

Taxonomy of rock-inhabiting fungi from James Ross Island, Antarctica

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Abstract

A total of 51 strains of rock-inhabiting fungi, which were isolated from rock fragments in the deglaciated area of James Ross Island, Antarctica, were studied and compared by sequencing the ITS rDNA region. Analysed strains were classified into two classes of the phylum *Ascomycota*. Specifically, 41 strains were classified within the families *Teratosphaeriaceae* (20) and *Extremaceae* (21), belonging to the order *Mycosphaerellales* of the class *Dothideomycetes*. The remaining *Dothideomycetes* strains were found to be related to species from the orders *Cladosporiales* and *Dothideales*. Additionally, only four strains were related to the order *Chaetothyriales*, which belongs to the class *Eurotiomycetes*. On the whole, 29 analysed strains were affiliated with four genera of typical rock-inhabiting fungi, namely *Oleoguttula*, *Rachicladosporium*, *Elasticomyces* and *Vermiconidia*. The genus *Vermiconidia* was represented by the highest number of isolates among the studied strains, suggesting that it represents a common component of the fungal community of the investigated area. Out of the 51 analysed strains, only seven were successfully identified as the species *Rachicladosporium antarcticum*, *Oleoguttula mirabilis*, and *Elasticomyces elasticus*. Further investigation and characterization of the majority of analysed strains are necessary to determine their taxonomic position and describe potentially new taxa.

Key words: Antarctica, black yeast, ITS rDNA region, *Dothideomycetes*, meristematic fungi, microcolonial fungi, rock-inhabiting fungi

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Introduction

Antarctic rocks represent an inhospitable environment that provides several ecological niches for extremotolerant microorganisms. While various fungi can colonize rocks, not all of them are the primary inhabitants of this niche. Black microcolo-

nial fungi are a specific group of microorganisms commonly referred to as "rock-inhabiting fungi" (RIF) (Sterflinger and Krumbein 1995, Ruibal et al. 2009). These fungi are able to survive in harsh environments worldwide and the habitats where

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RIF can be found are diverse, ranging from the dry, cold, and icy regions of Antarctica to hot and arid areas like the Mediterranean (Ruibal et al. 2009, Selbmann et al. 2005, 2008; Egidi et al. 2014). They can be found on mountain massifs, deserts, historical monuments (Wollenzien et al. 1995, Sert et al. 2007, 2012; Sterflinger et al. 2012, Egidi et al. 2014, Isola et al. 2015, Sun et al. 2020, Coleine et al. 2021), and even on solar panels or roof tiles (Martin-Sanchez et al. 2018, Ruibal et al. 2018).

The ability of RIF to thrive under extreme conditions and withstand various stress factors, such as nutrient deficiency, intense sunlight, high UV radiation, osmotic stress, temperature fluctuations or low water availability is attributed to their adaptation evolution resulting to an effective combination of their morphological and physiological characteristics (Sterflinger and Krumbein 1995, Sterflinger 1998, Selbmann et al. 2005, 2008). These unique traits have led to the use of different terminology in order to describe them. These fungi exhibit restricted growth, resulting in the formation of small, compact black colonies, therefore are referred to as "micro-colonial fungi" (MCF) (Staley et al. 1982). Another term used for this group of fungi is "black yeasts or black yeast-like fungi" (de Hoog and Hermanides-Nijhof 1977, Sterflinger 2006), which highlights the abundance of melanin pigment in their cell walls and the formation of daughter cells through multilateral or polar budding (de Hoog and Hermanides-Nijhof 1977, Selbmann et al. 2005). In such fungi, melanin serves as an essential protectant against UV radiation, desiccation and osmotic stress (Selbmann et al. 2015, Coleine et al. 2018). The presence of melanin increases cell turgor, aiding RIF to penetrate rocks more effectively and facilitate colonization (Coleine and Selbmann 2021). Moreover, the meristematic growth of RIF, characterized by isodiametric cell expansion, contributes to their resilience, enabling them

to survive desiccation and extreme temperatures (Wollenzien et al. 1995, Selbmann et al. 2005, Ruibal et al. 2009). This notable characteristic has led to the designation of these fungi as "meristematic fungi" (de Hoog and Hermanides-Nijhof 1977).

Phylogenetic analyses led to the discovery of numerous new genera and species of RIF over the past two decades (*see below*). The RIF is a polyphyletic group, with the majority of described genera belonging to the classes *Dothideomycetes* and *Eurotiomycetes* within the division *Ascomycota*, along with a smaller group of fungi closely related to the class *Arthoniomycetes* (Gueidan et al. 2008, Ruibal et al. 2009, Egidi et al. 2014, Quaedvlieg et al. 2014, Ametrano et al. 2019). Within the class *Eurotiomycetes*, RIF dominate the order *Chaetothyriales*. This order is well-known especially because of species causing opportunistic infections in humans, but the ecology of this order is much more diverse. Epilithic species of *Chaetothyriales* frequently inhabit the sites polluted with contaminants (Quan et al. 2020). They have been isolated from historical stone monuments in the Mediterranean regions (Wollenzien et al. 1995, Sert et al. 2007, Isola et al. 2015) and in various provinces of China (Sun et al. 2020) but as well as from natural rocks of Spain, Mallorca, Italian Alps or China (Ruibal et al. 2005, 2008, Hubka et al. 2014, Sun et al. 2020). On the other hand, RIF belonging to the class *Dothideomycetes* dominate the orders *Capnodiales*, *Dothideales* and *Pleosporales*, and they primarily colonize rocks in cold deserts in polar and high mountain regions (Sterflinger et al. 1999, Ruibal et al. 2009, Egidi et al. 2014). Recent phylogenetic study has led to the separation of the order *Capnodiales s. lat.* into *Capnodiales s. str.* and six other orders, namely *Cladosporiales*, *Comminutisporales*, *Mycosphaerellales*, *Neophaeothecales*, *Phaeothecales* and *Racodiales* (Abdollahzadeh et al. 2020). Consequently, some genera of RIF have been reclassified under the orders

Mycosphaerellales and *Cladosporiales* (Wijayawardene et al. 2022). The current classification and understanding of RIF are continuously evolving as new knowledge emerge. As scientific research progresses, our understanding of the taxonomy and diversity of RIF is likely to evolve, providing new insights into their complex relationships and expanding our knowledge of

their ecological roles and adaptations.

Fungal communities occurring at James Ross Island are being investigated as a part of the Masaryk University's Polar Research programme. This study aims to expand our understanding of the taxonomy, diversity, and ecology of rock-inhabiting fungi in this previously unexplored Antarctic region.

Material and Methods

Sampling and isolation

A group of RIF strains was isolated from rock fragments collected during the 2018-2020 polar expeditions in the deglaciated part of James Ross Island (64° 10' S, 57° 45' W) and from adjacent islands in the region (Table 1). The samples were aseptically collected into sterile plastic tubes and preserved at 8°C until they were analysed. Isolation of strains was performed by directly inoculating the rock fragments onto dichloran rose-bengal

chloramphenicol agar (DRBC, Difco™) and malt yeast extract agar (MYEA, Selbmann et al. 2005). The agar plates were aerobically incubated at 15°C and inspected weekly for a period of one month. Black colonies were then transferred onto 2% malt extract agar (MEA, Difco™). Obtained axenic cultures were subsequently preserved for long-term storage at -70°C.

