UNIVERSITY OF TUSCIA, ITALY

DEPARTMENT OF ECOLOGICAL AND BIOLOGICAL SCIENCES

PhD course in

Ecology and sustainable management of environmental resources

XXX Course

Microbial diversity of endolithic lichen-dominated communities in Victoria Land, Antarctica

(s.s.d. BIO/03)

PhD student

Claudia Coleine

Coordinator of PhD course

Prof. Roberta Cimmaruta

Tutors

Prof. Laura Selbmann

Prof. Laura Zucconi

A.Y. 2017/2018

“Your time is limited, so don't waste it living someone else's life. Don't let the noise of others' opinions drown out your own inner voice. And most important, have the courage to follow your heart and intuition. Don't lose faith. I'm convinced that the only thing that kept me going was that I loved what I did. You've got to find what you love. Your work is going to fill a large part of your life, and the only way to be truly satisfied is to do what you believe is great work. And the only way to do great work is to love what you do. As with all matters of the heart, you'll know when you find it. And, like any great relationship, it just gets better and better as the years roll on”.

Steve Jobs (1955-2011)

ABSTRACT

Endolithic communities, representing the predominant life-form in the ice-free areas of Continental Antarctica, are known to host among the most resistant and adapted microorganisms to extreme environmental pressure such as aridity, low temperatures, wide thermal fluctuations, low nutrient availability, seasonal increased UV radiation and geographical isolation. Despite the increasing interest in exploring these ecosystems at the edge of life, relevant for future investigations of extraterrestrial life, our knowledge on microbial diversity and composition is still limited.

The present thesis aimed to explore, by molecular surveys, the microbial diversity in the Antarctic lichen-dominated communities of Victoria Land, visited during the XXVI Italian Antarctic Expedition (2010-2011).

Denaturing Gel Gradient Electrophoresis (DGGE) approach was first performed on a large sampling of different types of rocks (e.g. quartz, dolerite, sandstone and granite) and then a meta-barcoding method on Illumina platform was implemented on a selection of sandstone samples, the best rock substratum for microbial colonization. We have also performed the whole-genome sequencing analysis on seven endolithic fungal strains to make comparative genomics based on genome sequences already available on NCBI/GenBank database. Overall, our findings indicated that these ecosystems harbor relatively low bacterial and fungal species diversity, compared with microbial communities inhabiting temperate environments. The eveness of the communities indicated a very high degree of specialization (F_o near to 100%) with a consequent low resilience; therefore, any possible perturbation may lead to irreversible consequences. Additionally, we demonstrated that the considered environmental parameters (e.g. altitude and sea distance) did not influence the biodiversity. We, therefore, concluded that other environmental parameters (e.g. North/South exposure and variations of micro- and nano-scales of environmental conditions) should be considered in a next sampling as potential driving factors for biodiversity composition and variation. The study represents the widest molecular survey to date and the first wide window on the actual biodiversity and composition of the Antarctic cryptoendolithic communities. Data obtained represent an important contribution for planning a more targeted sampling in the future in order to decipher the relation between environmental variables and Antarctic endolithic lifestyle and understand how these border communities may be influenced by Climate Change.

CONTENTS

CHAPTER 1. INTRODUCTION

1.1 Antarctica

The Antarctic continent hosts the coldest and most arid environments on the Earth (Onofri *et al*., 2004). Positioned asymmetrically around the South Pole and largely south of the Antarctic Circle, Antarctica is the southernmost continent, surrounded by the Southern Ocean. Antarctica is the fifth-largest continent, covering more than 14 million km^2; the Transantarctic Mountains, running from the Ross Sea to the Weddell Sea, divide the continent in two geographically and geologically distinct areas, East Antarctica and West Antarctica, the latter including the Antarctic Peninsula. The first is a carbonic rock body that covers 10.2 million square kilometres, roughly 73% of the continent; it is almost entirely buried by the East Antarctic Ice Sheet with an average thickness of 2200 reaching up to 4700 meters in some locations. West Antarctica is composed of small fragments of continental crust covered by ice sheet and lying primarily below sea level (Fig. 1.1).

Figure 1.1 Map of Antarctica

At the edge of the continent, strong katabatic winds often blow from the polar plateau at storm force. In the interior, wind speeds are typically moderate. While the vast majority of the continental land surface area is permanently covered by a multi-kilometer thick layer of ice, around 1-3% is ice-free for at least part of the year. These ice-free areas are largely confined to the perimeter of the continent at coastal sites

and regions cut off from the Antarctic Ice Sheet, but also include mountain peaks that protrude through the extensive ice cover of the Antarctic Plateau, including biotic habitats as lakes, glacial streams, flushes, ponds, open mineral soils and stable rock surfaces (Cowan and Ah Tow, 2004).

The Antarctic continent offer a unique combination of extreme climatic conditions which include extremely low temperatures, wide temperature fluctuations, low water availability, UV irradiation, long periods of darkness, and high periodic incident solar radiation. Climatic conditions are by no means homogenous across the continent and widely differing climatic regions exist; for example, temperatures and water availability differ between the glacial dome, peninsular, and Dry Valleys regions of the continent. Conditions across most of the continent are inhospitable to most plant and animal populations.

Antarctica includes three climatic regions: i) sub-Antarctic region, dominated by vascular plants at lower altitude and Bryophyta and lichens at higher altitude; ii) maritime Antarctic region, where lichens belonging to the genera *Acarospora*, *Buellia*, *Caloplaca*, *Lecanora*, *Lecidea* and *Rhizoplaca* predominate. Only two species, *Colobanthus quitensis* (*Caryophillaceae*) and *Deschampsia antartica* (*Poaceae*), are present as vascular plants and; iii) continental Antarctica, where biology is dominated, more than elsewhere on earth, by microorganisms (Nienow and Friedmann, 1993) due the extreme environmental conditions; here mosses and lichens are restricted to a small altitudinal range along coastal regions (Convey *et al*., 2008).

Mosses and lichens are the only conspicuous organisms that, together with cyanobacteria, typically represent the dominant phototrophs in Antarctica (Vincent, 2002). The complete absence of terrestrial vertebrates means that heterotrophic organisms are limited to invertebrates, together with microorganisms as protozoa, fungi, bacteria, and archaea.

1.1.1 Victoria Land

The largest expanses of ice-free land are found in the Victoria Land, the Transantarctic Mountains, and the Antarctic Peninsula, while the areas in East and West Antarctica are substantially smaller. To date, microbiological analyses have been most extensively undertaken on the Peninsula and in the Victoria Land. This Land covers a latitudinal gradient of 8° from Darwin Glacier to Cape Adare, positioned between the Polar plateau and the coast and exposed to a wide spectrum of climatic variation, including temperature and precipitation regimes (Barrett *et al*., 2006a) (Fig. 1.2).

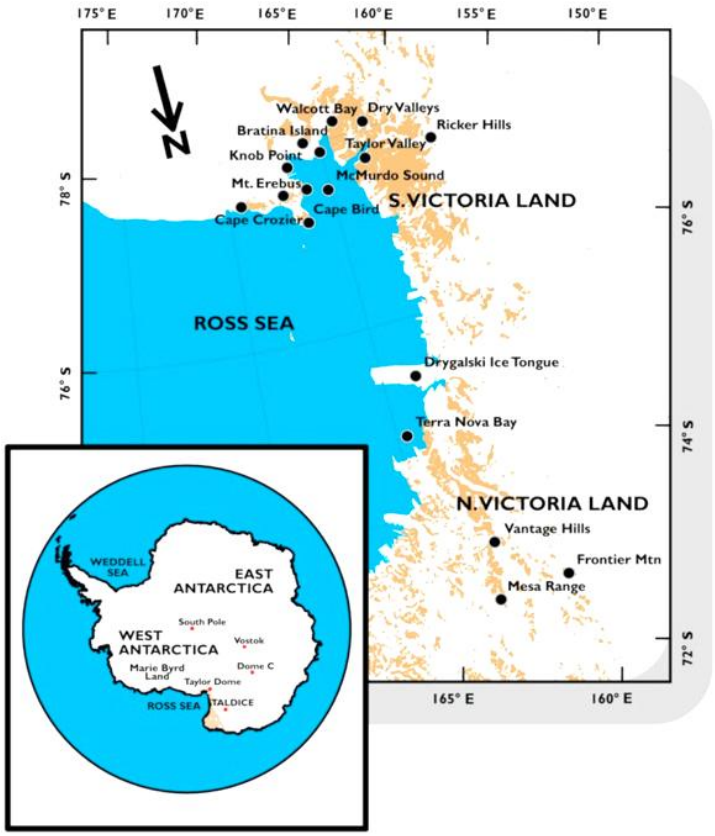


Figure 1.2 Victoria Land, Antarctica

Ice-free areas dominate the landscape of Southern Victoria Land and the high altitude locations of Northern Victoria Land, while low-elevation coastal soils of Northern Victoria Land see considerable marine and biological influence (Barrett *et al*., 2006b).

Figure 1.3 McMurdo Dry Valleys (Southern Victoria Land)

The McMurdo Dry Valleys (or Ross Desert), in the middle of the Transantarctic Mountains in South Victoria Land, constitute the most extensive ice-free area in Antarctica (Friedmann and Ocampo, 1976; Onofri *et al*., 2004), with an aspect of a true desert (Fig. 1.3). It covers an area of roughly 4800 km^2 (about 0.3% of the total land area of the continent) (Fox and Cooper, 1994) and is considered the coldest, driest and windiest desert on Earth (Wynn-Williams, 1990; Moorhead *et al*., 1999; Pointing *et al*., 2015). Indeed, the mean annual air temperatures ranges from -20 to -35°C, and mean precipitation from less than 10 to 100 mm (Bockheim and McLeod, 2008). Dry katabatic winds blowing above 100 km h^{-1} descend from the Antarctic ice plateau into the valleys and contribute to the maintenance of desert conditions (Friedmann, 1982; Doran *et al*., 2002). McMurdo Dry Valleys are dominated by microbial communities that colonize oligotrophic mineral soils (Cary *et al*., 2010) but mostly occur endolithically (de los Rìos *et al*., 2014) as a stress avoidance strategy (Pointing and Belnap, 2012; Pointing *et al*., 2014). The McMurdo Dry Valleys are considered the closest Martian analogous habitat on Earth, exhibiting extraordinary aridity, low temperatures, wide thermal fluctuations, low nutrient availability, seasonal increased UV radiation, and geographical isolation (Onofri *et al*., 2004).

1.2 Microbial life in dryland environments

Under extreme aridity, thermal and high solar radiation stress, endolithic ecological niches found in cold and hot deserts are considered to be environmental refuges for life and they are often the only visible life in such extreme terrestrial environments (Golubic *et al*., 1981; Friedmann, 1982; Walker and Pace, 2007; Pointing and Belnap, 2012; Wierzchos *et al*., 2012). Lithic-associated microhabitats are referred as lithobiontic ecological niches, and their communities' inhabitants are termed "lithobionts" (Golubic *et al*., 1981). Lithic niches are widely dispersed and allow microbial communities to withstand environmental stressors. These communities contribute significantly to the ecology and function of both hot and cold deserts and they are broadly divided into epilithic (on rock surface), hypolithic (underneath rock) and endolithic (inside rock) communities (Cowan and Ah Tow, 2004) (Fig. 1.4).

