008). Yet, the classes *Lichinomycetes* (with the single order *Lichinales*), *Eurotiomycetes* (with the orders *Pyrenulales* and *Verrucariales*), *Arthoniomycetes* (with the single order *Arthoniales*), and *Dothideomycetes* also include lichens. Although *Lichinales*, *Pyrenulales*, *Verrucariales* and *Arthoniales* are monophyletic lineages containing mostly lichenised species, lichens in *Dothideomycetes* seem to encompass a broader phylogenetic spectrum: the *Trypetheliaceae*, a family of mostly tropical bark-colonising lichens, forms a monophyletic group within *Dothideomycetes* (Del Prado *et al.* 2006, Nelsen *et al.* 2009, Schoch *et al.* 2009a). *Arthopyrenia salicis*, a corticolous, temperate lichen species nests within the order *Pleosporales* (Del Prado *et al.* 2006, Nelsen *et al.* 2009). Two melanised micro-filamentous lichens, *Cystocoleus ebeneus* and *Racodium rupestre*, were assigned to the order *Capnodiales* (Muggia *et al.* 2008, Nelsen *et al.* 2009). Finally, the two lichen families *Strigulaceae* (mostly leaf-colonising tropical species) and *Monoblastiaceae* (temperate and tropical species) are now shown to belong to *Dothideomycetes* (Nelsen *et al.* 2009; this volume).

Whether these lichen lineages, that are unrelated to *Lecanoromycetes*, originated from independent gains of lichenisation is not clear (Lutzoni *et al.* 2001, James *et al.* 2006, Gueidan *et al.* 2008, Arnold *et al.* 2009, Schoch *et al.* 2009a, b). Within *Eurotiomycetes*, phylogenetic data suggest that the lineage including *Pyrenulales* and *Verrucariales* possibly results from an independent gain of lichenisation (Gueidan *et al.* 2008, Schoch *et al.* 2009a). Phylogenetic data suggest that lichens in *Verrucariales* may have evolved from rock-inhabiting fungi (Gueidan *et al.* 2008), a result in agreement with experimental data demonstrating that some RIF and one melanised lichen-colonising fungus could form associations with lichen-associated algae (Gorbushina *et al.* 2005, Brunauer *et al.* 2007). This rock-inhabiting ancestor may have evolved associations with epilithic microalgae in order to get a more constant supply in nutrients. If the evolution of fungal-algal associations occurred in *Eurotiomycetes*, it most likely also occurred in different fungal groups. It is therefore interesting to see if in *Dothideomycetes*, where rock fungi are so diverse, similar transitions in lifestyles can be suggested.

Although many lichenised species in *Dothideomycetes* are either corticolous or only secondarily or occasionally saxicolous, *Cystocoleus ebeneus* and *Racodium rupestre* are true rock inhabitants. Amongst lichens in *Dothideomycetes*, these two species are the most likely to have evolved from a rock-inhabiting ancestor. They share substrate preference and some morphological features, such as their melanised hyphae, with RIF. Strikingly, in our result, *Cystocoleus ebeneus* is nested within a lineage comprising almost exclusively RIF (*Teratosphaeriaceae* 2, Fig. 3).

Racodium rupestre is also related to a RIF, but this relationship is not supported (Fig. 3). This result agrees with a rock-inhabiting ancestor for these two lichenised species, but further data will however be necessary to test this hypothesis. Also of interest is the close phylogenetic relationship between the lichen order *Arthoniales* and the lineage 1 of RIF (Figs 2, 4). Although mostly corticolous or foliicolous, *Arthoniales* also comprises saxicolous species (Ertz *et al.* 2009). Further data is needed to explore the relationships between saxicolous species of *Arthoniales* and RIF. In conclusion, these preliminary results suggest that there might be a link between rock-dwelling habit and lichenisation. However, additional taxon and gene sampling are needed to confirm the phylogenetic placements of some of the lichenised taxa and to clarify their relationships to RIF. Only then the hypothesis of RIF as ancestors of lichenised lineages can be adequately tested.

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SUPPLEMENTARY INFORMATION

Table 1. Taxon and gene sampling for the three- and five- gene analyses. Geographical origins are also mentioned for RIF. A dash indicates missing sequences. Newly produced sequences are shown in bold. A column also indicates if taxa were included in the three-gene (3) or in both three- and five-gene analyses (3 & 5).