Molecular analysis

To determine taxonomic affiliation and establish the evolutionary relationships among the isolated RIF strains, the internal transcribed spacer region of the ribosomal DNA (ITS rDNA region) was sequenced. This region is commonly used in molecular taxonomy as a standard marker for fungal identification and classification due to its high variability. The taxonomic affiliation of studied strains was determined based on the current fungal classification proposed by Wijayawardene et al. (2022).

Molecular analysis involved the isolation of genomic DNA according to the method described by Laichmanová (2020). The ITS rDNA region (ITS1-5.8S-ITS2)

was then amplified using two sets of universal primers: SR6R and LR1 (Vilgalys and Hester 1990) and ITS5 and ITS4 (White et al. 1990) following the protocols outlined by Marvanová and Laichmanová (2014). Resulting amplicons were purified using High Pure PCR Product Purification Kit (Roche, Germany). Sanger sequencing of purified PCR products was performed in Eurofins Genomics (Ebersberg, Germany). The obtained sequences were visually inspected and edited using the Chromas 2.6.6 visualization software (available at ^[1]). All final sequences were deposited in the NCBI GenBank nucleotide database (Table 1).

Strain No.	Locality	GPS coordinates	ITS *	Taxon
<i>Cladosporiaceae</i>				
P10353	Berry Hill, north slope, JRI	63°48'25"S, 57°50'12"W	MW222193	<i>Cladosporium</i> sp.
P10377	Sekyra Peak, JRI	63°54'38"S, 58°00'14"W	MW222195	<i>Rachicladosporium antarcticum</i>
P10413	Near Alfa Glacier, JRI	63°56'47"S, 57°52'05"W	MW222216	<i>Rachicladosporium antarcticum</i>
P11108	Cape Lachman, JRI	63°47'48"S, 57°48'53"W	OR200610	<i>Rachicladosporium</i> sp.
<i>Teratosphaeriaceae</i>				
P10109	Johnson Mesa, JRI	63°49'07"S, 57°54'02"W	MW222191	<i>Oleoguttula mirabilis</i>
P10386	Lachman Crags, JRI	63°51'05"S, 57°49'13"W	MW222203	unidentified
P10387	Lachman Crags, JRI	63°51'15"S, 57°49'36"W	MW222204	unidentified
P10397	Lachman Crags, JRI	63°51'05"S, 57°49'13"W	MW222227	unidentified
P10399	Berry Hill, JRI	63°48'28"S, 57°49'45"W	MW222207	unidentified
P10401	Berry Hill, north slope, JRI	63°48'25"S, 57°50'12"W	MW222208	unidentified
P10403	Berry Hill, north slope, JRI	63°48'25"S, 57°50'12"W	MW222209	unidentified
P10405	Berry Hill, north slope, JRI	63°48'25"S, 57°50'12"W	MW222210	unidentified
P10406	Near Panorama Pass, JRI	63°49'01"S, 57°50'50"W	MW222211	<i>Oleoguttula mirabilis</i>
P10407	Near Panorama Pass, JRI	63°49'01"S, 57°50'50"W	MW222212	<i>Oleoguttula mirabilis</i>
P10444	Johnson Mesa, JRI	63°49'07"S, 57°54'02"W	MW222225	<i>Oleoguttula mirabilis</i>
P10463	Devils Rocks, JRI	63°51'14"S, 57°49'04"W	MW222221	unidentified
P10481	Near JGM station, JRI	63°48'00"S, 57°52'46"W	MW222223	unidentified
P11013	Johnson Mesa, JRI	63°49'00"S, 57°54'00"W	OR200611	unidentified
P11099	JRI	63°47'34"S, 57°48'30"W	OR200612	unidentified
P11100	JRI	63°47'34"S, 57°48'30"W	OR200613	unidentified
P11101	JRI	63°47'48"S, 57°48'53"W	OR200614	unidentified
P11106	Near JGM station, JRI	63°48'10"S, 57°53'54"W	OR200615	unidentified
P11109	JRI	63°47'48"S, 57°48'53"W	OR200616	<i>Elasticomyces elasticus</i>
P11215	JRI	63°50'15"S, 57°49'13"W	OR200617	unidentified
<i>Extremaceae</i>				
P10439	Berry Hill, JRI	63°48'28"S, 57°49'45"W	MW222197	<i>Vermiconidia</i> sp.
P10376	Johnson Mesa, JRI	63°49'00"S, 57°54'00"W	OR200618	<i>Vermiconidia</i> sp.

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P10378	Monolith Lake, JRI	63°54'40"S, 57°57'32"W	MW222196	<i>Vermiconidia</i> sp.
P10379	Monolith Lake, JRI	63°54'40"S, 57°57'32"W	MW222198	<i>Vermiconidia</i> sp.
P10385	The Naze, JRI	63°55'52"S, 57°30'01"W	MW222202	<i>Vermiconidia</i> sp.
P10391	Lachman Crag, JRI	63°50'09"S, 57°51'02"W	MW222205	<i>Vermiconidia</i> sp.
P10392	Berry Hill, JRI	63°48'28"S, 57°49'45"W	MW222230	<i>Vermiconidia</i> sp.
P10396	The Naze, JRI	63°55'52"S, 57°30'29"W	MW222206	<i>Vermiconidia</i> sp.
P10410	Botany Bay, Trinity Peninsula	63°40'43"S, 57°50'32"W	MW222213	<i>Vermiconidia</i> sp.
P10411	Tail Island	63°48'48"S, 57°50'02"W	MW222214	<i>Vermiconidia</i> sp.
P10412	JRI	63°51'14"S, 57°49'04"W	MW222215	<i>Vermiconidia</i> sp.
P10415	Berry Hill, north slope, JRI	63°48'25"S, 57°50'12"W	MW222217	<i>Vermiconidia</i> sp.
P10417	Berry Hill, north slope, JRI	63°48'25"S, 57°50'12"W	MW222218	<i>Vermiconidia</i> sp.
P10418	Berry Hill, north slope, JRI	63°48'20"S, 57°49'57"W	MW222219	<i>Vermiconidia</i> sp.
P10419	Near JGM station, JRI	63°48'00"S, 57°52'46"W	MW222220	<i>Vermiconidia</i> sp.
P10459	Sekyra Peak, JRI	63°54'38"S, 58°00'14"W	MW222226	<i>Vermiconidia</i> sp.
P10469	Lachman Crag, JRI	63°50'09"S, 57°51'02"W	MW222231	<i>Vermiconidia</i> sp.
P10470	Berry Hill, JRI	63°48'28"S, 57°49'45"W	MW222229	<i>Vermiconidia</i> sp.
P10479	Near Alfa Glacier, JRI	63°56'47"S, 57°52'05"W	MW222222	<i>Vermiconidia</i> sp.
P11010	Berry Hill, north slope, JRI	63°48'25"S, 57°50'12"W	OR200619	<i>Vermiconidia</i> sp.
P11098	Near JGM station, JRI	63°48'07"S, 57°53'43"W	OR200620	<i>Vermiconidia</i> sp.
<i>Sacrotheciaceae</i>				
P10184	Lachman Crag, JRI	63°51'23"S, 57°50'19"W	MW222192	unidentified
P11107	Cape Lachman, JRI	63°47'34"S, 57°48'30"W	OR200621	unidentified
<i>Herpotrichiellaceae</i>				
P10380	Sekyra Peak, JRI	63°54'38"S, 58°00'14"W	MW222199	unidentified
P10382	Near JGM station, JRI	63°48'33"S, 57°53'10"W	MW222200	unidentified
P10384	Near JGM station, JRI	63°48'33"S, 57°53'10"W	MW222201	unidentified
P10464	Near Bohemian Stream, JRI	63°48'36"S, 57°54'03"W	MW222228	unidentified