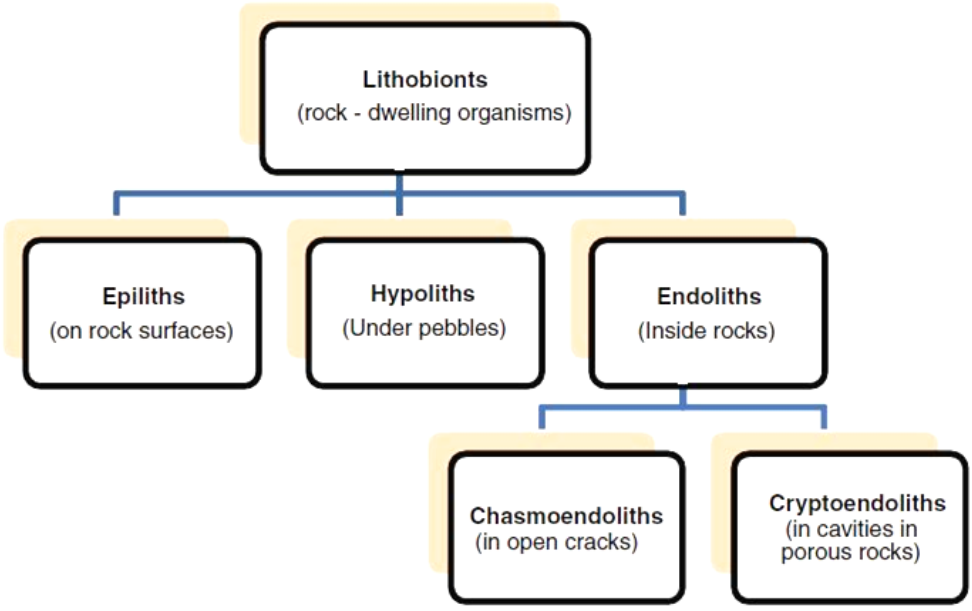


Figure 1.4 Classification of lithobiont niches inhabited by desert microorganisms

Ecosystems of arid and hyper-arid environments are highly vulnerable, including the microbial communities supporting these biomes. Under extreme water deficit and high solar radiation, endolithic (inhabiting rock) habitats are considered environmental refuges for life (Friedmann, 1982; Cary *et al*., 2010; Pointing and Belnap, 2012; Wierzchos *et al*., 2012). In these microbial ecosystems, the rock substrate provides protection from incident UV, excessive solar radiation, and freeze-thaw events while providing physical stability, and enhanced moisture availability (Walker and Pace, 2007; Chan *et al*., 2012). The establishment of endoliths is often associated with moss and lichen communities on the rock surfaces (Pointing and Belnap, 2012; Nash *et al*., 1977), although heterotrophic fungi and cyanobacteria

also appear to be key constituents (Staley *et al*., 1982; Gorbushina, 2007). However, the colonization pattern of rock is strictly dependent on climatic conditions; indeed, towards the interior of the continent and at higher altitudes, conditions become more severe and the microbial colonization progressively decreases as reported by Zucconi and colleagues (2016).

Epilithic colonization is perhaps the most vulnerable of the lithic colonization, as it is exposed to strong winds, ultraviolet radiation, desiccation and other perturbations (Cockell *et al*., 2008; Wynn-Williams and Edwards, 2000); as a consequence, in continental Antartica epi-lithobionts are often restricted to the coastal Antarctic regions (Broady, 1981; Wynn-Williams and Edwards, 2000), and when epilithic growth becomes rare until complete disappearance, endolithic colonization predominates (Nienow and Friedmann, 1993).

Instead, endolithic microbial communities colonize the interior of rocks by adapting to the different ecological sub-niches within the lithic substrate (Golubic *et al*., 1981). Depending on the micromorphological and structural properties of the rock, endoliths can be found as interstitial habitats of cracks and fissures (chasmoendoliths or chasmoliths) and also in spaces between mineral grains (cryptoendolithic) (Golubic *et al*., 1981; Nienow and Friedmann, 1993; Budel *et al*., 2009; Cowan and Ah Tow, 2004). Chasmoliths are commonly found in siliceous rocks, but also have been reported in granite, marble, silicified sandstone, gypsum crusts and anorthosite (Friedmann, 1982; Broady, 1981; de los Rìos *et al*., 2005). In contrast, cryptoendoliths are principally associated with porous sandstone rocks (Bell, 1993; Friedmann and Ocampo- Friedmann 1984; Nienow *et al*., 2003; Zucconi *et al*., 2016), but could be found also in other mineral types including granite, gneiss, limestone, marble, gypsum, halite and evaporites (Cockell *et al*., 2002; Nienow and Friedmann, 1993; Boison *et al*., 2004; Wierzchos *et al*., 2006). In the overall, endolithic lifestyle dominates in the most inhospitable terrestrial climates and is regarded as the borderline adaptation of life just before its extinction (Friedmann, 1982; Nienow and Friedmann, 1993; Onofri *et al*., 2007).

1.2.1 Antarctic cryptoendolithic lichen-dominated communities

In Antarctica, more than in any other continent, microorganisms play a dominant role in the terrestrial environment and rocks are one of the main substrata for colonization (Nienow and Friedmann, 1993). The susceptibility of rocks to endolithic colonization depends mainly on the physical and chemical properties of the substrate. These properties are essential to provide the microbial community with enough light for photosynthesis, appropriate moisture retention from water collection during snow or fog events, a heat sink and reflector to help regulate temperature, and protection against harmful UV radiation (Friedmann and Ocampo-Friedmann, 1984; Nienow, 2009; Wierzchos *et al*., 2012).

Different endolithic communities have been described in Antarctica, depending on the nature and proportions of primary producers involved, including lichens and cyanobacteria. In the McMurdo Dry Valleys (Southern Victoria Land), the most widely distributed community is the one dominated by cryptoendolithic lichens described by Friedmann (1982). Since the earliest reports of endolithic colonization (Friedmann and Ocampo, 1976; Friedmann, 1982), the lichen-dominated community has been recognized as a clearly visible layered community with different coloured bands occurring at different depths within the rock (Wierzchos *et al*., 2012). The near-surface layer appears black due to the presence of melanized fungi, a deeper white layer is attributed to lichen growth, and the deepest green layer has been identified as supporting free-living chlorophyte algae and cyanobacteria (Friedmann, 1982; de los Rìos *et al*., 2014). The bands, or "zones," are usually comprised of a 1 mm thick upper black zone, a 2-4 mm thick middle white zone, and a lower green zone of similar size (Friedmann, 1982) (Fig.1.5).

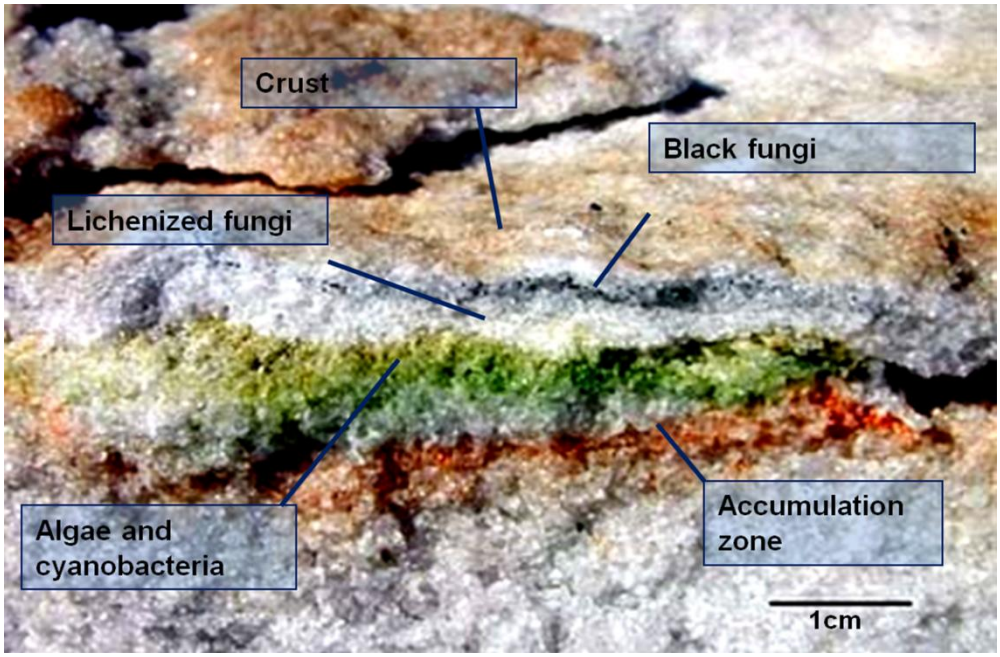


Figure 1.5 Cryptoendolithic lichen dominated community colonizing sandstone

Molecular methods showed a single green algal species, *Trebouxia jamesii* to be dominant (de la Torre *et al.*, 2003; Domaschke *et al*., 2012), known as component of lichen associations, such as the highly adapted Antarctic lichen *Lecidea cancriformis* (Ruprecht *et al*., 2012). Instead, among the prokaryotic members, phylotypes related to Actinobacterial, alpha-Proteobacterial, c-Proteobacterial, and Planctomycetales species are predominant (de la Torre *et al*., 2003). Seven new genera and 12 new species, most of which belonging to the ecological group of the "Black Yeasts" known for their

extraordinaly tolerance to stresses, have been described to date from these communities with molecular phylogenetic approaches (Selbmann *et al*., 2005; 2008; 2014; Egidi *et al*., 2014).

1.3 Next Generation Sequencing approach to study microbial diversity

The advent of High-Throughput Sequencing (HTS) technologies (e.g. Ion Torrent, Illumina) (Logares *et al*., 2012) is enabling to develop molecular tools to resolve community molecular diversity greater than that was previously appreciated and analyze microbial diversity at an unprecedented scale culture-independent approach. The recent emergence of Next-Generation Sequencing (NGS) platforms (Shendure and Ji, 2008; Glenn, 2011) can provide billions of sequence reads in a single experiment, which corresponds to an improvement of at least five orders of magnitude when compared to traditional Sanger DNA sequencing.

Using -omics approaches is now economically feasible given the constant reduction in high-throughput sequencing costs, having the advantage of retrieving deeply more accurate and detailed DNA/RNA information. Among the several NGS approaches, DNA metabarcoding has emerged as having potential to provide speedy assessment of community structure from environmental samples. Metabarcoding is transforming ecology (Creer *et al.*, 2010; Bik *et al.*, 2012), especially of cryptic biodiversity as the scarcely known Antarctic endolithic communities. Previously, studies had been impeded by the difficulty of measuring very high levels of diversity of very small *taxa*, and metabarcoding technology has mostly resolved this limit, in the same way that microbiome biology has been unlocked by next-generation sequencing (Committee on Metagenomics, 2007).

Despite the known biases introduced during PCR Sequencing of DNA polymerase chain reaction (PCR), amplicon sequencing is the most common approach for investigating environmental prokaryotic and eukaryotic diversity, to describe the taxonomic diversity and structure of microbial communities (Tedersoo *et al*., 2015; Smith and Peay, 2014).

1.4 Aim of the thesis and chapters description

The present thesis aims to describe the microbial diversity in Antarctic lichen-dominated communities. Knowledge on diversity of these commuities is still scant, and data on eukaryotes are even more fragmentary if compared to prokaryotes.

We first utilized Denaturing Gel Gradient Electrophoresis (DGGE) and then Next Generation Sequencing approaches. DGGE method was performed to analyze a selection of colonized rock samples collected during the XXVI Italian Antarctic Expedition (2010-2011), for investigating the microbial diversity focused on fungal component. Samples of different typologies of rocks (sandstone, granite, dolerite and quartz) were collected in the Victoria Land. Basing on these first results, sandstone has been chosen as model, representing the best rock substratum for microbial colonization, and sandstone samples only were analyzed by Next Generation Sequencing approaches. Meta-barcoding analyses targeting both fungal and bacterial components were performed to describe in more detail microbial composition and community structure. In addition, we used whole-genome sequencing approach and obtained genome sequences from seven Antarctic fungal strains with the aim to make comparative genomics analyses using genome sequences described in this thesis and genome sequences available on NCBI/GenBank database.

The first chapter includes an introduction of Antarctica, in particular of the Victoria Land, describing then the lithic communities, focusing on Antarctic cryptoendolitic ones. The chapter ends with a focus on Next Generation Sequencing, being the method mostly used in this thesis. The second chapter describes the DGGE analysis performed on a wide selection of rock (sandstone, quartz, dolerite and granite) samples; chapter ends with the crucial selection of only sandstone as best rock substratum, basing on that our further analysis. The following two chapters (3 and 4) show analyses using meta-barcoding approach targeting fungal and bacterial components, respectively focusing on taxonomic, alpha- and beta- diversity data and correlation between biodiversity data and the considered environmental parameters (e.g. altitude and distance from the sea). Chapter 5 includes the whole genome sequencing approach and describes the seven fungal strains studied. Also, draft assemblies and annotations were provided and the accession numbers for NCBI/GenBank database were deposited.