Taxon	Collection #	Additional information	Order	LSU	SSU	mtSSU	RPB2	RPB1	Analysis
<i>Hyphozyma lignicola</i>	CBS 325.93	Outgroup		AF353595	AJ496239	-			3
<i>Symbiotaphrina buchneri</i>	CBS 6902	Outgroup, AFTOL 1836		FJ176887	FJ176831	-	FJ238370	FJ238442	3 & 5
<i>Symbiotaphrina kochii</i>	CBS 250.77	Outgroup, AFTOL 1902		AY227719	FJ176833	-	GU597369	FJ238443	3 & 5
Arthoniomycetes									
<i>Arthonia caesia</i>	-	AFTOL 775	Arthoniales	FJ469668	-	FJ469671	FJ469670	FJ772241	3 & 5
<i>Dendrographa leucophaea</i>	-	AFTOL 308	Arthoniales	AY548810	AY548803	AY548811	EU704017	-	3 & 5
<i>Dendrographa minor</i>	-	AFTOL 355	Arthoniales	AF279382	AF279381	GU561843	AY641034	GU561849	3 & 5
<i>Lecanactis abietina</i>	-	AFTOL 305	Arthoniales	AY548812	AY548805	AY548813	AH013900	GU561850	3 & 5
<i>Opegrapha dolomitica</i>	-	AFTOL 993	Arthoniales	-	DQ883706	-	DQ883714	DQ883717	3 & 5
<i>Roccella fuciformis</i>	-	AFTOL 126	Arthoniales	AY584654	AY584678	EU704082	DQ782866	-	3 & 5
<i>Roccellographa cretacea</i>	-	AFTOL 93	Arthoniales	DQ883696	DQ883705	FJ772240	DQ883713	DQ883716	3 & 5
<i>Schismatomma decolorans</i>	-	AFTOL 307	Arthoniales	AY548815	AY548809	AY548816	DQ883715	-	3 & 5
<i>Simonyella variegata</i>	-	AFTOL 80	Arthoniales	-	AY584669	AY584631	DQ782861	DQ782819	3 & 5
Dothideomycetes									
<i>Botryosphaeria dothidea</i>	CBS 115476	AFTOL 946	Botryosphaeriales	DQ678051	DQ677998	FJ190612	DQ677944	EU186063	3 & 5
<i>Guignardia bidwellii</i>	CBS 237.48	AFTOL 1618	Botryosphaeriales	DQ678085	DQ678034	-	DQ677983	-	3 & 5
<i>Macrophomina phaseolina</i>	CBS 227.33	AFTOL 1783	Botryosphaeriales	DQ678088	DQ678037	FJ190645	DQ677986	-	3 & 5
<i>Neofusicoccum ribis</i>	CBS 115475	AFTOL 1232	Botryosphaeriales	DQ678053	DQ678000	-	DQ677947	-	3 & 5
<i>Capnodium coffeae</i>	CBS 147.52	AFTOL 939	Capnodiales, Capnodiaceae	DQ247800	DQ247808	FJ190609	DQ247788	DQ471162	3 & 5
<i>Capnodium salicinum</i>	CBS 131.34	AFTOL 937	Capnodiales, Capnodiaceae	DQ678050	DQ677997	-	-	-	3
<i>Microxyphium citri</i>	CBS 451.66		Capnodiales, Capnodiaceae	GU301848	GU296177	-	GU371727	GU357750	3 & 5
<i>Scorias spongiosa</i>	CBS 325.33	AFTOL 1594	Capnodiales, Capnodiaceae	DQ678075	DQ678024	FJ190643	DQ677973	-	3 & 5
<i>Cladosporium cladosporioides</i>	CBS 170.54	AFTOL 1289	Capnodiales, Davidiellaceae	DQ678057	DQ678004	FJ190628	DQ677952	EU186064	3 & 5
<i>Cladosporium sp.</i>	CBS 180.53	AFTOL 1035	Capnodiales, Davidiellaceae	AY016367	AY016351	AY350576	DQ677945	-	3 & 5
<i>Davidiella tassiana</i>	CBS 399.80	AFTOL 1591	Capnodiales, Davidiellaceae	DQ678074	DQ678022	-	DQ677971	-	3 & 5
<i>Cercospora beticola</i>	CBS 116456	AFTOL 1788	Capnodiales, Mycosphaerellaceae	DQ678091	DQ678039	FJ190647	-	-	3
<i>Mycosphaerella fijjensis</i>	OSC 100622	AFTOL 2021	Capnodiales, Mycosphaerellaceae	DQ678098	DQ676652	FJ190656	DQ677993	-	3 & 5
<i>Mycosphaerella graminicola</i>	CBS 292.38	AFTOL 1615	Capnodiales, Mycosphaerellaceae	DQ678084	DQ678033	DQ677982	DQ677982	-	3 & 5
<i>Mycosphaerella punctiformis</i>	CBS 113265	AFTOL 942	Capnodiales, Mycosphaerellaceae	DQ470968	DQ471017	FJ190611	DQ470920	DQ471165	3 & 5

Table 1. (Continued).