Table 1. List of studied strains.

Notes: JRI – James Ross Island, Antarctica; JGM – Johann Gregor Mendel Czech Antarctic Station; *GenBank accession number of ITS rDNA sequences.

Phylogenetic analysis

To determine the taxonomic position of the isolated RIF strains, the obtained sequences were compared within the GenBank database using the MegaBLAST software (Morgulis et al. 2008). A total of 49 reference ITS rDNA sequences of the closest related taxa were selected based on the results of this comparison. Phylogenetic analysis of a total of 100 ITS rDNA sequences was conducted using the MEGA version X software (Kumar et al. 2018). To align the sequences, the ClustalW algorithm (Thompson et al. 1994) was employed. After the initial alignment, manual inspection and editing were carried out. The phylogenetic tree (Fig. 1) was constructed using the maximum likelihood (ML) method (Nei and Kumar 2000) with GTR+G+I substitution model. To assess

the robustness of the phylogenetic inference, the bootstrap method (Felsenstein 1985) with 1000 replications was employed. Additionally, Bayesian posterior probability values (PP) were determined using the MrBayes 3.2.7a program (Huelsenbeck and Ronquist 2001). For the Bayesian analysis the model of evolution was estimated by the programs PAUP 4.0a (Swofford 2003) and MrModeltest2 v.2.4 (Nylander 2004) using the Akaike Information Criterion (AIC). Analyses of two parallel runs with four Markov chains were conducted for 1 000 000 generations and trees were sampled every 100th generation. The initial 25% of the trees were discarded as burn-in. The tree was rooted with *Saccharomyces cerevisiae* CBS 1171^T (NR_111007).

Results and Discussion

The taxonomic position of the 51 studied strains was determined by comparing their ITS rDNA sequences and conducting a phylogenetic comparison with 49 reference sequences of the closest related taxa. The phylogenetic analysis presented in the study indicated that the majority of the analysed strains belonged to the class *Dothideomycetes*. Specifically, 41 strains (representing 80% of the total number of studied RIF strains) were classified within the families *Teratosphaeriaceae* and *Extremaceae*, belonging to the order *Mycosphaerellales*. The significant presence of Antarctic RIF strains within the taxonomic group *Dothideomycetes* is in agreement with previous research conducted by Egidi et al. (2014). In addition to the *Mycosphaerellales* order, the strains belonging to the class *Dothideomycetes* were related to species from the family *Cladosporiaceae* within the order *Cladosporiales* and the family *Sacotheciaceae* within the order *Dothideales*. The remain-

ing strains were classified within the family *Herpotrichiellaceae*, which belongs to the order *Chaetothyriales* in the class *Eurotiomycetes*.

The phylogenetic analysis divided the studied strains into five distinct clades. Each clade represented a different family within the *Ascomycota* phylum. This finding suggests the presence of significant genetic diversity and evolutionary divergence among the studied strains. The depicted phylogenetic tree (Fig. 1) was divided into two parts. The first part of the tree represented the families *Cladosporiaceae* and *Teratosphaeriaceae*, while the second part included the families *Extremaceae*, *Sacotheciaceae* and *Herpotrichiellaceae*.

Totally, four RIF strains of the family *Cladosporiaceae* were classified into two genera. These include *Cladosporium* (P10353) and *Rachicladosporium* (P10413, P10377, P11108). Two of the strains, each isolated from different locations, were iden-

tified as *Rachicladosporium antarcticum* due to their high sequence similarity (99.6% - 99.7%) to *Rachicladosporium antarcticum* CCFEE 5527^T (NR_144970) as reported by Laichmanová (2020). Strain P11108 had highest similarity (99.6%) to *Rachicladosporium monterosium* CCFEE 5398^T (NR_144968). Although these percentage similarities of ITS sequences are significant, phylogenetic analysis grouped strain P11108 in a tight cluster with *R. mcmurdoi* and *R. monterosium*. Therefore, it is not possible to reliably assign this strain to a species level. *Rachicladosporium antarcticum* and *Rachicladosporium mcmurdoi* were previously described from rocks in the Antarctic Peninsula (Egidi et al. 2014). Furthermore, *Rachicladosporium aridum*, another Antarctic species was proposed by Selbmann and Coleine in a recent study (Wijayawardene et al. 2021). Apart from Antarctica, other *Rachicladosporium* species have been reported from mountainous regions in Italy. *Rachicladosporium monterosium* (Ruibal et al. 2009, Egidi et al. 2014) is one of them. According to the MycoBank database^[2] (as of June 23, 2023), the genus *Rachicladosporium* includes a total of 14 species. These species encompass not only rock-inhabiting microfungi but also epiphytic species. This may suggest that the genus *Rachicladosporium* exhibits a diverse range of ecological preferences and habitats.

A total of 20 strains represented the family *Teratosphaeriaceae*. Among these, four strains (P10109, P10406, P10407, P10444) were identified as *Oleoguttula mirabilis* based on their high sequence similarity (99.2%) to the reference strain *Oleoguttula mirabilis* CCFEE 5523^T (KF309973) as reported by Laichmanová (2020). For the other eight strains, *O. mirabilis* was the closest taxon with sequence similarities ranging from 94.0% to 95.7%. These strains formed two dis-