General conclusions and synthesis are presented in chapter 6.

CHAPTER 2.

EFFECT OF ENVIRONMENTAL PARAMETERS ON BIODIVERSITY OF THE FUNGAL COMPONENT IN THE LITHIC ANTARCTIC COMMUNITIES

Abstract

A wide sampling of rocks, colonized by microbial epi- and endolithic communities, was performed along an altitudinal gradient from sea level to 3600 m a.s.l. and sea distance from the coast to 100 km inland along the Victoria Land coast, Antarctica. Seventy-two rock samples of different typology, representative of the entire survey, were selected and studied using DGGE (Denaturing Gradient Gel Electrophoresis) to compare variation in fungal diversity according to environmental conditions along this altitudinal and sea distance transect. Lichenized fungi were largely predominant in all the samples studied and the biodiversity was heavily influenced even by minimal local variations. The n-MDS analysis showed that altitude and sea distance affect fungal biodiversity but in a much lesser extent than rock typology/porosity since sandstone allows the communities to maintain high biodiversity indices. The Pareto Lorenz curves indicate that all the communities analyzed are highly adapted to extreme conditions but scarcely resilient, so any external perturbation may have irreversible effects on these fragile ecosystems.

Key words: Antarctic, Climate change, DGGE, Endolithic communities, Fungi

Selbmann, L., Onofri, S., Coleine, C., Buzzini, P., Canini, F., and Zucconi, L. (2017) Effect of environmental parameters on biodiversity of the fungal component in lithic Antarctic communities. *Extremophiles*, 1-12.

2.1 Introduction

Rock represents the earliest terrestrial niche for life at the time when microbes were the only life-forms on Earth. At present, when conditions are too harsh for complex organisms, microbes remain the only settlers and even withdraw inside rocks if external environmental parameters become incompatible for active life. Airspace within rocks offers microbiota a protected and buffered microenvironment and, actually, endolithic development allows life to expand into different extreme conditions, i.e. hot and cold deserts or geothermal environments (Friedmann and Ocampo 1976; Friedmann, 1982; Bell, 1993; Walker *et al.*, 2005). Rocks are the prevailing substratum for life in Antarctica (Nienow and Friedmann, 1993); soils generated in the distant past, when the continent was positioned at higher latitudes, were eroded by ice and winds once continental drift moved Antarctica into its present position at the South Pole (Selbmann *et al*., 2015). Highly oligotrophic soils of the McMurdo Dry Valleys support relatively low biomass: an autotrophic component is lacking, prokaryotes are dominated as Actinobacteria while eukaryotic assemblages are largely dominated by fungi, both filamentous and yeasts (Pointing *et al*., 2009; Rao *et al.*, 2011; Lee *et al.*, 2012). Rocky outcrops support the highest standing biomass in the Antarctic ice-free desert (Cowan and Tow, 2004; Cary *et al.*, 2010; Cowan *et al.*, 2014).

Some ice-free areas of continental Antarctica are considered the best analogues for a Martian environment; there, the conditions approach the limits of tolerability for most life forms, and endolithic life-style is the most widespread type of colonization and often the sole possibility for survival (Friedmann and Ocampo, 1976; Friedmann, 1982). These communities offer important opportunities for study in many different fields; their reduced complexity and the absence of higher organisms, as plants or animals, make them excellent systems to investigate and elucidate the evolution of microbial function and process without the influence of any external perturbation (Vincent, 2000). A number of new and possible endemic genera and species that evolved exclusively in these peculiar niches have been described in the last decade (Selbmann *et al*., 2005, 2008, 2015; Egidi *et al.*, 2014). Antarctic endolithic communities host among the most extremo-tolerant organisms known to date; the black meristematic fungus *Cryomyces antarcticus*, repeatedly selected for experiments in simulated and real Outer Space conditions is a notable example (Onofri *et al.*, 2012, 2015; Selbmann *et al.*, 2015a; Pacelli *et al.*, 2017).

As border ecosystems, endolithic Antarctic communities are expected to be very sensitive to external variations. Antarctica has experienced the most rapid changes in mean air temperatures on Earth over the past 50 years; in some Antarctic areas up to 5 times the mean rate of global warming was recorded (Steig *et al*., 2009). Even in the McMurdo Dry Valleys, the rising levels of the permanently frozen Lake Bonney in Taylor Valley indicate a recent change in the local climate (Bomblies *et al*., 2001). To monitor future variations related to climate change, the distribution of epi- and endolithic colonization, up to the limit of extinction, has recently been mapped in North Victoria Land, Antarctica (Zucconi *et al*., 2016).

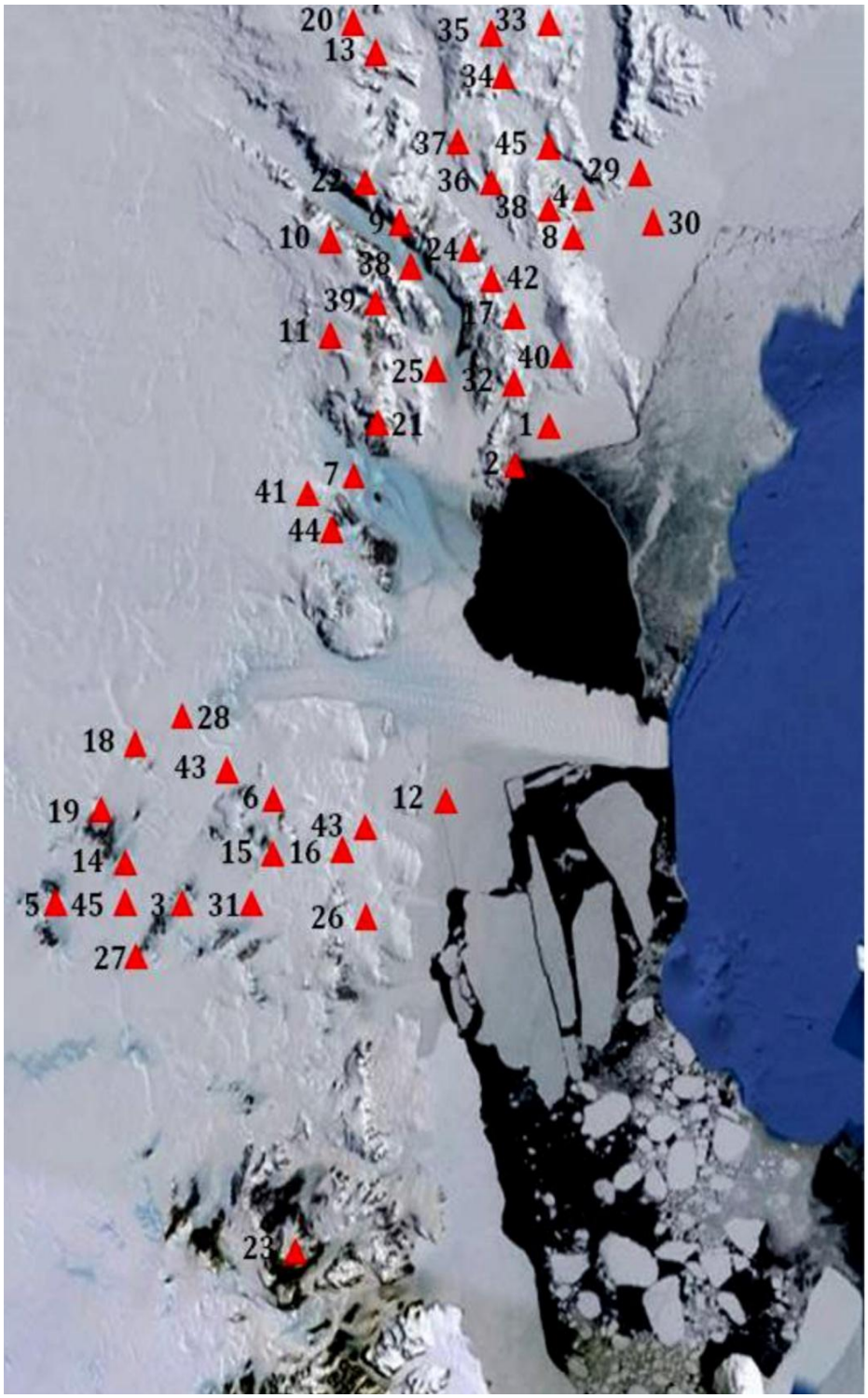
Despite recent advances, data on biodiversity amplitude in these communities remain scarce because of only limited or scattered investigations on a few rock samples or on different samples from a single location (de La Torre *et al*., 2003; Pointing *et al*., 2009; Archer *et al*., 2016). These studies also have focused almost exclusively on the bacterial compartment (Wei *et al*., 2016). Moreover, nothing is known on how and if biodiversity in these communities is affected by differing environmental pressures. Since it is virtually certain that the progression of current global warming will continue and intensify in the future, we can expect a direct influence on the delicate equilibrium in these border ecosystems, including by introduction of much more competitive allochthonous species (Frenot *et al*., 2005; Farrell *et al*., 2011; Olech and Chwedorzewska, 2011; Selbmann *et al*., 2012). Therefore, it is of utmost importance to expand our knowledge on terrestrial Antarctic ecosystems before any external impacts alter their natural equilibrium.

This study is based on extensive rock sampling along a latitudinal and sea distance transect in Northern Victoria Land, Antarctica. Our objective in this survey was to elucidate fungal biodiversity and composition of Antarctic lithic microbial communities, its variation and community structure, and changes with differing environmental variables. Since rock porosity is hypothesized to influence microbial diversity (Cockell *et al*., 2003; Cámara *et al*., 2014), rocks of different typologies were included in the study. Our data also provide insight for monitoring and predicting any possible future variation in fungal biodiversity in relation to climate change (Hogg and Wall, 2011).

2.2 Material and Methods

2.2.1 Samples Collection

Sampling sites were distributed along a latitudinal transect ranging from 73°26’46’’S (Chisholm Hills, Cosmonaut Glacier) to 76°54’36’’S (Battleship Promontory), and an altitudinal gradient from sea level (Kay Island, Vegetation, Island, Inexpressible Island, Prior Island) up to about 3600 m a.s.l. (Mt. Adamson), and along a ~100 km distance from the coast to the interior of the continent (Chisholm Hills, Archambault Ridge, Ambalada Peak, Trio Nunatak, Ricker Hills, Bobby Rocks). The study was conducted on a selection of 72 samples from 46 localities, based on results obtained in a previous study and representative of a much more extensive sampling effort (Zucconi *et al*., 2016) (Fig. 2.1).



1. Campo Icaro
2. Vegetation Island
3. Pudding Butte
4. Harrow Peak
5. Ambalada Peak
6. Mt Howard
7. Mt. Larsen
8. Random Hills
9. Timber Peak
10. Mt. New Zealand
11. Thern Promontory
12. Prior Island
13. Mt. McGee
14. Mt. Billing
15. Bobby Rocks
16. Mt. Bowen
17. Mt. Dickason
18. Ford Peak
19. Ricker Hills
20. Archambault Ridge
21. Anderson Ridge
22. Shafer Peak
23. Battleship Promontory
24. Mt. Burrows
25. Mt. Nansen
26. Starr Nunatak
27. McLea Nunatak
28. Trio Nunatak
29. Inexpressible Island
30. Kay Island
31. Richard Nunatak
32. Mt. Keinath
33. Stewart Heights
34. Mt. Adamson
35. Chisholm Hills
36. Pinkard Table
37. Bier Point
38. Mt. Meister
39. Eskimo Point
40. Whishbone Ridge
41. Reed Nunatak
42. Olson Nunatak
43. Mt. Stephen
44. Unnamed site
45. Tinker Glacier

Figure 2.1 Map of the sampling sites. All samples were collected in Victoria Land, Antarctica. Localities have triangle symbol-code and are listed on the right of the map

Rock sampling was performed from December 2010 to February 2011 in different sites of Victoria Land, continental Antarctica, by L. Zucconi and L. Selbmann (Fig. 2.2). Rock samples were collected aseptically using a sterile chisel and preserved in sterile plastic bags at -20°C. Rocks of different typology

(sandstone, granites, dolerites, quarts and lava dike) were collected; the degree of porosity was determined by Zucconi *et al*., (2016); localities and sampling details are presented in Table 2.1.