<i>Dothideomycetes</i>	Collection #	Additional Information	Order	LSU	SSU	mtSSU	RPB2	RPB1	Analysis
<i>Capnobotryella renispora</i>	CBS 214.90		Capnodiales, Teratosphaeriaceae	EU019248	Y18698	-	-	-	3 & 5
<i>Catenulostroma abietis</i>	CBS 459.93	AFTOL 2210	Capnodiales, Teratosphaeriaceae	DQ678092	DQ678040	FJ190648	-	GU357797	3 & 5
<i>Catenulostroma microsporium</i>	CBS 110890; CPC 1832		Capnodiales, Teratosphaeriaceae	EU019255	GU214520	-	-	-	3
<i>Hortaea werneckii</i>	CBS 107.67	mtSSU from CBS 708.76	Capnodiales, Teratosphaeriaceae	EU019270	Y18693	GU561844	-	-	3
<i>Teratosphaeria associata</i>	CBS 112224	ex <i>Teratosphaeria fibrillosa</i>	Capnodiales, Teratosphaeriaceae	GU301874	GU296200	-	-	GU357744	3 & 5
<i>Teratosphaeria destructans</i>	CBS 111370		Capnodiales, Teratosphaeriaceae	GU214702	GU214702	-	-	-	3
<i>Teratosphaeria juvenalis</i>	CBS 110906		Capnodiales, Teratosphaeriaceae	AY720715	FJ493217	-	-	-	3
<i>Capnodiales</i> sp. 1	CBS 101364	ex <i>Anisomeridium consobrinum</i>	Capnodiales, incertae sedis	GU323215	GU561840	-	GU561853	-	3 & 5
<i>Devriesia streitziiae</i>	CBS 122379		Capnodiales, incertae sedis	GU296146	GU301810	GU561845	GU371738	-	3 & 5
<i>Mycosphaerella eurypotami</i>	JK 5586J		Capnodiales, incertae sedis	GU301852	GU479761	-	GU371722	GU561851	3 & 5
<i>Tripospermum myrtil</i>	CBS 437.68		Capnodiales, incertae sedis	GU323216	-	GU561846	GU561854	GU561852	3 & 5
<i>Columnosphaeria fagi</i> 1	CBS 171.93	AFTOL 1582	Dothideales	AY016359	AY016342	-	DQ677966	-	3 & 5
<i>Columnosphaeria fagi</i> 2	CBS 584.75	AFTOL 912	Dothideales	DQ470956	DQ471004	FJ13608	DQ470906	DQ471148	3 & 5
<i>Delphinella strobiligena</i>	CBS 735.71	AFTOL 1257	Dothideales	DQ470977	DQ471029	-	DQ677951	DQ471175	3 & 5
<i>Dothidea insculpta</i>	CBS 189.58	AFTOL 921	Dothideales	DQ247802	DQ247810	FJ190602	AF107800	DQ471154	3 & 5
<i>Dothiora cannabinae</i>	CBS 737.71	AFTOL 1359	Dothideales	DQ470984	DQ479933	FJ190636	DQ470936	DQ471182	3 & 5
<i>Stylocthis puccinioides</i>	CBS 193.58	AFTOL 902	Dothideales	AY004342	AY016353	-	-	FJ238427	3 & 5
<i>Sydowia polyspora</i>	CBS 116.29	AFTOL 1300	Dothideales	DQ678058	DQ678005	FJ190631	DQ677953	-	3 & 5
<i>Gloniopsis praelonga</i>	CBS 112415		Hysteriales	FJ161173	FJ161134	-	-	-	3 & 5
<i>Rhytidhysterium rufulum</i>	CBS 306.38		Hysteriales	FJ469672	AF164375	-	-	FJ238444	3 & 5
<i>Eisinoë centrolobi</i>	CBS 222.50	AFTOL 1854	Myriangiales	DQ678094	DQ678041	FJ190651	-	-	3 & 5
<i>Eisinoë phaseoli</i>	CBS 165.31	AFTOL 1855	Myriangiales	DQ678095	DQ678042	FJ190652	-	-	3 & 5
<i>Myriangium duriaei</i>	CBS 260.36	AFTOL 1304	Myriangiales	DQ678059	AY016347	AY571389	DQ677954	-	3 & 5
<i>Phaeosclera dematoides</i>	CBS 157.81		Myriangiales	GU301858	GU296184	-	-	GU357764	3 & 5
<i>Lophium mytilinum</i>	CBS 269.34	AFTOL 1609	Mytiliniidiales	DQ678081	DQ678030	GU456342	DQ677979	-	3 & 5
<i>Mytilinidion resinicola</i>	CBS 304.34		Mytiliniidiales	FJ161185	FJ161145	-	FJ161101	-	3 & 5
<i>Hysteropatella clavispora</i>	CBS 247.34	AFTOL1305	Patellariales	AY541493	DQ678006	AY571388	DQ677955	-	3 & 5
<i>Hysteropatella elliptica</i>	CBS 935.97	AFTOL 1790	Patellariales	DQ6767657	EF495114	FJ190649	DQ676647	-	3 & 5
<i>Patellaria atrata</i>	CBS 958.97		Patellariales	GU301855	GU296181	-	DQ676647	GU357749	3 & 5
<i>Arthopyrenia salicis</i>	CBS 368.94	mtSSU from GenBank	Pleosporales	AY538339	AY538333	AY538345	-	FJ941893	3 & 5

Table 1. (Continued).