tinct highly supported lineages, suggesting the presence of two potentially novel taxa. Therefore, further investigations and additional analyses are required to clarify the taxonomic status of this group of strains. Currently, the genus *Oleoguttula* is represented by only one species originally described from rock in Antarctica (Egidi et al. 2014) but additionally, it has been reported from the Greenland ice sheet (Perini et al. 2019). Strain P11109 was identified as *Elasticomyces elasticus*. Sequence similarity of this strain to *Elasticomyces elasticus* CBS 122538^T (NR_137023) was 98.0%. The affiliation of this strain to *E. elasticus* was supported by its high sequence similarity, which showed a close match to many other strains of the same species in the GenBank database. The genus *Elasticomyces* currently consists of a single species *Elasticomyces elasticus*, initially isolated from rocks in Antarctica and subsequently discovered in other regions such as the Andes or the Himalayas (Selbmann et al. 2008, Egidi et al. 2014). Its occurrence appears to be associated with lichens, as it is frequently found on stones in the presence of lichen communities (*see below*). The type culture of *E. elasticus* CBS 122538^T was even isolated directly from the thallus of the Antarctic lichen *Usnea antarctica* (Selbmann et al. 2008). Other isolates of *E. elasticus* were reported from lichens of the genus *Lecanora* (Selbmann et al. 2013). Additionally, it has been observed as part of the Antarctic lichen-dominated cryptoendolithic community, as described by Selbmann et al. (2008). Strains P10386 and P10397 demonstrated high sequence similarity of 99.4% to the type strain *Neophaeotheceoides proteae* CBS 114129^T (NR_157417), which is known to be associated with leaf spot in the *Proteaceae* family (Crous et al. 2008, Quaedvlieg et al. 2014).

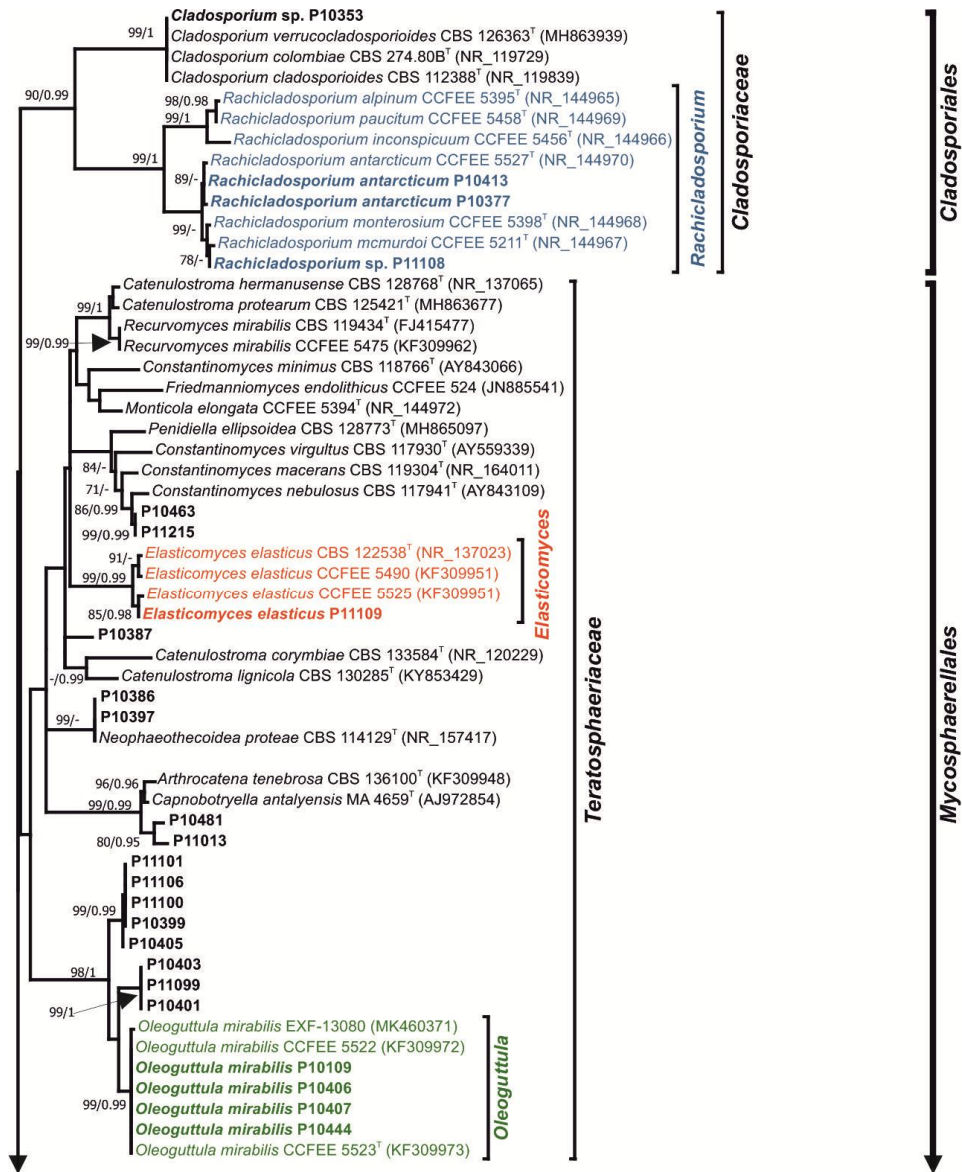


Fig. 1. Phylogenetic tree based on ITS rDNA sequences of 51 studied strains (in bold) and 49 representatives of the phylogenetically closest taxa. Phylogenetic relationships were inferred using the maximum likelihood method (ML) and the GTR+G+I model of evolution. ML bootstrap support $\geq 70\%$ and Bayesian posterior probabilities ≥ 0.95 are given for the respective nodes. *Saccharomyces cerevisiae* CBS 1171^T was used as outgroup. The scale bar indicates 0.5 expected changes per site.