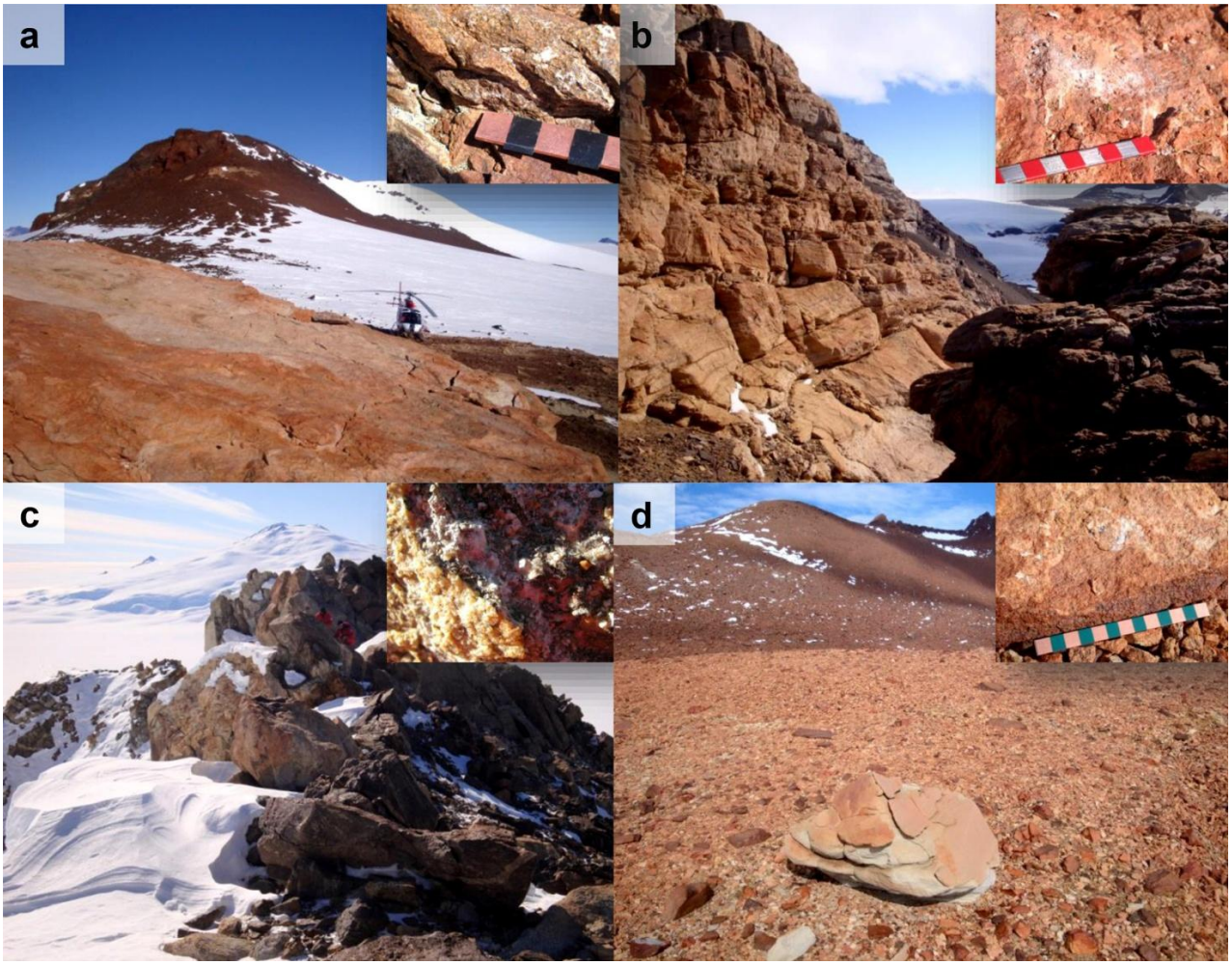


Figure 2.2 A selection of sampling sites in Northern Victoria Land. Dolerite substrates for endolithic microbial colonization at Bobby Rocks (a); sandstone cliff in Richard Nunatak and sample collected in the site (b); view of Mt. Dickason and endolithic growth in granite (c); erratic sandstone boulder on valley floor of Ford Peak and a magnification of the colonization (d). Subscale bar 1 cm.

Table 2.1 Table 1S includes samples and characteristics of visited localities: altitude (m a.s.l.), sea distance (km), coordinates, type of rocks collected (CS, conglomeratic sandstone; D, dolerite; G, granite; S, sandstone; Q, quartz) and rock porosity (l, low; m, medium; h, high) are reported. For each locality the corresponding lane number of the DGGE gel is also reported.

Lanes	Sample	Altitude (m a.s.l.)	Sea Distance (km)	Coordinates	Rocks	Porosity
1	Campo Icaro sample 1	60	0.1	74°42′57″S 164°06′41″E	G	l
2	Vegetation Island sample 4	200	0	74°47′03″S 163°39′35″E	G	l
3	Pudding Butte site 2	1.600	58.6	75°52′02″S 159°58′58″E	CS	m
4	Harrow Peak	1230	6.5	74°04′33″S 164°48′32″E	G	m
5	Ambalada Peak sample 3	1800	105.5	75°57′05″S 158°24′25″E	CS	h
6	Mt Howard site 2 sample 1	1352	33.8	75°40′50″S 161°16′15″E	CS	m
7	Mt Larsen sample 2	935	10	74°53′03″S 162°09′54″E	G	m
8	Random Hills sample 2	1700	13	74°06′11″S 164°22′53″E	G	l
9	Timber Peak site 1 sample 3	2800	49,5	74°10′13″S 162°25′31″E	CS	m
10	Mt New Zealand sample 4	2888	47	74°10′46″S 162°31′01″E	S	m
11	Thern Promontory	1500	29	74°33′S 162°04′E	S	m
12	Mt Howard site 2 sample 2	1352	33.8	75°40′50″S 161°16′15″E	CS	m
13	Prior Island sample 1	50	0	75°41′31″S 162°52′49″E	G	l
14	Mt McGee site 2	2725	19	73°44′26″S 162°40′32″E	G	l
15	Mt Billing sample 2	1300	44	75°42′12″S 160°54′28″E	CS	m
16	Bobby Rocks sample 1	1680	91	75°48′35″S 159°11′15″E	S	h
17	Mt Bowen sample 1	1874	39.5	75°45′24″S 161°03′46″E	S	h
18	Mt Dickason site 1 sample 5	1840	21	74°23′58″S 164°00′21″E	G	l
19	Ford Peak sample 3	1190	57	75°41′29″S 160°25′57″E	CS	m
20	Timber Peak site 2 sample 4	2800	50	74°10′11″S 162°25′34″E	CS	m
21	Trio Nunatak site 4 sample 3	1400	84.5	75°28′59″S 159°35′21″E	S	m
22	Ricker Hills site 3 sample 2	1820	91.5	75°43′25″S 159°10′55″E	S	l
23	Archambault Ridge site 1 sample 4	3200	90	73°41′06″S 162°25′50″E	CS	hm
24	Anderson Ridge sample 2	500	4.7	74°42′51″S 162°37′04″E	G	m
25	Shafer Peak site 1	3100	59	74°02′19″S 162°37′16″E	CS	m
26	Battleship Promontory sample 9	1000	33.5	76°54′36″S 160°56′05″E	S	lm
27	Mt Burrows	2000	26.6	74°18′S 163°39′ E	G	l
28	Mt Nansen site 1 sample 4	2240	12.5	74°37′43″S 162°35′38″E	G	l
29	Starr Nunatak sample 2	1420	3.2	75°53′56"S 162°35′38"E	G	l
30	McLea Nunatak	2100	75.6	75°59′47″S 159°30′05″E	S	lm
31	McLea Nunatak	2100	75.6	75°59′47″S 159°30′05″E	LD	l
32	Trio Nunatak site 3 sample 5	1000	82	75°30′02″S 159°40′28″E	S	lm
33	Inexpressible Island sample 9	0	0	74°53′21″S 163°44′43″E	Q	l
34	Olson nunatak sample 3	200	2	74°55′55″S 162°24′08″E	S	mh
35	Archambault Ridge site 3 sample 1	3400	87	73°40′09″S 162°35′37″E	CS	m
36	Kay Island sample 1	80	0	74°04′13″S 165°18′57″E	Q	m
37	Mt Nansen site 2 sample 1	2349	20	74°32′11″S 162°33′55″E	S	h
38	Inexpressible Island sample 3	0	0	74°53′21″S 163°44′43″E	G	l
39	Richard Nunatak site 3 sample 2	2000	70.2	75°56′00″S 159°47′38″E	CS	lm

Lanes	Sample	Altitude (m a.s.l.)	Sea Distance (km)	Coordinates	Rocks	Porosity
40	Pudding Butte site 3 sample 1	1600	64	75°52′51″S 160°08′56″E	S	m
41	Mt Keinath	385	5.2	74°33′03″S 164°03′92″E	G	l
42	Archambault Ridge site 2 sample 3	2725	80	73°44′26″S 162°40′32″E	G	l
43	Mt Howard site 1 sample 8	1200	33.8	75°40′22″S 161°17′01″E	CS	m
44	Richard Nunatak site 3 sample 1	2000	70.2	75°56′00″S 159°47′38″E	CS	lm
45	Stewart Heights sample 3	2670	74	73°29′26″S 163°54′44″E	CS	m
46	Mt Adamson	3600	60	73°55′59″S 162°58′43″E	S	l
47	Stewart Heights sample 4	2670	74	73°29′26″S 163°54′44″E	G	m
48	Ricker Hills site 1 sample 1	1115	96	75°38′39″S 159°01′42″E	S	m
49	Mt Nansen site 3	1200	23.2	74°34′44″S 162°14′37″E	CS	l
50	Chisholm Hills sito 1 sample 2	2500	81	73°26′46″S 163°18′50″E	S	m
51	Chisholm Hills site 1 sample 1	2500	81	73°26′46″S 163°18′50″E	D	m
52	Pinkard Table	1550	13.5	74°04′30″S 164°03′05″E	G	m
53	Bier Point	1460	8	74°08′11″S 164°08′00″E	G	l
54	Mt Meister	1632	41	74°12′38″S 162°44′53″E	G	l
55	Eskimo Point	1816	37	74°16′14″S 162°36′33″E	Q	l
56	Wishbone Ridge	1396	17	74°26′24″S 163°58′01″E	G	m
57	Campo Icaro	60	0.1	74°42′57″S 164°06′41″E	Q	l
58	Reed Nunatak	1133	0	74°49′19″S 161°58′31″E	G	l
59	Olson nunatak sample 4	200	2	74°55′55″S 162°24′08″E	G	l
60	Mt Howard site 1	1200	33.8	75°40′22″S 161°17′01″E	D	l
61	Mt Howard site 1	1200	33.8	75°40′22″S 161°17′01″E	Q	l
62	Prior Island	50	0	75°41′31″S 162°52′49″E	Q	m
63	Mt Stephen	532	20.3	75°41′49″S 161°45′12″E	LD	l
64	Mt Billing	1300	44	75°42′12″S 160°54′28″E	D	l
65	Bobby Rocks	1680	91	75°48′35″S 159°11′15″E	D	l
66	Unnamed Site	2700	40.5	74°15′13″S 162°30′52″E	S	l
67	Tinker Glacier	600	27	73°56′08″S 164°24′09″E	LD	l
68	Mt McGee site 3 sample 1	230	13	74°01′42″S 164°44′45″E	G	l
69	Shafer Peak site 2	3300	48	74°02′38″S 162°36′46″E	G	l
70	Mt Dickason site 2	1586	21.5	74°23′29″S 164°01′13″E	G	m
71	Battleship Promontory sample 8	1000	33.5	76°54′36″S 160°56′05″E	S	lm
72	Vegetation Island sample 1	200	0	74°47′03″S 163°39′35″E	G	l

2.2.2 Rocks preparation and DNA extraction

Seventy-two rock samples (500 mg) were ground under sterile conditions using an MM 400 RETSCH grinder (Verder Scientific, Bologna, Italy). Total DNA was extracted using a NucleoSpin® Plant II Kit (Macherey-Nagel, Gmbh & Co. KG, Duren, Germany) and Quant-iT dsDNA HS assay kit (Invitrogen molecular probes- Eugene, Oregon, USA) was performed to quantify extracted DNA.