Dothideomycetes	Collection #	Additional Information	Order	LSU	SSU	mtSSU	RPB2	RPB1	Analysis
<i>Bimuria novae-zealandiae</i>	CBS 107.79	AFTOL 931	Pleosporales	AY016356	AY016338	FJ190605	DQ470917	DQ471159	3 & 5
<i>Dendryphiella arenaria</i>	CBS 181.58	AFTOL 995	Pleosporales	DQ470971	DQ471022	FJ190617	DQ470924	DQ842036	3 & 5
<i>Leptosphaeria maculans</i>	DAOM 229267	AFTOL 277	Pleosporales	DQ470946	DQ470993	-	DQ470894	DQ471136	3 & 5
<i>Pleospora herbarum</i>	CBS 541.72	AFTOL 940	Pleosporales	DQ247804	DQ247812	FJ190610	DQ247794	DQ471163	3 & 5
<i>Preussia terricola</i>	DAOM 230091	AFTOL 282	Pleosporales	AY544686	AY544726	AY544754	DQ470895	DQ471137	3 & 5
<i>Sirodesmium olivaceum</i>	CBS 395.59		Pleosporales	GU250894	GU250915	GU250904	GU250947	GU250958	3 & 5
<i>Westerdykella cylindrica</i>	CBS 454.72	AFTOL 1037	Pleosporales	AY004343	AY016355	AF346430	DQ470925	DQ471168	3 & 5
<i>Pleosporales</i> sp. 1	CBS 101277	ex <i>Thielarella luridella</i>	Pleosporales	-	GU456309	-	GU456361	-	3 & 5
<i>Pleosporales</i> sp. 2	AFTOL 101	ex <i>Anisomeridium polypori</i>	Pleosporales	-	DQ782877	-	DQ782864	DQ782822	3 & 5
<i>Astrothelium cinnamomeum</i>	AFTOL 110	ex <i>Trypethelium</i> sp.	Trypetheliaceae	AY584652	AY584676	AY584632	AY584690	DQ782824	3 & 5
<i>Laurera megasperma</i>	AFTOL 2094		Trypetheliaceae	FJ267702	GU561841	GU561847	GU561855	-	3 & 5
<i>Trypethelium nitidiusculum</i>	AFTOL 2099		Trypetheliaceae	FJ267701	GU561842	GU561848	GU561856	-	3 & 5
<i>Helicomyces roseus</i>	CBS 283.51	AFTOL 1613	Tubeufiaceae	DQ678083	DQ678032	-	DQ677981	-	3 & 5
<i>Tubeufia cerea</i>	CBS 254.75	AFTOL 1316	Tubeufiaceae	DQ470982	DQ471034	FJ190634	DQ470934	DQ471180	3 & 5
<i>Tubeufia paltudosa</i>	CBS 245.49	AFTOL 1580	Tubeufiaceae	DQ767654	DQ767649	-	DQ767643	-	3 & 5
<i>Cystocleus ebeneus</i>	L348	RPB2 from L344; RPB1 from L343	Dothideomycetes, incertae sedis	EU048580	EU048573	EU048586	GU214293	GU214204	3 & 5
<i>Fanlowiella carmichaelina</i>	CBS 206.36	AFTOL 1787	Dothideomycetes, incertae sedis	AY541492	AY541482	-	DQ677989	-	3 & 5
<i>Kirschsteiniothelia aethiops</i> 1	CBS 109.53	AFTOL 925	Dothideomycetes, incertae sedis	AY016361	AY016344	FJ190604	DQ470914	DQ471157	3 & 5
<i>Kirschsteiniothelia aethiops</i> 2	DAOM 231155	AFTOL 273	Dothideomycetes, incertae sedis	DQ678046	DQ677996	FJ190590	DQ677940	-	3 & 5
<i>Phaeotrichum benjamini</i>	CBS 541.72	AFTOL 1184	Dothideomycetes, incertae sedis	AY004340	AY016348	-	DQ677946	-	3 & 5
<i>Racodium rupestre</i>	L424	RPB1 from L341	Dothideomycetes, incertae sedis	EU048582	EU048577	EU048589	-	GU214205	3 & 5
<i>Sarcinomyces crustaceus</i>	CBS 156.89		Dothideomycetes, incertae sedis	GU250893	-	GU250905	GU250948	GU250959	3 & 5
<i>Tyramosorus pinicola</i>	CBS 124.88	AFTOL 1235	Dothideomycetes, incertae sedis	DQ470974	DQ471025	FJ190620	DQ470928	DQ471171	3 & 5
Rock-inhabiting fungi									
<i>Coniosporium apollinis</i>	CBS 362.97	ex-type strain	Dothideomycetes, incertae sedis	GU250895	GU250916	GU250906	GU250949	-	3 & 5
<i>Coniosporium apollinis</i>	CBS 100213		Dothideomycetes, incertae sedis	GU250896	GU250917	GU250907	GU250950	GU250960	3 & 5
<i>Coniosporium apollinis</i>	CBS 100214		Dothideomycetes, incertae sedis	GU250897	GU250918	GU250908	GU250951	-	3 & 5
<i>Coniosporium apollinis</i>	CBS 100218		Dothideomycetes, incertae sedis	GU250898	GU250919	GU250909	GU250952	GU250961	3 & 5
<i>Coniosporium apollinis</i>	CBS 109860		Dothideomycetes, incertae sedis	GU250899	GU250920	GU250910	GU250953	GU250962	3 & 5

Table 1. (Continued).