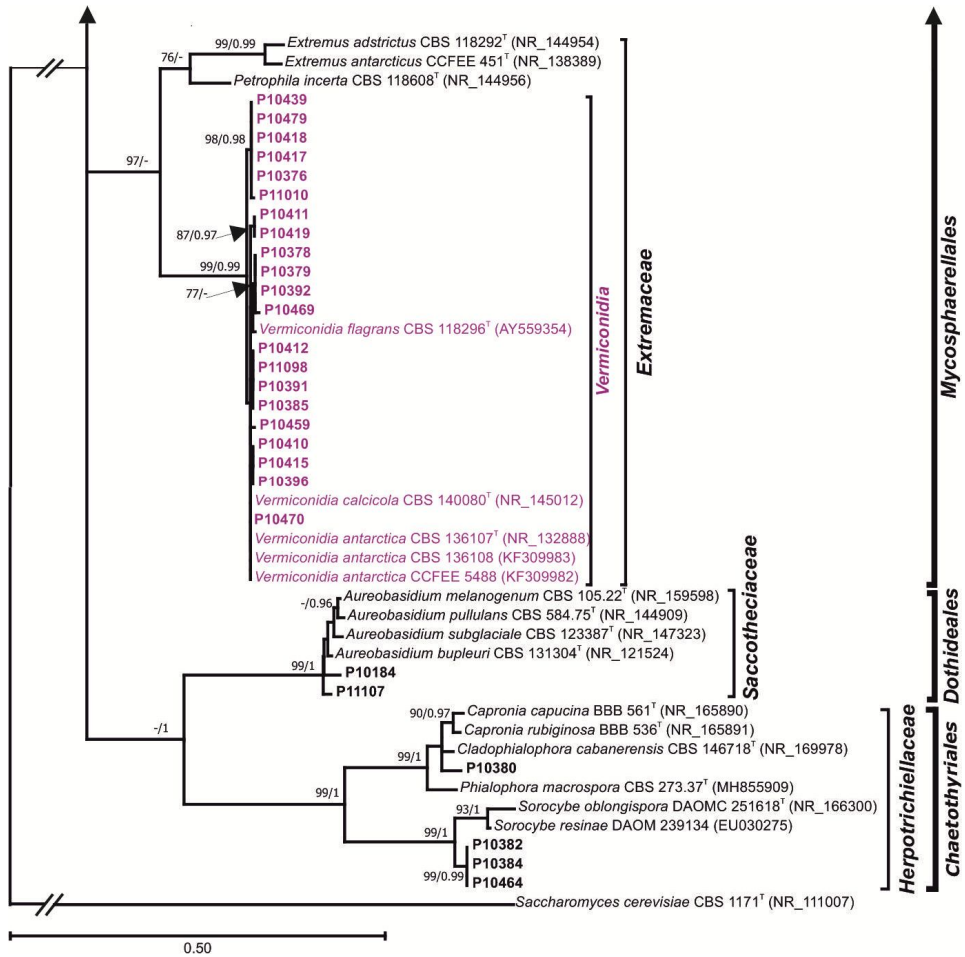


Fig. 1. (Continued)

Although, the ITS sequence similarity between the studied strains and *N. proteae* is high, it is important to conduct further taxonomic investigations to classify and describe these isolates. This is particularly important because the strains and *N. proteae* occupy different ecological habitats. Strains P10463, P11215 and P10387 were grouped together within a low-supported clade *Teratosphaeriaceae*. This clade includes the majority of Antarctic RIF genera. The topology of this cluster is not reliable. Strain P10387 exhibited a sequence similarity of 90.1% to *Recurvo-*

myces mirabilis CBS 119434^T (FJ415477), which was described from rocks in continental Antarctica and also found in the Italian Alps (Selbmann et al. 2008). Currently, *Recurvomyces* is represented only by this species. Strains P10463 and P11215 exhibited a sequence similarity of 94.3% to *Penidiella ellipsoidea* CBS 128773^T (MH865097), a species isolated from leaf bracts of *Phaenocoma prolifera* in South Africa (Crous and Groenewald 2011). Additionally, these strains showed a sequence similarity in the range of 92.6% to 95.8% to species within the ge-

nus *Constantinomyces*. This genus is represented by four rock fungal species isolated in the Central Mountain System in Spain and Mallorca (Ruibal et al. 2008, Egidi et al. 2014, Crous et al. 2019a). The remaining two strains (P10481 and P11013) within the clade *Teratosphaeriaceae* showed a close relationship with *Arthrocatena tenebrosa* CBS 136100^T (NR_144971), a rock-inhabiting fungus described from the Italian Alps (Ruibal et al. 2009, Egidi et al. 2014, Crous et al. 2019a). The sequence similarity between these strains and *A. tenebrosa* ranged from 93.5% to 95.0%. According to the current fungal classification *A. tenebrosa* is assigned between genera *incertae sedis* within the order *Mycosphaerellales* (Wijayawardene et al. 2022). Therefore, the affiliation of our strains within the family *Teratosphaeriaceae* is uncertain.

Approximately 50% of the studied strains were assigned within the family *Extremaceae*. The strains revealed highly similar ITS sequences and were grouped in a well supported clade within the genus *Vermiconidia*. They were isolated from various locations on James Ross Island as well as from Tail Island and the Trinity Peninsula. The majority of the species within *Extremaceae* are known to be rock-inhabiting fungi. Indeed, two species that have been described from Antarctica are *Extremus antarcticus* and *Vermiconidia antarctica* (Egidi et al. 2014, Crous et al. 2019a). Additionally, *Vermiconidia* comprises three other RIF species reported from different localities, such as Mallorca, the Italian Alps and Italian monuments (Ruibal et al. 2005, 2009; Egidi et al. 2014, Isola et al. 2015, Crous et al. 2019a).

Two strains, namely P10184 and P11107 were assigned to the family *Sacotheciaceae* and exhibited the highest sequence similarity (ranging from 93.0% to 97.5%) to species within the genus *Aureobasidium*. This indicates a close genetic relationship between these RIF strains and *Aureobasidium* species. However, fur-

ther investigation is needed to accurately classify these strains within the *Sacotheciaceae* family and determine their taxonomic position within the genus *Aureobasidium*. This genus includes black yeast-like species with *Aureobasidium pululans* being well-known biotechnologically utilized species (Prasongsuk et al. 2018). It is a widespread, oligotrophic organism capable of thriving in diverse extreme environments (Gostinčar et al. 2014). It has been isolated from various locations, including Antarctic soil and rocks (Connell and Staudigel 2013, Coleine et al. 2018). *Aureobasidium subglaciale* is another species within the *Aureobasidium* genus that has been described from cold regions, specifically glacial habitats in Svalbard, Norway (Zalar et al. 2008, Gostinčar et al. 2014). This species demonstrates the ability of the *Aureobasidium* genus to adapt and thrive in extreme environments, such as those found in glaciated areas.

Four analysed strains were divided into two well-supported clades within the family *Herpotrichiellaceae*. The family *Herpotrichiellaceae* comprises both well-known human opportunistic pathogens (de Hoog et al. 2020) and species associated with various other habitats such as soil, plants, lichens, ants and rocks (Nepel et al. 2014, Isola et al. 2015, Muggia et al. 2016, Tsurykau and Etayo 2017, Vasse et al. 2017, Costa et al. 2020, Sun et al. 2020, Muggia et al. 2021). Strain P10380 showed a close genetic relationship with species of the genus *Capronia*, which are associated with plants originating from the Andean Patagonian forests (Sánchez et al. 2019). Additionally, it exhibited sequence similarities ranging from 86.0% to 92.8% with medically important genera such as *Cladophialophora* and *Phialophora*. Strains P10382, P10384, and P10464 exhibited the closest similarity (90.6%) to *Sorocybe oblongispora* DAOMC 251618^T (NR_166300), a resinicolous fungus proposed by Tanney and Seifert in Crous et

al. (2019b). The formation of a separate, highly supported lineage by these strains suggested that they are genetically dis-

tinct from the other analysed strains and may represent a novel genus within the *Herpotrichiellaceae* family.