2.2.3 PCR-DGGE (Denaturing Gel Gradient Electrophoresis)

For DGGE analysis a semi-nested PCR was performed using primers with a GC-clamp (Muyzer *et al.*, 1993). Fungal ITS rRNA was amplified from total DNA with primers ITS1F and ITS4 (*Tao et al.*, 2008) in semi-nested PCR as reported in Coleine *et al*. (2015). Amplicons were purified using NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel, Gmbh & Co. KG, Düren, Germany). One hundred ng of final DNA concentration was loaded into each well for DGGE and runs were performed on DGGE-1 System (ELETTROFOR s.a.s., Scientific Instruments, Rovigo, Italy).

Gel at 7.5% polyacrylamide (37.5:1 acrylamide:bisacrylamide) was run with 1 x TAE (Tris-Acetate-EDTA) buffer at 200 V and 60°C for 5 h. Optimum denaturing gradient for band separation was 20-60% formamide and urea. Bands were visualized by staining for 40 min with GelRed solution (Biotiuminc, CA, USA) (1.34 g NaCl, 66.7 pi GelRed and 200 mL dw). DGGE reference marker was constructed and composed by mixing equal amounts of amplicons obtained from seven fungal and yeast species from the CCFEE (Culture Collection of Fungi From Extreme Environments): *Cryomyces antarcticus, Friedmanniomyces endolithicus, Elasticomyces elasticus, Debaryomyces hansenii, Naganishia* (formerly *Cryptococcus*) *vishniacii, Recurvomyces mirabilis* and *Saxomyces penninicus.*

Predominant bands were excised and re-amplified with appropriate primers; amplicons were purified, sequenced and compared in the public domain using BLASTN algorithm (http://www.ncbi.nlm.nih.gov/).

2.2.4 Analysis of fungal community fingerprints

Scanned gels were analyzed with Phoretix 1D Pro software (TotalLab Ltd, Newcastle, UK). A dendrogram, relating band pattern similarities, was calculated with UPGMA algorithms (Unweighted Pair Group Method with Arithmetic Mean).

After matching bands from independent tracks, pairwise DICE's coefficients of similarity were calculated. Diversity indices were calculated from the DGGE profiles. Richness index (S) indicates the number of bands detected in each lane.

The Shannon–Weaver index of general diversity, *H*' (Shannon and Weaver, 1963) was calculated using the following function:

$H' = -\Sigma p_i \ln p_i$

where P_i is the importance probability of the bands in a track. *H'* was calculated on the basis of the bands on the gel tracks using the intensity of the bands as judged by peak heights in the densitometric curves.

The Simpson index of dominance (1-D) (Simpson, 1949) was calculated using the following function:

$1\text{-}D = 1 - \Sigma p_i^2$

where p is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N).

The Pielou Evenness index (E) (Pielou, 1966) which reflects the relative importance of each taxon within the entire assemblage, was calculated as:

$E = (-\Sigma P_i \ln P_i)/\ln S$

where P_i is the relative coverage of species *i* and *S* is species richness.

To graphically represent the evenness of this community, Lorenz distribution curves were established based on the DGGE profiles. For each DGGE lane, the respective bands are ranked from high to low based on their intensities. Consecutively, the cumulative proportions of species are used as the *x* axis, and the *y* axis is represented by their respective cumulative proportions of intensities. Mathematically, this yields a convex curve, analyzing the functional organization index (F_o). In this study, the Lorenz curves were also evaluated based on the Pareto principle (Pareto, 1897).

2.2.5 Statistical Analysis

Multivariate statistical analyses were performed to determine the effects of different environmental parameters on fungal diversity using PAST software v2.17 (PAleontological Statistics) (Hammer *et al*., 2001). To address non-linear relationships, non-metric Multidimensional Scaling (n-MDS) was performed; Bray–Curtis similarity matrix was calculated and used to generate a 2D plot with the n-MDS (Clarke, 1993).

2.3 Results

2.3.1 DNA extraction

DNA was efficiently extracted from 67 rock samples out of the 72 analyzed. Most of the samples from which DNA extraction was not successful were collected at very high altitudes (e.g. Mt. Adamson 3600 m a.s.l.; Archambault Ridge 3400 m a.s.l.) or in locations very distant from the sea (Bobby Rocks, 91 km from the sea).

2.3.2 DGGE Analysis

Duplicates from all the samples gave identical DGGE patterns, validating the experimental procedure (data not shown). Forty different phylotyes were identified by imaging analysis and 40 bands, representative of the entire biodiversity were excised from the DGGE gels; re-amplification by nested PCR and sequencing was successful for 29 of the bands analyzed (Fig. 2.3). Sequences were compared in the public domain using NCBI algorithm Blastn search (Table 2.2).

Table 2.2 Taxonomic identification of excised, re-amplified and sequenced DGGE bands. DGGE excised bands numbers are reported in Fig. 3.

DGGE Bands	DGGE lanes	Taxonomic identification	Identity (%)	Accession Number
1	72	*Buellia* sp.	94	MF554782
2	72	Uncultured *Herpotrichiellaceae*	87	MF554783
3	6	Uncultured fungus	97	MF554784
4	4	*Lecanora fuscobrunnea*	100	MF554785
5	4	*Lecanora polytropa*	99	MF554786
6	8	*Buellia* sp.	93	MF554807
7	36	*Lecanoromycetidae* sp.	92	MF554787
8	36	*Candelariella* cfr. *antennaria*	82	MF554808
9	15	*Rhizoplaca* cfr. *macleanii*	93	MF554788
10	11	Uncultured fungus	96	MF554789
11	1	*Rhizoplaca* cfr. *macleanii*	93	MF554790
12	2	*Buellia frigida*	100	MF554791
13	9	*Lecidea cancriformis*	99	MF554792
14	47	*Buellia* sp.	94	MF554793
15	40	*Parmeliaceae* sp.	94	MF554809
16	37	*Parmeliaceae* sp.	95	MF554794
17	37	*Tremellales*	84	MF554795
18	37	*Rhizoplaca* cfr. *macleanii*	93	MF554796
19	24	*Friedmanniomyces endolithicus*	98	MF554797
20	12	Uncultured fungus	97	MF554798
21	42	*Lecidea cancriformis*	99	MF554799
22	10	*Umbilicaria* sp.	90	MF554810
23	3	*Rhizoplaca* cfr. *macleanii*	94	MF554800
24	14	*Acarospora* sp.	95	MF554801
25	13	*Lecidea cancriformis*	100	MF554802
26	5	*Rhizoplaca* cfr. *macleanii*	93	MF554803
27	50	*Naganishia friedmannii*	100	MF554804
28	34	*Buellia* sp.	93	MF554805
29	28	*Lecidea cancriformis*	99	MF554806

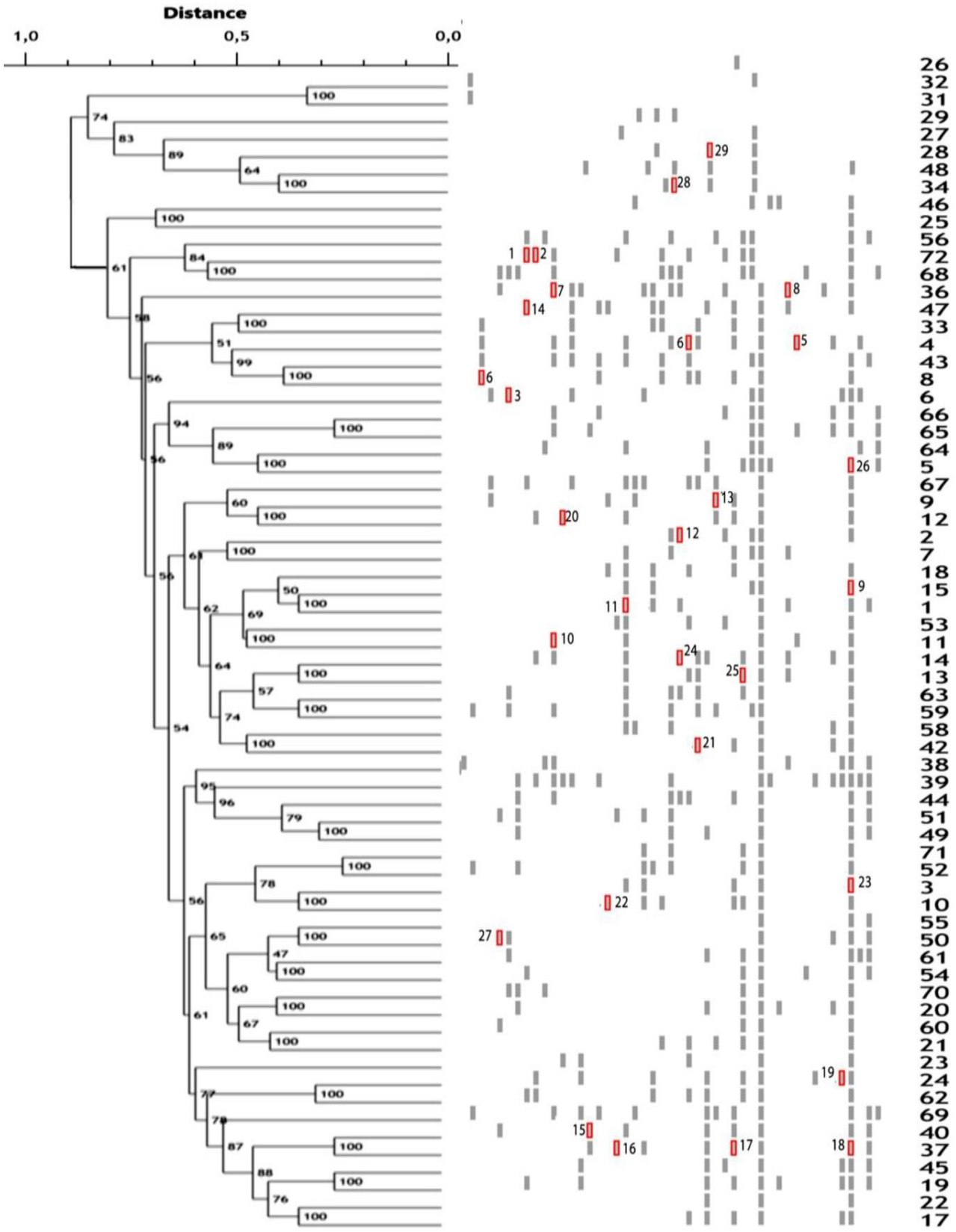


Figure 2.3 Dendrogram of DGGE profiles based on DICE coefficient. Comparison of fungal soil communities of 67 different samples. Numbers of DGGE profiles are listed in Table 1S. Squares from 1 to 29: bands excised, reamplified and sequenced; results from the DNA sequence analyses are summarized in Table 2.2

Nine bands were identified to species and six to Genus. Band 19 from lane 24, corresponding to Anderson Ridge, yielded 98% similarity with the endemic Antarctic cryptoendolithic fungus *Friedmanniomyces endolithicus* and band 27 from Chisholm Hills (lane 50) was identified 100% as the psychrophilic yeast species *Naganishia* (former *Cryptococcus*) *friedmannii*. Band 2 (lane 72) from Vegetation Island, had a lower match, 87% similarity, with an unidentified black fungus in the Family Herpotrichiellaceae. Seven bands were identified with certainty as lichen species: the endemic Antarctic species *Lecanora fuscobrunnea* (band 4, lane 4), and *Lecanora polytropa* (band 5, lane 4) both from Harrow Peak, *Buellia frigida* (band 12, lane 2) from Vegetation Island, *Lecidea cancriformis* (bands 13, 21, 25, 29) from Timber Peak, Trio Nunatak, Prior Island, and Mt. Nansen respectively. Lanes 3, 10 and 20, corresponding to Mt. Howard site 2 sample 1, Thern Promontory and Mt. Howard site 2 sample 2 respectively, were not possible to identify at higher taxonomic ranks since they yielded 96-97% similarity with three different uncultured clones of unidentified fungi. All the other sequences were not possible to identify at species level because of the low match in the GenBank; however, all belonged to lichen genera in the Order Lecanorales (*Buellia*, *Candelariella*, *Rhizoplaca*, *Umbilicaria*, *Acarospora*).