Rock-inhabiting fungi	Collection #	Additional Information	Order	LSU	SSU	mtSSU	RPB2	RPB1	Analysis	Locality
<i>Coniosporium apollinis</i>	CBS 109865		<i>Dothideomycetes, incertae sedis</i>	GU250900	GU250921	GU250911	GU250954	GU250963	3 & 5	Greece
<i>Coniosporium apollinis</i>	CBS 109867		<i>Dothideomycetes, incertae sedis</i>	GU250901	-	GU250912	GU250955	GU250964	3 & 5	Greece
<i>Coniosporium uncinatum</i>	CBS 100212		<i>Dothideomycetes, incertae sedis</i>	GU250902	GU250922	GU250913	GU250956	-	3 & 5	Italy
<i>Coniosporium uncinatum</i>	CBS 100219	ex-type strain	<i>Dothideomycetes, incertae sedis</i>	GU250903	GU250923	GU250914	GU250957	GU250965	3 & 5	France, Paris
rock isolate TRN 5	CBS 118762	Ruibal et al. (2008)	<i>Capnodiales, Teratosphaeriaceae</i>	GU323956	GU323988	GU324017	-	GU324051	3 & 5	Central Spain
rock isolate TRN 11	CBS 118281	Ruibal et al. (2008)	<i>Dothideales</i>	GU323957	-	GU324018	-	GU324052	3 & 5	Central Spain
rock isolate TRN 42	CBS 117958	Ruibal et al. (2008)	<i>Capnodiales, Davidiellaceae</i>	GU323958	-	GU324019	-	GU324053	3 & 5	Central Spain
rock isolate TRN 43	CBS 117950	Ruibal et al. (2008)	<i>Capnodiales, Davidiellaceae</i>	GU323959	GU323989	GU324020	-	-	3	Central Spain
rock isolate TRN 44	CBS 118324	Ruibal et al. (2008)	<i>Capnodiales, Davidiellaceae</i>	GU323960	GU323990	GU324021	-	-	3	Central Spain
rock isolate TRN 49	-	Ruibal et al. (2008)	<i>Pleosporales</i>	-	AY843233	-	-	-	3	Central Spain
rock isolate TRN 62	CBS 118305	Ruibal et al. (2005)	<i>Capnodiales, incertae sedis</i>	GU323961	GU323991	GU324022	-	GU324054	3 & 5	Mallorca
rock isolate TRN 66	CBS 118306	Ruibal et al. (2005)	<i>Capnodiales, incertae sedis</i>	GU323962	GU323992	GU324023	-	GU324055	3 & 5	Mallorca
rock isolate TRN 77	CBS 118287	Ruibal et al. (2005)	<i>Capnodiales, incertae sedis</i>	GU323963	GU323993	GU324024	GU324066	GU324057	3 & 5	Mallorca
rock isolate TRN 79	CBS 117930	Ruibal et al. (2005)	<i>Capnodiales, Teratosphaeriaceae</i>	GU323964	GU323994	GU324025	-	-	3	Mallorca
rock isolate TRN 80	CBS 118286	Ruibal et al. (2005)	<i>Capnodiales, incertae sedis</i>	GU323965	GU323995	GU324026	-	GU324056	3 & 5	Mallorca
rock isolate TRN 87	CBS 118290	Ruibal et al. (2005)	<i>Capnodiales, Capnodiaceae</i>	GU323966	GU323996	GU324027	-	GU324058	3 & 5	Mallorca
rock isolate TRN 111	CBS 118294	Ruibal et al. (2005)	<i>Capnodiales, incertae sedis</i>	GU323967	GU323997	GU324028	-	GU324059	3 & 5	Mallorca
rock isolate TRN 119	CBS 118250	Ruibal et al. (2005)	<i>Capnodiales, incertae sedis</i>	GU323968	-	GU324029	-	-	3	Mallorca
rock isolate TRN 122	CBS 117931	Ruibal et al. (2005)	<i>Capnodiales, Teratosphaeriaceae</i>	GU323969	GU323998	GU324030	-	-	3	Mallorca
rock isolate TRN 123	CBS 117932	Ruibal et al. (2005)	<i>Capnodiales, Teratosphaeriaceae</i>	GU323970	GU323999	GU324031	GU324067	GU324060	3 & 5	Mallorca
rock isolate TRN 124	CBS 118283	Ruibal et al. (2005)	<i>Capnodiales, Teratosphaeriaceae</i>	GU323971	GU324000	GU324032	-	GU324061	3 & 5	Mallorca
rock isolate TRN 129	CBS 117933	Ruibal et al. (2005)	<i>Capnodiales, Teratosphaeriaceae</i>	GU323972	GU324001	GU324033	-	-	3	Mallorca
rock isolate TRN 137	CBS 118300	Ruibal et al. (2005)	<i>Capnodiales, incertae sedis</i>	GU323973	GU324002	GU324034	-	GU324062	3 & 5	Mallorca
rock isolate TRN 138	CBS 118301	Ruibal et al. (2005)	<i>Capnodiales, incertae sedis</i>	GU323974	GU324003	GU324035	GU324068	GU324063	3 & 5	Mallorca
rock isolate TRN 142	CBS 118302	Ruibal et al. (2005)	<i>Capnodiales, incertae sedis</i>	GU323975	GU324004	GU324036	GU324069	-	3 & 5	Mallorca
rock isolate TRN 152	CBS 118346	Ruibal et al. (2005)	<i>Capnodiales, incertae sedis</i>	GU323976	GU324005	GU324037	-	-	3	Mallorca