Conclusions

This study presents the results of the classification of 51 RIF strains isolated from rocks in Antarctic region. On the whole, 30 strains analysed in this study were affiliated with five different genera. These included *Cladosporium* and *Rachicladosporium* from the family *Cladosporiaceae*, *Elasticomyces* and *Oleoguttula* from the family *Teratosphaeriaceae*, and *Vermiconidia* from the family *Extremaceae*. The genus *Vermiconidia* was represented by the highest number of isolates among the studied strains. This indicates the significant presence and abundance of *Vermiconidia* in the studied area, suggesting their ecological importance and adaptation to the rocky environment. The strains within the family *Saccotheciaceae*, order *Dothideales*, were closely related to the genus *Aureobasidium*. The strains belonging to the *Herpotrichiellaceae* family and 13 strains within the *Teratosphaeriaceae* family displayed the sequence similarity values lower than 96% to reference sequences of type strains available in the GenBank database. This indicates that these isolates may represent novel taxa that have not been previously described. Out of the 51 analysed strains, only seven were successfully identified as the species *Rachicladosporium antarcticum*, *Oleogut-*

tula mirabilis, and *Elasticomyces elasticus*. It is worth noting that these species were described from the Antarctic region, providing additional support for their occurrence in the study area.

The findings of this study suggest that the melanised microscopic fungi inhabiting the rocks of James Ross Island form a polyphyletic group with the dominance of representatives of the class *Dothideomycetes*. Notably, the genus *Vermiconidia* was the most abundant, indicating that it is a common component of the microbial flora of the investigated area. These results contribute to our understanding of the fungal diversity and microbial ecology in this particular Antarctic environment and point out the presence of unique and unexplored fungal taxa in this Antarctic region.

Further investigation and characterization of the majority of the analysed strains are necessary for the designation of their taxonomic position in the fungal classification, for a description of new taxa and for a better understanding of their ecological significance in the Antarctic environment. This would involve molecular analyses of additional genes, but also a thorough examination of their morphological and physiological characteristics.

References

- ABDOLLAHZADEH, J., GROENEWALD, J. Z., COETZEE, M. P. A., WINGFIELD, M. J. and CROUS, P. W. (2020): Evolution of lifestyles in *Capnodiales*. *Studies in Mycology*, 95: 381-414. doi: 10.1016/j.simyco.2020.02.004
- AMETRANO, C. G., KNUDSEN, K., KOCOURKOVÁ, J., GRUBE, M., SELBMANN, L. and MUGGIA, L. (2019): Phylogenetic relationships of rock-inhabiting black fungi belonging to the widespread genera *Lichenothelia* and *Saxomyces*. *Mycologia*, 111(1): 127-160. doi: 10.1080/00275514.2018.1543510

- COLEINE, C., SELBMANN, L. (2021): 2.1 Black fungi inhabiting rock surfaces. *In*: B. Büdel, T. Friedl (eds.): *Life at Rock Surfaces: Challenged by Extreme Light, Temperature and Hydration Fluctuations*. Berlin, Boston: De Gruyter, pp. 57–86. doi: 10.1515/9783110646467-003
- COLEINE, C., STAJICH, J. E., DE LOS RÍOS, A. and SELBMANN, L. (2021): Beyond the extremes: Rocks as ultimate refuge for fungi in drylands. *Mycologia*, 113(1): 108-133. doi: 10.1080/00275514.2020.1816761
- COLEINE, C., ZUCCONI, L., ONOFRI, S., POMBUBPA, N., STAJICH, J. and SELBMANN, L. (2018): Sun exposure shapes functional grouping of fungi in cryptoendolithic Antarctic communities. *Life*, 8(2): 19. doi: 10.3390/life8020019
- CONNELL, L., STAUDIGEL H. (2013): Fungal diversity in a Dark Oligotrophic Volcanic Ecosystem (DOVE) on Mount Erebus, Antarctica. *Biology*, 2(2): 798-809. doi: 10.3390/biology2020798.
- COSTA, F. F., DA SILVA, N. M., VOIDALESKI, M. F., WEISS, V. A., MORENO, L. F., SCHNEIDER, G. X., NAJAFZADEH, M. J., SUN, J., GOMES, R. R., RAITZ, R. T., CASTRO, M. A. A., DE MUNIZ, G. B. I., DE HOOG, G. S. and VICENTE, V. A. (2020): Environmental prospecting of black yeast-like agents of human disease using culture-independent methodology. *Scientific Reports*, 10(1): 14229. doi: 10.1038/s41598-020-70915-0
- CROUS, P. W., GROENEWALD, J. Z. (2011): Why eternities don't last. *Persoonia*, 26: 70-84.
- CROUS, P. W., SCHUMACHER, R. K., AKULOV, A., THANGAVEL, R., HERNÁNDEZ-RESTREPO, M., CARNEGIE, A. J., CHEEWANGKON, R., WINGFIELD, M. J., SUMMERELL, B. A., QUAEDEVLIET, W., COUTINHO, T. A., ROUX, J., WOOD, A. R., GIRALDO, A. and GROENEWALD, J. Z. (2019a): New and interesting fungi. 2. *Fungal Systematics and Evolution*, 3: 57-134.
- CROUS, P. W., SUMMERELL, B. A., MOSTERT, L. and GROENEWALD, J. Z. (2008): Host specificity and speciation of *Mycosphaerella* and *Teratosphaeria* species associated with leaf spots of *Proteaceae*. *Persoonia*, 20: 59-86.
- CROUS, P. W., WINGFIELD, M. J., LOMBARD, L., ROETS, F., SWART, W. J., ALVARADO, P., CARNEGIE, A. J., MORENO, G., LUANGSAARD, J., THANGAVEL, R., ALEXandrova, A. V., BASEIA, I. G., BELLANGER, J. M., BESSETTE, A. E., BESSETTE, A. R., DE LA PEÑA-LASTRA, S., GARCÍA, D., GENÉ, J., PHAM, T. H. G., HEYKOOP, M. *et al.* (2019b): Fungal Planet description sheets: 951–1041. *Persoonia*, 43: 223-425. doi: 10.3767/persoonia.2019.43.06
- DE HOOG, G. S., GUARRO, J., GENÉ, D. J., AHMED, S. A., AL-HATMI, A. M. S., FIGUERAS, M. J. and VITALE, R. G. (2020): Atlas of clinical fungi. 4th edition. Hilversum: Foundation Atlas of Clinical Fungi, 779 p.
- DE HOOG, G. S., HERMANIDES-NIJHOF, E. (1977): The black yeasts and allied Hyphomycetes. *Studies in Mycology*, 15: 1-222.
- EGIDI, E., DE HOOG, G. S., ISOLA, D., ONOFRI, S., QUAEDEVLIET, W., DE VRIES, M., VERKLEY, G. J. M., STIELOW, J. B., ZUCCONI, L. and SELBMANN, L. (2014): Phylogeny and taxonomy of meristematic rock-inhabiting black fungi in the *Dothideomycetes* based on multilocus phylogenies. *Fungal Diversity*, 65: 127-165.
- FELSENSTEIN, J. (1985): Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39: 783-791.
- GOSTINČAR, C., OHM, R. A., KOGEJ, T., SONJAK, S., TURK, M., ZAJC, J., ZALAR, P., GRUBE, M., SUN, H., HAN, J., SHARMA, A., CHINIQUY, J., NGAN, C. Y., LIPZEN, A., BARRY, K., GRIGORIEV, I. V. and GUNDE-CIMERMAN, N. (2014): Genome sequencing of four *Aureobasidium pullulans* varieties: Biotechnological potential, stress tolerance, and description of new species. *BMC genomics*, 15: 549. doi: 10.1186/1471-2164-15-549
- GUEIDAN, C., VILLASEÑOR, C. R., DE HOOG, G. S., GORBUSHINA, A. A., UNTEREINER, W. A. and LUTZONI, F. (2008): A rock-inhabiting ancestor for mutualistic and pathogen-rich fungal lineages. *Studies in Mycology*, 61: 111-119. doi: 10.3114/sim.2008.61.11
- HUBKA, V., RÉBLOVÁ, M., REHULKA, J., SELBMANN, L., ISOLA, D., DE HOOG, G. S. and KOLAŘÍK, M. (2014): *Bradomyces* gen. nov. (*Chaetothyriales*, *Trichomeriaceae*), a new ascomycete genus accommodating poorly differentiated melanized fungi. *Antonie van Leeuwenhoek*, 106(5): 979-992. doi: 10.1007/s10482-014-0267-4
- HUELSENBECK, J. P., RONQUIST, F. (2001): MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17: 754-755.