To perform a clustering analysis of the DGGE profiles the DICE coefficient was calculated according to the presence/absence of each band in the profile using the Phoretix 1D software (Fig. 2.3). This analysis did not highlight any direct relation between the biodiversity, the sampling sites, rock typology and environmental parameters, since sometimes the biodiversity varied considerably even in different samples from the same locality but also between two rock samples of the same site. For example, two sandstone conglomerates collected both from site 2 of Mt. Howard (lane 6 e 12 respectively) where DGGE profiles were 38% different.

The number of bands obtained in the DGGE from the 66 rock samples (S) ranged from 1 to 15 (Fig. 3). Two samples yielded the richest profiles, collected at Kay Island (lane 36, 14 bands) and Richard Nunatak (lane 39, 15 bands); one band only was obtained from Shafer Peak (lane 25), above 3000 m a.s.l., and McLea Nunatak (lane 31), at 2100 m a.s.l. and 75 km sea distance. Surprisingly, the highest number of bands was obtained from a sample collected at Richard Nunatak, 2000 m a.s.l. and 70.2 km sea distance. Besides, in the same locality and same rock typology, a remarkable variability among different rocks was observed, as for Mt. Howard (lanes 12, 7 bands and 43, 11 bands). Along with the fungal diversity derived from DGGE banding patterns (S), the Shannon-Weaver (H'), Simpson (1-D) and Pielou (J') diversity indices were calculated to consider not only richness value, but evenness and community homogeneity (relative species abundance); H' ranged from 0.51 to 2.45, 1-D from 0.33 to 0.9 and J' from 0.71 to 0.99 (Table 2.3).

Table 2.3 The diversity indices values were calculated for each lane number: Richness index (S), Shannon-Weaver index (H'), Simpson index of dominance (1-D) and Pielou Evenness index (J'). The relation among these indexes was displayed as nMDS ordinations, reported in Fig.4.

DGGE Lanes	***S***	***H'***	***1-D***	***J'***
1	7	1.64	0.76	0.84
2	6	1.47	0.74	0.82
3	5	1.40	0.74	0.87
4	12	2.11	0.85	0.85
5	7	1.69	0.78	0.87
6	9	2.02	0.85	0.92
7	6	1.44	0.69	0.80
8	8	1.75	0.79	0.84
9	7	1.26	0.75	0.75
10	7	1.69	0.77	0.87
11	5	1.23	0.66	0.77
12	7	1.53	0.78	0.78
13	7	1.58	0.75	0.81
14	11	2.17	0.87	0.91
15	5	1.14	0.62	0.71
16	-	-	-	-
17	6	1.66	0.80	0.93
18	7	1.67	0.78	0.86
19	9	1.88	0.78	0.85
20	7	1.73	0.81	0.89
21	6	1.62	0.78	0.90
22	3	0.97	0.58	0.89
23	5	1.36	0.69	0.84
24	9	1.87	0.81	0.85
25	1	-	-	-
26	-	-	-	-
27	2	0.61	0.41	0.87
28	3	1.02	0.62	0.93
29	3	1.09	0.66	0.99
30	-	-	-	-
31	1	-	-	-
32	2	0.51	0.33	0.74
33	8	1.92	0.83	0.92
34	4	1.27	0.69	0.92
35	-	-	-	-
36	14	2.17	0.85	0.82
37	8	1.78	0.80	0.86
38	8	1.90	0.83	0.91
39	15	2.45	0.90	0.90
40	8	1.63	0.76	0.78

41	-	-	-	-
42	5	1.42	0.72	0.88
43	11	1.98	0.81	0.83
44	9	1.94	0.83	0.88
45	7	1.91	0.85	0.98
46	5	1.18	0.63	0.73
47	11	2.17	0.86	0.90
48	6	1.38	0.71	0.77
49	6	1.47	0.73	0.82
50	6	1.59	0.77	0.89
51	8	1.71	0.78	0.82
52	8	1.82	0.80	0.88
53	6	1.61	0.77	0.90
54	6	1.56	0.75	0.87
55	3	1.04	0.63	0.94
56	9	1.79	0.79	0.82
57	-	-	-	-
58	6	1.55	0.77	0.86
59	10	1.93	0.81	0.84
60	4	1.24	0.67	0.90
61	7	1.70	0.79	0.88
62	8	1.91	0.83	0.92
63	8	1.81	0.81	0.87
64	7	1.68	0.77	0.87
65	8	1.57	0.74	0.75
66	8	1.50	0.72	0.72
67	11	2.13	0.86	0.89
68	12	2.24	0.87	0.90
69	12	2.15	0.86	0.91
70	6	1.58	0.77	0.88
71	5	1.28	0.69	0.80
72	10	2.11	0.87	0.92

2.3.3 nMDS ordination

The relation among the diversity indices calculated (S, H', D and J') was displayed as nMDS ordinations where all the environmental parameters considered (altitude, sea distance, rock porosity) were included (Fig. 2.4). The non-metric Multi-Dimensional Scaling organizes data spatially and identifies similarities and differences between data sets. A good fitness score, called stress, for data points was found. In fact, a stress value below 0.1 indicates a reliable ordination of data, without a real probability of misinterpretation (Clarke, 1993). In this analysis,the stress value was 0.03, well below the minimum for

ideal ordination. In the n-MDS map, obtained using the Bray-Curtis similarity index, four significantly different groups were recognized (1-way NPMANOVA, p <0.0001).

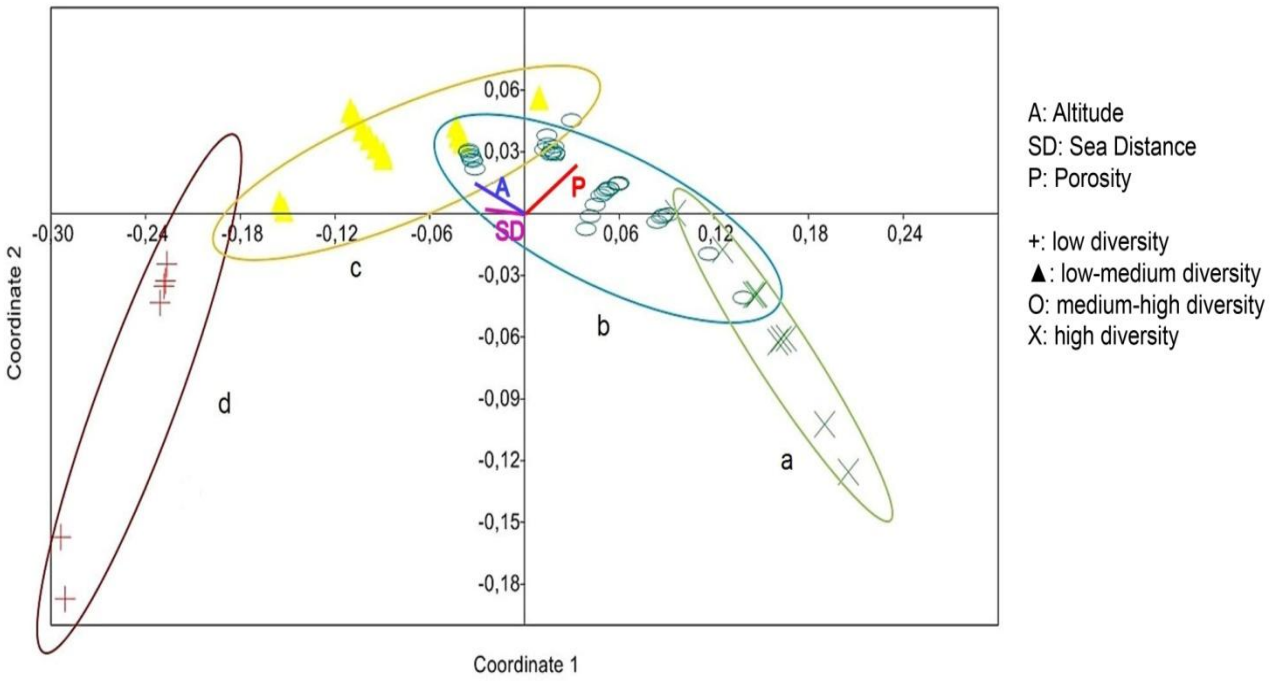


Figure 2.4 Non metric MultiDimensional Scaling analysis (n-MDS). All samples cluster in four groups (a-b-c-d). Sea distance, altitude and porosity parameters were considered.

An altitudinal and sea distance gradient taking into account the average values for each group was found. Low biodiversity indices (groups C, D) were related to higher altitude and longer sea distance values than samples with medium to high diversity indices. This trend was also evident in relation to the porosity of rock whose average values were higher in the cluster with medium to high diversity (group A, B) (Fig. 2.4).

2.3.4 Pareto Lorenz

To assess the interspecies abundance ratios, Pareto-Lorenz curve distribution patterns of the DGGE profiles were plotted based on the numbers of bands and their intensities, in different rock substrata: granite, sandstone, conglomerate sandstone, quartz and dolerite (Fig. 2.5a-e). F_o values were rather high in all cases, ranging from 63 to 75%, with highest values in sandstone and dolerite-lava dike samples. These values indicate that fungal lithic communities were dominated by a few abundant and specialized

species and some other rare species. This community organization denotes a high degree of adaptation and specialization, leading to efficient functionality in extreme conditions.

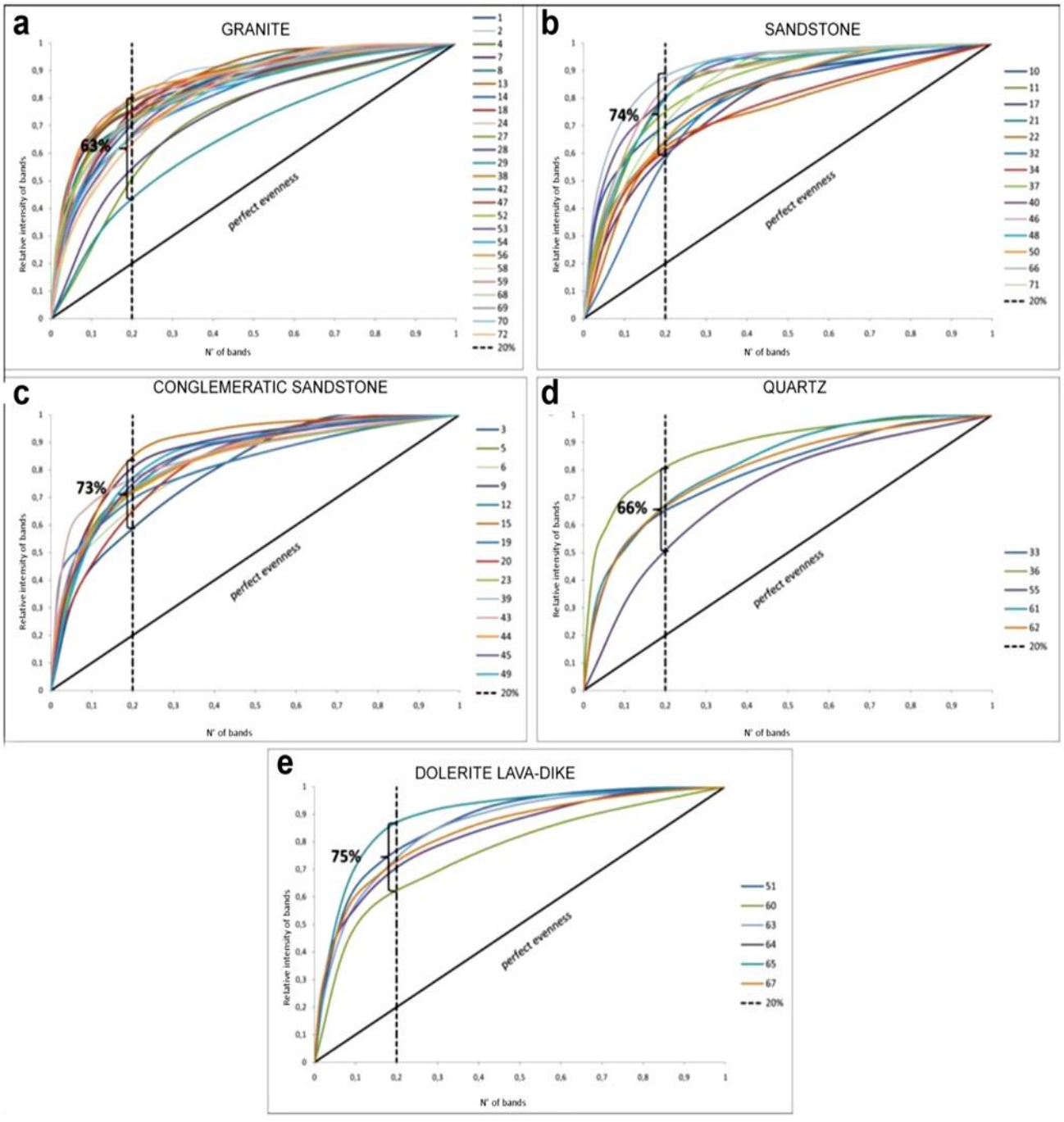


Figure 2.5 Pareto-Lorenz curves. The analysis was based on number and relative intensities of the bands in the DGGE profiles and performed for each rock typology: granite (a), sandstone (b), conglomeratic sandstone (c),quartz (d) and dolerite-lava dike (e). Theoretical higher evenness (perfect evenness line) is characterized by a curve close to the 45°C diagonal.