Table 1. (Continued).

Rock-inhabiting fungi	Collection #	Additional Information	Order	LSU	SSU	mtSSU	RPB2	RPB1	Analysis	Locality
rock isolate TRN 153	CBS 118330	Ruibal et al. (2005)	Caprocliales, <i>incertae sedis</i>	GU323977	GU324006	GU324038	GU324070	-	3 & 5	Mallorca
rock isolate TRN 211	CBS 117937	Ruibal et al. (2008)	Capnodiales, <i>Teratosphaeriaceae</i>	GU323978	GU324007	GU324039	-	-	3	Central Spain
rock isolate TRN 213	-	Ruibal et al. (2008)	related to Arthoniales	-	GU324008	GU324040	-	-	3	Central Spain
rock isolate TRN 221	-	Ruibal et al. (2008)	Pleosporales	-	AY843241	-	-	-	3	Central Spain
rock isolate TRN 235	CBS 118605	Ruibal et al. (2008)	Myriangiiales	GU323979	-	GU324041	GU324071	-	3 & 5	Central Spain
rock isolate TRN 245	CBS 117940	Ruibal et al. (2008)	Caprocliales, <i>Teratosphaeriaceae</i>	GU323980	GU324009	GU324042	-	-	3	Central Spain
rock isolate TRN 267	CBS 118769	Ruibal et al. (2008)	Dothideomycetes, <i>incertae sedis</i>	-	GU324010	GU324043	GU324072	-	3 & 5	Central Spain
rock isolate TRN 268	CBS 119305	Ruibal et al. (2008)	Dothideales	GU323981	-	GU324044	-	-	3 & 5	Central Spain
rock isolate TRN 279	CBS 117943	Ruibal et al. (2008)	Caprocliales, <i>Teratosphaeriaceae</i>	GU323983	GU324012	GU324046	-	-	3	Central Spain
rock isolate TRN 434	-	Ruibal et al. (2008)	Pleosporales	-	AY843260	-	-	-	3	Central Spain
rock isolate TRN 437	CBS 118327	Ruibal et al. (2008)	Dothideomycetes, <i>incertae sedis</i>	GU323984	GU324013	GU324047	-	-	3	Central Spain
rock isolate TRN 452	-	Ruibal et al. (2008)	related to Arthoniales	GU323985	GU324014	GU324048	-	-	3	Central Spain
rock isolate TRN 456	-	Ruibal et al. (2008)	related to Arthoniales	GU323986	GU324015	GU324049	-	GU324065	3 & 5	Central Spain
rock isolate TRN 499	-	Ruibal et al. (2008)	Pleosporales	-	AY843278	-	-	-	3	Central Spain
rock isolate TRN 529	-	Ruibal et al. (2008)	related to Arthoniales	GU323987	GU324016	GU324050	-	-	3 & 5	Central Spain
rock isolate A6	-	Gorbushina (unpublished)	Dothideomycetes, <i>incertae sedis</i>	GU250924	GU250932	-	GU250939	-	3 & 5	Turkey
rock isolate A35	CBS 123158	Gorbushina (unpublished)	Coniosporium uncinatum	GU250925	GU250933	-	-	GU250943	3 & 5	Crimea
rock isolate A73	-	Gorbushina (unpublished)	Capnodiales, <i>incertae sedis</i>	GU250926	GU250934	-	GU250940	GU250944	3 & 5	Greece
rock isolate AN1	-	Gorbushina (unpublished)	Capnodiales, <i>Davidiellaceae</i>	GU250927	GU250935	-	GU250941	-	3 & 5	Israel, Negev
rock isolate AN13	CBS 125207	Gorbushina (unpublished)	Dothideomycetes, <i>incertae sedis</i>	GU250928	GU250936	-	GU250942	GU250945	3 & 5	Israel, Negev
rock isolate S2	-	Gorbushina (unpublished)	Caprocliales, <i>incertae sedis</i>	GU250931	-	-	-	GU250946	3 & 5	Slovenia
rock isolate DVA4	-	Staley et al. (1982)	Dothideomycetes, <i>incertae sedis</i>	GU250929	GU250937	-	-	-	3	U.S.A., Arizona
rock isolate DVA7	-	Staley et al. (1982)	Dothideomycetes, <i>incertae sedis</i>	GU250930	GU250938	-	-	-	3	U.S.A., Arizona
rock isolate CCFEE 451	-	Selbmann et al. (2005, 2008)	Caprocliales, <i>incertae sedis</i>	GU250360	GU250314	GU250403	-	-	3	Antarctica
rock isolate CCFEE 453	-	Selbmann et al. (2005, 2008)	<i>Cryomyces antarcticus</i>	GU250361	GU250315	GU250404	-	-	3	Antarctica
rock isolate CCFEE 456	-	Selbmann et al. (2005, 2008)	<i>Cryomyces antarcticus</i>	-	GU250316	GU250405	-	-	3	Antarctica