- ISOLA, D., ZUCCONI, L., ONOFRI, S., CANEVA, G., DE HOOG, G. S. and SELBMANN, L. (2015): Extremotolerant rock inhabiting black fungi from Italian monumental sites. *Fungal Diversity*, 76: 75-96.
- KUMAR, S., STECHER, G., LI, M., KNYAZ, C. and TAMURA, K. (2018): MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35: 1547-1549.
- LAICHMANOVÁ, M. (2020): New records of *Oleoguttula mirabilis* and *Rachicladosporium antarcticum* from James Ross Island, Antarctica. *Czech Polar Reports*, 10(2): 226-235. doi: 10.5817/CPR2020-2-17
- MARTIN-SANCHEZ, P. M., GEBHARDT, C., TOEPEL, J., BARRY, J., MUNZKE, N., GÜNSTER, J. and GORBUSHINA, A. A. (2018): Monitoring microbial soiling in photovoltaic systems: A qPCR-based approach. *International Biodeterioration and Biodegradation*, 129: 13-22.
- MARVANOVÁ, L., LAICHMANOVÁ, M. (2014): *Campylospora leptosoma* sp. nov. and characteristics of *Campylospora* spp., based on morphology and on ITS sequences. *Mycosphere*, 5(2): 245-261.
- MORGULIS, A., COULOURIS, G., RAYTSELIS, Y., MADDEN, T. L., AGARWALA, R. and SCHÄFFER, A. A. (2008): Database indexing for production MegaBLAST searches. *Bioinformatics*, 24(16): 1757-1764. doi: 10.1093/bioinformatics/btn322
- MUGGIA, L., FLEISCHHACKER, A., KOPUN, T. and GRUBE, M. (2016): Extremotolerant fungi from alpine rock lichens and their phylogenetic relationships. *Fungal Diversity*, 76: 119-142. doi: 10.1007/s13225-015-0343-8
- MUGGIA, L., QUAN, Y., GUEIDAN, C., AL-HATMI, A. M. S., GRUBE, M. and DE HOOG, S. (2021): Sequence data from isolated lichen-associated melanized fungi enhance delimitation of two new lineages within *Chaetothyriomycetidae*. *Mycological Progress*, 20: 911-927. doi: 10.1007/s11557-021-01706-8
- NEI, M., KUMAR, S. (2000): *Molecular Evolution and Phylogenetics*. Oxford University Press. Oxford, 333 p.
- NEPEL, M., VOGLMAYR, H., SCHÖNENBERGER, J. and MAYER, V. E. (2014): High diversity and low specificity of chaetothyrlean fungi in carton galleries in a neotropical ant-plant association. *PLoS One*, 9(11): e112756. doi: 10.1371/journal.pone.0112756
- NYLANDER, J. A. A. (2004): MrModeltest v2. Program distributed by the author.
- PERINI, L., GOSTINČAR, C., ANESIO, A. M., WILLIAMSON, C., TRANTER, M. and GUNDE-CIMERMAN, N. (2019): Darkening of the Greenland Ice Sheet: Fungal abundance and diversity are associated with algal bloom. *Front Microbiology*, 10: 557. doi: 10.3389/fmicb.2019.00557
- PRASONGSUK, S., LOTRAKUL, P., ALI, I., BANKEEREE, W. and PUNNAPAYAK, H. (2018): The current status of *Aureobasidium pullulans* in biotechnology. *Folia Microbiologica*, 63(2): 129-140. doi: 10.1007/s12223-017-0561-4
- QUAEDVLIEG, W., BINDER, M., GROENEWALD, J. Z., SUMMERELL, B. A., CARNEGIE, A. J., BURGESS, T. I. and CROUS, P. W. (2014): Introducing the Consolidated Species Concept to resolve species in the *Teratosphaeriaceae*. *Persoonia Molecular Phylogeny and Evolution of Fungi*, 33: 1-40.
- QUAN, Y., MUGGIA, L., MORENO, L. F., WANG, M., AL-HATMI, A. M. S., DA SILVA MENEZES, N., SHI, D., DENG, S., AHMED, S., HYDE, K. D., VICENTE, V. A., KANG, Y., STIELOW, J. B. and de Hoog, S. (2020): A re-evaluation of the *Chaetothyriales* using criteria of comparative biology. *Fungal Diversity*, 103: 47-85. doi: 10.1007/s13225-020-00452-8
- RUIBAL, C., GUEIDAN, C., SELBMANN, L., GORBUSHINA, A. A., CROUS, P. W., GROENEWALD, J. Z., MUGGIA, L., GRUBE, M., ISOLA, D., SCHOCH, C. L., STALEY, J. T., LUTZONI, F. and DE HOOG, G. S. (2009): Phylogeny of rock-inhabiting fungi related to *Dothideomycetes*. *Studies in Mycology*, 64: 123-133.
- RUIBAL, C., PLATAS, G. and BILLS, G. F. (2005): Isolation and characterization of melanized fungi from limestone formation in Mallorca. *Mycological Progress*, 4: 23-38.
- RUIBAL, C., PLATAS, G. and BILLS, G. F. (2008). High diversity and morphological convergence among melanized fungi from rock formations in the Central Mountain System of Spain. *Persoonia*, 21: 93-110. doi: 10.3767/003158508X371379