2.4 Discussion

This study is based on the largest Antarctic sampling to date of rocks hosting lithic communities and includes 72 specimens from 46 different localities in Victoria Land. Along with rock typology and porosity, a number of parameters including altitude and sea distance have been considered in the analyses to verify their effect on biodiversity and community structure. Here, our objective was to focus on the fungal compartment of these communities. Fungi play a pivotal role in these ecosystems, both as consumers, and for their protective role. In Antarctic cryptoendolithic communities, melanized fungi form a black barrier just above the photobiont stratification that may play an essential role in photobionts protection against damaging solar radiation (Selbmann *et al.,* 2013). Moreover, in association with algal photobionts they form lichen thalli spreading on and inside the rocks on continental Antarctica, thus representing the dominant primary producers (Nienow and Friedmann, 1993; Cannone and Seppelt, 2008). Fungi, including yeasts, are also the dominant components of eukaryotic assemblages in Antarctica (Pointing *et al*., 2009; Rao *et al*., 2011).

Among the species identified by DGGE band sequencing, the Antarctic endemic black fungus *Friedmanniomyces endolithicus* was recorded from Anderson Ridge. This fungus to date has been isolated exclusively to Victoria Land (Selbmann *et al*., 2005; Egidi *et al*., 2014) and is always found in lithic habitats developing both endolithically or associated with epilithic lichens (Selbmann *et al*., 2013). This fungus is also one of the most frequently isolated species in rock samples analysed here (unpublished data), *Naganishia friedmannii* was first described (as *Cryptococcus friedmannii*) from cryptoendolithic Antarctic communities (Vishniac. 1985), but later found worldwide in cold habitats in Alaska, Arctic permafrost, Iceland soil and Alpine glaciers (Faizutdinova *et al.,* 2005; Arenz *et al.,* 2006; Vishniac, 2006 a,b; Turchetti *et al*., 2013). Here, a sequence 100% similar to this species was found in a rock sample from Chisolm Hills, from a sandstone outcrop about 80 km distant from the sea, 2500 m a.s.l. The prevalence of the polyphyletic phenotypic basidiomycetous Genus *Cryptococcus* in worldwide cold habitats has been recently reported (Buzzini *et al*., 2012; Connell *et al*., 2014; Zalar and Gunde-Cimerman, 2014). Some studies also speculated that this Genus (together with *Rhodotorula*) may express a more efficient adaptation to selective pressures that are typical of cold ecosystems if compared to ascomycetous yeasts (Vishniac, 2006b; Connel *et al*., 2008; Selbmann *et al.,* 2014a). However, recent taxonomic revision of the Pucciniomycotina and Tremellomycetes (Liu *et al*., 2015; Wang *et al*., 2015) positioned species of *Cryptococcus* into many new *taxa*, thus modifying the known taxonomic composition and consequently ecological significance of yeast distribution in cold ecosystems, including Antarctica (Buzzini *et al*., 2017).

One of the sequences from a rock sample collected at Vegetation Island was 87% similar with an unidentified fungus in the Herpotrichiellaceae. Fungi in this Family are melanized environmental

organisms, often associated with polluted environments (Isola *et al*., 2013) or both cold and warm-blooded animals where they may adapt and develop as opportunists (Badali *et al*., 2009; de Hoog *et al*., 2011; Prenafeta-Boldú *et al*., 2006). However, they were rarely isolated from Antarctic rocks; *Exophiala* sp., for instance, was isolated from rock collected at Kay Island (unpublished). Their occurrence in coastal sites could be associated to the presence of seabirds, which transport fungal spores in their gut even over long distances.

Almost 80% of the amplicons sequenced here belonged to lichen *taxa*, all invariably in the Order Lecanorales. This high frequency is not surprising since lichen symbioses are the most successful obligate association of fungi and algae facilitating their colonization with associated organisms into the harshest environments on Earth, including the Antarctic desert (Onofri *et al*., 2007).

Crustose lichen genera were the most recurrent in our analyses and their distribution spans from sea level to longer sea distances and higher altitudes. This range is not surprising since lichens with this thallus organization are the best adapted to extreme conditions and are predominant even at the highest mountain elevations. Some of them were identified with certainty as *Lecanora fuscobrunnea* and *Buellia frigida*, two Antarctic endemic species, *Lecanora polytropa* and *Lecidea cancriformis*. Others were identified as undescribed species of *Acarospora*, *Buellia*, *Lecidea* and *Rhizoplaca* (90-95% similarity). Species in the Family Parmeliaceae were found in lanes 37 and 40 obtained from rock samples collected at Mt. Nansen (20 km from the sea) and Pudding Butte (60 km far from the sea), respectively. *Umbilicaria* sp. was found once; its heavily melanized thallus supplies a protection from solar irradiation and rapid warming. and in fact this lichen is rather recurrent in Antarctica, particularly along coastal sites (Øvstedal and Lewis Smith, 2001). Here it was found in a sample from Mt. New Zealand.

The DGGE profiles were highly variable and the species richness in terms of number of bands obtained varied between 1 and 15 among the samples analyzed, independently from the locality, altitude or sea distance. The highest number of bands (15, lane 39) was found at Richard Nunatak, 2000 m a.s.l. and over 70 km sea distance. A variability in biodiversity was evident also in two samples collected at Mt. Howard for lanes 6 and 12 (9 and 7 bands, respectively). For this reason, the cluster analysis did not yield a linear relation between biodiversity and sampling sites (Fig. 2.3).

The same result was found in areas near the coast, where environmental conditions are less harsh; a high variation in fungal biodiversity was found in close sampling sites at Inexpressible Island (lanes 33 and 38, 7 and 8 bands respectively) and Kay Island (lane 36, 13 bands) directly on the coast, and at Starr Nunatak (lane 29, 3 bands) or Harrow Peak (lane 4, 12 bands) at 3.2 and 6.5 km from the sea, respectively. These data suggest that fungal biodiversity (i.e. number of *taxa*, as well as what genera and species have been found in a given site) might not be related to locality, altitude or distance from the sea only, but some other factors may be determinant and must be taken into consideration for future investigations, namely

water availability, rock temperature and sun exposure. In border environments, as in the ice-free areas of continental Antarctica, all these factors may strongly affect the micro- and nano-climatic conditions. Even slight variation in these conditions, due also to the topology of the rock surface, may allow or avoid moisture accumulation with crucial consequences for life processes of fungi and yeasts occurring in the cryptoendolithic communities.

Along with species richness, the relative abundance of different fungal species in the communities was also considered and Shannon-Weaver (H'), Simpson (1-D) and Pielou's (J)' diversity indices were calculated. The relationship between diversity indices obtained from individual profiles of fungal communities and environmental parameters was studied and graphically represented in a map through a non-Metric Multi-Dimensional Scaling analysis. The reliability of data ordination in the map was supported by the very low stress value, 0.03, and statistical analysis (1-way NPMANOVA. $p < 0.0001$). The grouping clearly shows that low indices expressing the structure of fungal communities in different sites were related with altitude and sea distance while high biodiversity indices correlated with rock porosity (Fig. 2.4).

The pivotal role of rock substrate in terms of porosity is evident. This role is consistent with other recent observations; sandstone has a much more homogeneous distribution of pores compared to other rock typologies and this may favor microbial endolithic growth and biodiversity (Cockell *et al.*, 2003). The typical cryptoendolithic colonization (Friedmann, 1982), invariably associated with sandstone. extends to higher altitudes and greater distance from the sea than endoliths associated to other rock typologies (Zucconi *et al.*, 2016). Rock porosity may also counteract, in certain extent, the negative effect of other environmental parameters on endolithic growth, allowing settlers to move throughout higher altitudes and longer sea distances (Zucconi *et al.*, 2016).

The functionality (F_o) of the communities is graphically represented by the Pareto-Lorenz curves (Fig. 2.5). The values obtained in all cases were far from the theoretical perfect uniformity, represented by a line with a slope of 45° (F_o= 25%), indicating that all species in the community have the same number of individuals. F_o value of 45% indicates a community where few species are dominant and more suited to environmental conditions, while an F_o value of 80% represents a highly specialized community where a small number of species dominate and all others are present in a small numbers, with a large difference between the two groups, F_o values here calculated for the different types of rock substratum ranged from 63 to 75%, indicating a high degree of specialization; besides this high functional organization, these communities have low resilience and are prone to external perturbations and a long time may be required to recover after perturbation events (Marzorati *et al.*, 2008).

This study represents a first approach to investigate the effect of environmental parameters of lithic communities living in border Antarctic ecosystems. Results indicate a high degree of specialization of the

fungal communities, thus reflecting a specialized adaptations but low resilience. External perturbations may lead to irreversible consequence for these fragile communities with possible extinction events and genetic loss. Our data suggest that altitude and sea distance affect the communities while rock porosity has a protective role for endoliths. Our study also provides important insights for future investigations: i) more research is needed to further elucidate complex relations between environmental variables and microbial lithic biodiversity in sandstone, herein confirmed as the best substratum for endolithic life; ii) data obtained were extremely variable even within the same sampling site, suggesting that a thorough definition is necessary of what would be the minimal sampling for each site to be representative of the actual biodiversity; iii) some other parameters, along with altitude and sea distance, should be considered as potentially important variables for explaining biodiversity. In addition to north and south exposures, variations at micro- and nano-scales of water availability, Photosynthetic Active Radiation (PAR) and temperature is likely significant for these microbial communities.

For more complete and reliable analyses, future research should be extended to other biological compartments of the communities, both eukaryotes and prokaryotes. A more informative approach such as next generation sequencing, based on a more restricted and targeted selection of rock samples is desirable. A complete understanding on how these communities respond to increasing environmental pressures will provide insights on the effects of climate change on these unique, border ecosystems and their threatened biodiversity. Moreover, understanding how the combination of environmental parameters influences the habitability of edge environments is relevant for future exploration of extraterrestrial life (Cockell *et al*., 2016).

CHAPTER 3.

ANTARCTIC CRYPTOENDOLITHIC FUNGAL COMMUNITIES ARE HIGHLY ADAPTED AND DOMINATED BY LECANOROMYCETES AND DOTHIDEOMYCETES.