Table 1. (Continued).

Rock-inhabiting fungi	Collection #	Additional Information	Order	LSU	SSU	mtSSU	RPB2	RPB1	Analysis	Locality
rock isolate CCFEE 502	-	Selbmann <i>et al.</i> (2005, 2008)	Capnodiales, <i>Teratosphaeriaceae</i>	GU250363	GU250318	GU250406			3	Antarctica
rock isolate CCFEE 514	-	Selbmann <i>et al.</i> (2005, 2008)	<i>Cryomyces antarcticus</i>	-	GU250319	GU250407			3	Antarctica
rock isolate CCFEE 515	-	Selbmann <i>et al.</i> (2005, 2008)	<i>Cryomyces antarcticus</i>	-	GU250320	GU250408			3	Antarctica
rock isolate CCFEE 524	-	Selbmann <i>et al.</i> (2005, 2008)	<i>Friedmanniomyces endolithicus</i>	GU250364	DQ066715	GU250409	-	-	3 & 5	Antarctica
rock isolate CCFEE 534	-	Selbmann <i>et al.</i> (2005, 2008)	<i>Cryomyces antarcticus</i>	-	DQ066713	GU250410			3	Antarctica
rock isolate CCFEE 536	-	Selbmann <i>et al.</i> (2005, 2008)	<i>Cryomyces antarcticus</i>	GU250365	GU250321	GU250411	-	-	3 & 5	Antarctica
rock isolate CCFEE 670	-	Selbmann <i>et al.</i> (2005, 2008)	<i>Friedmanniomyces endolithicus</i>	GU250366	GU250322	GU250412			3	Antarctica
rock isolate CCFEE 690	-	Selbmann <i>et al.</i> (2005, 2008)	<i>Cryomyces antarcticus</i>	-	GU250323	GU250413			3	Antarctica
rock isolate CCFEE 5018	-	Selbmann <i>et al.</i> (2005, 2008)	Capnodiales, <i>Davidiellaceae</i>	-	GU250324	GU250414			3	Antarctica
rock isolate CCFEE 5176	-	Selbmann <i>et al.</i> (2005, 2008)	related to Arthoniales	-	GU250325	-			3	Antarctica
rock isolate CCFEE 5180	-	Selbmann <i>et al.</i> (2005, 2008)	<i>Friedmanniomyces endolithicus</i>	GU250367	GU250326	GU250415			3	Antarctica
rock isolate CCFEE 5184	-	Selbmann <i>et al.</i> (2005, 2008)	<i>Friedmanniomyces simplex</i>	GU250368	DQ066716	GU250416			3	Antarctica
rock isolate CCFEE 5187	CBS 116302	Selbmann <i>et al.</i> (2005, 2008)	<i>Cryomyces minteri</i>	GU250369	DQ066714	GU250417	-	-	3 & 5	Antarctica
rock isolate CCFEE 5205	-	Selbmann <i>et al.</i> (2005, 2008)	Capnodiales, <i>incertae sedis</i>	GU250370	GU250327	GU250418			3	Antarctica
rock isolate CCFEE 5211	-	Selbmann <i>et al.</i> (2005, 2008)	Capnodiales, <i>Davidiellaceae</i>	GU250371	GU250328	GU250419	-	-	3 & 5	Antarctica
rock isolate CCFEE 5264	-	Selbmann <i>et al.</i> (2008)	<i>Recurvomyces mirabilis</i>	GU250372	GU250329	-			3	Antarctica
rock isolate CCFEE 5284	-	Selbmann (unpublished)	related to Arthoniales	GU250373	GU250330	-			3	Antarctica
rock isolate CCFEE 5299	-	Selbmann (unpublished)	Capnodiales, <i>Davidiellaceae</i>	GU250374	-	-			3	Antarctic Peninsula
rock isolate CCFEE 5303	-	Selbmann (unpublished)	related to Arthoniales	-	GU250331	-			3	Antarctica
rock isolate CCFEE 5319	-	Selbmann <i>et al.</i> (2008)	<i>Elasticomyces elasticus</i>	GU250375	GU250332	-			3	Antarctica on lichens
rock isolate CCFEE 5320	CBS 122540	Selbmann <i>et al.</i> (2008)	<i>Elasticomyces elasticus</i>	GU250376	GU250333	GU250420	-	-	3 & 5	Antarctica on lichens
rock isolate CCFEE 5322	-	Selbmann (unpublished)	Capnodiales, <i>incertae sedis</i>	GU250377	GU250334	-			3	Antarctica on lichens
rock isolate CCFEE 5388	-	Selbmann (unpublished)	Capnodiales, <i>Davidiellaceae</i>	GU250380	GU250337	GU250422			3	Alps
rock isolate CCFEE 5389	-	Selbmann (unpublished)	Capnodiales, <i>incertae sedis</i>	GU250381	GU250338	GU250423			3	Alps
rock isolate CCFEE 5398	-	Selbmann (unpublished)	Capnodiales, <i>Davidiellaceae</i>	GU250382	GU250339	-			3	Alps
rock isolate CCFEE 5401	-	Selbmann (unpublished)	Capnodiales, <i>Teratosphaeriaceae</i>	GU250383	GU250340	GU250424			3	Alps