- RUIBAL, C., SELBMANN, L., AVCI, S., MARTIN-SANCHEZ, P. and GORBUSHINA, A. (2018): Roof-inhabiting cousins of rock-inhabiting fungi: Novel melanized microcolonial fungal species from photocatalytically reactive subaerial surfaces. *Life*, 8(3): 30. doi: 10.3390/life8030030
- SÁNCHEZ, R. M., MILLER, A. N. and BIANCHINOTTI, M. V. (2019): New species of *Capronia* (*Herpotrichiellaceae*, *Ascomycota*) from Patagonian forests, Argentina. *Plant and Fungal Systematics*, 64(1): 81-90.
- SELBMANN, L., DE HOOG, G. S., MAZZAGLIA, A., FRIEDMANN, E. I. and ONOFRI, S. (2005): Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert. *Studies in Mycology*, 51: 1-32.
- SELBMANN, L., DE HOOG, G. S., ZUCCONI, L., ISOLA, D., RUISI, S., GERRITS VAN DEN ENDE, A. H., RUIBAL, C., DE LEO, F., URZI, C. and ONOFRI, S. (2008): Drought meets acid: Three new genera in a dothidealean clade of extremotolerant fungi. *Studies in Mycology*, 61: 1-20.
- SELBMANN, L., GRUBE, M., ONOFRI, S., ISOLA, D. and ZUCCONI, L. (2013): Antarctic epilithic lichens as niches for black meristematic fungi. *Biology*, 2(2): 784-797. doi: 10.3390/biology2020784
- SELBMANN, L., ONOFRI, S., ZUCCONI, L., ISOLA, D., ROTTIGNI, M., GHIGLIONE, C., PIAZZA, P., ALVARO, M. C. and SCHIAPARELLI, S. (2015): Distributional records of Antarctic fungi based on strains preserved in the Culture Collection of Fungi from Extreme Environments (CCFEE) Mycological Section associated with the Italian National Antarctic Museum (MNA). *MycKeys*, 10: 57-71. doi: 10.3897/mycokeys.10.5343
- SERT, H. B., SÜMBÜL, H. and STERFLINGER, K. (2007): Microcolonial fungi from antique marbles in Perge/Side/Termessos (Antalya/Turkey). *Antonie Van Leeuwenhoek*, 91: 217-227.
- SERT, H. B., WUCZKOWSKI, M. and STERFLINGER, K. (2012): *Capnobotryella isilogluei*, a new rock-inhabiting fungus from Austria, *Turkish Journal of Botany*, 36(4): 401-407. doi: 10.3906/bot-1102-3
- STALEY, J. T., PALMER, F. and ADAMS, J. B. (1982): Microcolonial fungi: Common inhabitants on desert rocks? *Science*, 215 (4536): 1093-1095. doi: 10.1126/science.215.4536.1093.
- STERFLINGER, K. (1998): Temperature and NaCl- tolerance of rock-inhabiting meristematic fungi. *Antonie Van Leeuwenhoek*, 74: 271-281.
- STERFLINGER, K. (2006): Black yeasts and meristematic fungi: ecology, diversity and identification. In: G. Péter, C. A. Rosa (eds.). *Biodiversity and Ecophysiology of Yeasts*. The Yeast Handbook. Springer, Berlin, Heidelberg, pp. 501-514.
- STERFLINGER, K., DE HOOG, G. S. and HAASE, G. (1999): Phylogeny and ecology of meristematic ascomycetes. *Studies in Mycology*, 43: 5-22.
- STERFLINGER, K., KRUMBEIN, W. E. (1995): Multiple stress factors affecting growth of rock-inhabiting black fungi. *Botanica Acta*, 108(6): 490-496. doi: 10.1111/j.1438-8677.1995.tb00526.x
- STERFLINGER, K., TESEI, D. and ZAKHAROVA, K. (2012): Fungi in hot and cold deserts with particular reference to microcolonial fungi. *Fungal Ecology*, 5(4): 453-462. doi: 10.1016/j.funeco.2011.12.007
- SUN, W., SU, L., YANG, S., SUN, J., LIU, B., FU, R., WU, B., LIU, X., CAI, L., GUO, L. and XIANG, M. (2020): Unveiling the hidden diversity of rock-inhabiting fungi: *Chaetothyriales* from China. *Journal of Fungi*, 6(4): 187. doi: 10.3390/jof6040187
- SWOFFORD, D. L. (2003): PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland.
- THOMPSON, J. D., HIGGINS, D. G. and GIBSON, T. J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22: 4673-4680.
- TSURYKAU, A., ETAYO, J. (2017): *Capronia suijsae* (*Herpotrichiellaceae*, Eurotiomycetes), a new fungus on *Xanthoria parietina* from Belarus, with a key to the lichenicolous species growing on *Xanthoria* s. str. *The Lichenologist*, 49(1): 1-12. doi: 10.1017/S0024282916000530
- VASSE, M., VOGLMAYR, H., MAYER, V., GUEIDAN, C., NEPEL, M., MORENO, L., DE HOOG, S., SELOSSE, M-A, MCKEY, D. and BLATRIX, R. (2017): A phylogenetic perspective on the association between ants (*Hymenoptera: Formicidae*) and black yeasts (*Ascomycota*:

- Chaetothyriales*). *Proceedings of the Royal Society B*, 284: 20162519. doi: 10.1098/rspb.2016.2519
- VILGALYS, R., HESTER, M. (1990): Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, 172: 4238-4246.
- WHITE, T. J., BRUNS, T., LEE, S. and TAYLOR, J. W. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White (eds.): PCR protocols: A guide to methods and applications, Academic Press, Inc. New York, pp. 315–322.
- WIJAYAWARDENE, N. N., HYDE, K. D., DAI, D. Q., SANCHEZ-GARCIA, M., GOTO, B. T., SAXENA, R. K., ERDOGDU, M., SELCUK, F., RAJESHKUMAR, K. C., APTROOT, A. *et al.* (2022): Outline of Fungi and fungus-like taxa – 2021. *Mycosphere*, 13(1): 53-453. doi: 10.5943/mycosphere/13/1/2
- WIJAYAWARDENE, N. N., PHILLIPS, A. J. L., TIBPROMMA, S., DAI, D. Q., SELBMANN, L., MONTEIRO, J. S., APTROOT, A., FLAKUS, A., RAJESHKUMAR, K.C., COLEINE, C., PEREIRA, D.S., FAN, X., ZHANG, L., MAHARACHCHIKUMBURA, S.S.N., SOUZA, M. F., KUKWA, M., SUWANNARACH, N., RODRIGUEZ-FLAKUS, P., ASHTEKAR, N., DAUNER, L., TANG, L.Z., JIN, X. C. and KARUNARATHNA, S. C. (2021): Looking for the undiscovered asexual taxa: Case studies from lesser studied life modes and habitats. *Mycosphere*, 12(1): 1290-1333.
- WOLLENZIEN, U., DE HOOG, G. S., KRUMBEIN, W. E. and URZÍ, C. (1995): On the isolation of microcolonial fungi occurring on and in marble and other calcareous rocks. *Science of the Total Environment*, 167: 287-294.
- ZALAR, P., GOSTINČAR, C., DE HOOG, G. S., URŠIČ, V., SUDHADHAM, M. and GUNDE-CIMERMAN, N. (2008): Redefinition of *Aureobasidium pullulans* and its varieties. *Studies in Mycology*, 61: 21-38. doi: 10.3114/sim.2008.61.02

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[1] <http://technelysium.com.au/wp/chromas/>

[2] <https://www.mycobank.org/>