Table 1. (Continued).

Rock-inhabiting fungi	Collection #	Additional Information	Order	LSU	SSU	mtSSU	RPB2	RPB1	Analysis	Locality
rock isolate CCFEE 5410	–	Selbmann (unpublished)	Capnodiales, <i>incertae sedis</i>	GU250384	GU250341	GU250425			3	Andes
rock isolate CCFEE 5413	–	Selbmann (unpublished)	Dothideomycetes, <i>incertae sedis</i>	GU250385	GU250342	GU250426			3	Alps
rock isolate CCFEE 5414	–	Selbmann (unpublished)	Capnodiales, <i>Davidiellaceae</i>	GU250386	GU250343	–			3	Alps
rock isolate CCFEE 5416	–	Selbmann (unpublished)	Dothideomycetes, <i>incertae sedis</i>	GU250387	GU250344	GU250427			3	Alps
rock isolate CCFEE 5456	–	Selbmann (unpublished)	Capnodiales, <i>Davidiellaceae</i>	GU250388	GU250345	GU250428			3	Alps
rock isolate CCFEE 5457	–	Selbmann (unpublished)	Capnodiales, <i>Teratosphaeriaceae</i>	GU250389	GU250346	GU250429			3	Alps
rock isolate CCFEE 5458	–	Selbmann (unpublished)	Capnodiales, <i>Davidiellaceae</i>	–	GU250347	GU250430			3	Alps
rock isolate CCFEE 5459	–	Selbmann (unpublished)	Capnodiales, <i>incertae sedis</i>	GU250390	GU250348	GU250431			3	Alps
rock isolate CCFEE 5460	–	Selbmann (unpublished)	Dothideomycetes, <i>incertae sedis</i>	GU250391	GU250349	GU250432			3	Alps
rock isolate CCFEE 5466	–	Selbmann (unpublished)	Dothideomycetes, <i>incertae sedis</i>	GU250392	GU250350	GU250433			3	Alps
rock isolate CCFEE 5467	–	Selbmann (unpublished)	Capnodiales, <i>Teratosphaeriaceae</i>	GU250393	GU250351	–			3	Alps
rock isolate CCFEE 5476	–	Selbmann (unpublished)	close to <i>Cryomyces</i>	GU250394	GU250352	GU250434			3	Alps
rock isolate CCFEE 5489	–	Selbmann (unpublished)	Capnodiales, <i>incertae sedis</i>	GU250395	–	GU250435			3	Antarctica
rock isolate CCFEE 5490	–	Selbmann (unpublished)	<i>Elasticomyces elasticus</i>	–	GU250353	–			3	Antarctica
rock isolate CCFEE 5499	–	Selbmann (unpublished)	Capnodiales, <i>Teratosphaeriaceae</i>	GU250398	GU250355	GU250436			3	Alps
rock isolate CCFEE 5501	–	Selbmann (unpublished)	Capnodiales, <i>Teratosphaeriaceae</i>	GU250399	GU250356	GU250437			3	Aconcagua, Andes
rock isolate CCFEE 5502	–	Selbmann (unpublished)	Capnodiales, <i>incertae sedis</i>	GU250400	GU250357	GU250438			3	Aconcagua, Andes
rock isolate CCFEE 5508	–	Selbmann (unpublished)	Capnodiales, <i>Teratosphaeriaceae</i>	GU250401	GU250358	–			3	Aconcagua, Andes
rock isolate D007 09	–	Selbmann (unpublished)	related to <i>Arthoniales</i>	GU250402	GU250359	–			3	Antarctica