Abstract

Endolithic growth is one of the most spectacular microbial adaptations to extreme environmental constraints and the predominant life-form in the ice-free areas of Continental Antarctica. Although Antarctic endolithic microbial communities are known to host among the most resistant and extreme-adapted organisms, our knowledge on microbial diversity and composition in this peculiar niche is still limited. In this study, we investigated the diversity and structure of the fungal assemblage in the cryptoendolithic communities inhabiting sandstone using a meta-barcoding approach targeting the fungal Internal Transcribed Sequence region 1 (ITS1). Samples were collected from 14 sites in the Victoria Land, along an altitudinal gradient ranging from 1,000 to 3,300 m a.s.l. and from 29 to 96 km sea distance. Our study revealed a clear dominance of a 'core' group of fungal *taxa* consistently present across all the samples, mainly composed of lichen-forming and Dothideomycetous fungi. Pareto-Lorenz curves indicated a very high degree of specialization (F_0 approximately 95%), suggesting these communities are highly adapted but have limited ability to recover after perturbations. Overall, both fungal community biodiversity and composition did not show any correlation with the considered abiotic parameters, indicating that the Antarctic rock mycobiome may be shaped by fluctuations in environmental conditions occurring at the local scale.

Keywords: Antarctica, Endolithic communities, Extremophiles, Fungi, ITS Meta-barcoding

Coleine, C., Stajich, J.E., Zucconi, L., Onofri, S., Pombubpa, N., Egidi, E., Franks, A., Buzzini, P., and Selbmann, L. (2018). Antarctic cryptoendolithic fungal communities are highly adapted and dominated by Lecanoromycetes and Dothideomycetes. *Submitted to Frontiers in Microbiology Journal.*

3.1 Introduction

The Victoria Land region in Antarctica encompasses a latitudinal gradient of 8°, from the Darwin Glacier (79°S) in the South, to Cape Adare (71°S) in the North. Along with the widest area of the McMurdo Dry Valleys of the Southern Victoria Land, mountain tops and Nunataks hanging from the polar plateau in the Northern Victoria Land represent the ice free-areas with exposed naked rocks, the main substratum for life (Nienow and Friedmann, 1993). The dramatic temperature fluctuation, extremely low relative humidity, and scarce liquid water availability in this area constrain terrestrial ecosystem processes, leading to the predominance of microbial life, more than anywhere else on Earth (Nienow and Friedmann, 1993; Vincent, 2000; Zucconi *et al*., 2016). Given the harsh conditions due to osmotic, thermal and UV stresses throughout the interior of the Antarctic continent, microbial communities are forced to develop in cryptic habitats as a stress avoidance strategy (Pointing and Belnap, 2012). Therefore, except for the coastal sites, where the most permissive climatic conditions favor epilithic growth, life in the inland and high altitudes is predominantly present as endolithic colonization (Friedmann and Ocampo,1976; Zucconi *et al*., 2016).

Studies focused on the isolation, identification, evolution and adaptation of microbial *taxa* from Antarctic rock communities revealed the occurrence of a surprising diversity of both prokaryotic and eukaryotic microorganisms, some of which are exclusive to this habitat (Adams *et al.*, 2006; Selbmann *et al*., 2005, 2008, 2013, 2014a; Egidi *et al*., 2014). The taxonomic and functional diversity of bacteria from soil and hypolithic communities of the Miers Valley, in the McMurdo Dry Valleys of Antarctica, have been recently characterized (Wei *et al*., 2016), revealing a cyanobacteria-dominated community with a relatively high degree of functional redundancy, while fungal diversity still remains largely unexplored (Archer *et al*., 2016; Zucconi *et al*., 2016). In particular, Zucconi and colleagues (2016) highlighted the importance of environmental parameters, mainly rock typology and, to a lesser extent, altitude and sea distance, in shaping microbial colonization of the lichen-dominated lithic communities, which are exceptionally widespread in the Victoria Land region. Cryptoendoliths prefer porous rocks and readily colonize sandstone. The structure of the fungal component of these communities has been investigated using a fingerprinting approach, that revealed a high predominance of few fungal species. This organization denounces a high degree of specialization of the community, with a consequent high resistance to stresses, but a poor resilience so that external perturbations may easily lead to possible extinctions (Selbmann *et al*., 2017).

Despite a long history of collections, culturing, and *taxa* descriptions, the fungal diversity of the Antarctic cryptoendolithic communities have been primarily investigated using culture-dependent approaches (Selbmann *et al*., 2005, 2008; Egidi *et al.*, 2014). With the development of culture-independent molecular

methods, such as DNA meta-barcoding, a more accurate census of the microbial community is possible, giving robust and effective results both at global and local scales (Ji *et al*., 2013; Smith and Peay, 2014; Hiergeist *et al*., 2015; Tedersoo *et al*., 2015; Valentini *et al.*, 2016). Commonly occurring organisms, that are shared among communities from the same habitat, are likely to play a crucial functional role in that particular assemblage (Shade and Handelsman, 2011), and the DNA meta-barcoding approach can be conveniently applied to have a better understanding of biodiversity and ecology of the 'core' fungal members.

In this study, we utilized an ITS meta-barcoding strategy to investigate the diversity, composition and distributional patterns of the fungal communities colonizing sandstone samples from 12 localities and 14 sites spanning from North to South Victoria Land, Antarctica. This research aimed to (i) explore the fungal diversity and community composition related to an altitudinal (m a.s.l.) and distance from the sea (km) gradient, and (ii) define the 'core' group of fungal *taxa* associated with such communities.

A better understanding of the biodiversity and structure of such extreme-adapted communities represents a key step for improving the accuracy of predictions on the effects of climate change on polar microbial diversity and potentially aids in developing strategies to preserve the biodiversity of these unique ecosystems.

3.2 Material and Methods

3.2.1 Study area

Sampling sites are sandstone outcrops distributed along a latitudinal transect ranging from 73°29'26"S (Stewart Heights, Northern Victoria Land) to 76°54'36''S (Battleship Promontory, McMurdo Dry Valleys, Southern Victoria Land), from 1000 m a.s.l. (Battleship Promontory) to 3300 m a.s.l. (Shafer Peak site 2), and from 29 km (Thern Promontory) to 96 km (Ricker Hills) sea distance (Table 3.1; Figs. 3.1, 3.2). All sites were located in Northern Victoria Land, except for Battleship Promontory, located in Southern Victoria Land.

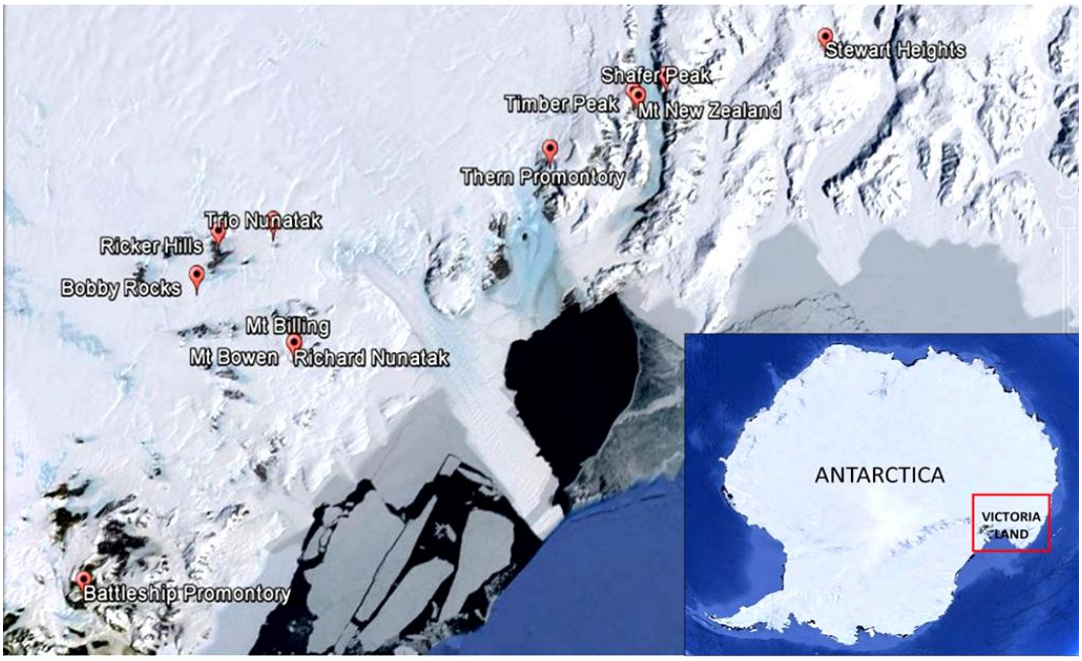


Figure 3.1 Map of the study area showing the location of the sampling sites. All locations visited were in Northern Victoria Land, except for Battleship Promontory, the only one location visited in Southern Victoria Land. Victoria Land is indicated with a red square in the Antarctic continent map.

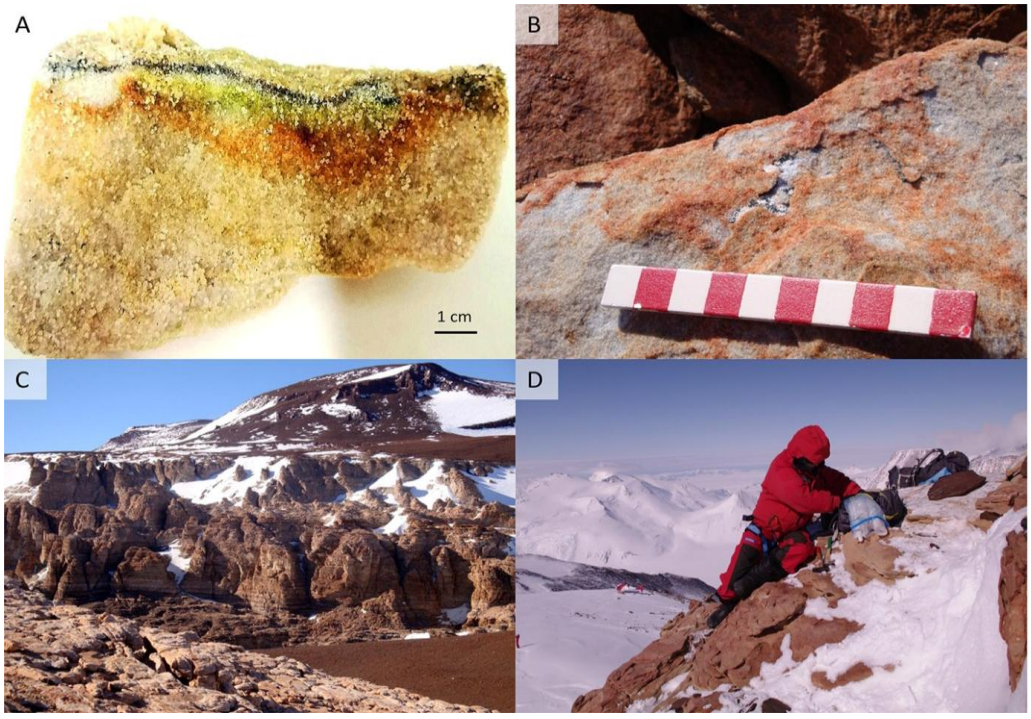


Figure 3.2 A) Cryptoendolithic lichen dominated community colonizing a sandstone sample at Battleship Promontory; **B)** A diffuse endolithic colonization at Mt Billing; **C)** Sandstone outcrops at Battleship Promontory; **D)** Laura Selbmann sampling at Mt New Zealand.

All samples were collected during the XXVI Italian Antarctic Expedition (2010-2011); the presence of lithic colonization was assessed by direct observation *in situ* using magnification lenses. Rock samples were excised using a geological hammer and sterile chisel, placed in sterile bags, stored and transported at -20°C at the Tuscia University (Viterbo, Italy) and stored until downstream analysis. Rocks are part of a larger set of different sandstone, granites, dolerites, quarts and lava-dike samples collected during the same expedition and analyzed using DGGE approach (Selbmann *et al*., 2017).

Table 3.1 List of sampling sites following an altitudinal gradient, with altitudes, sea distances and geographic coordinates.

Locations	Altitude (m a.s.l.)	Sea distance (km)	Coordinates
Battleship Promontory	1000	33.5	76°54'36"S 160°56'05"E
Trio Nunatak site 1	1000	82	75°30'02"S 159°40'28"E
Ricker Hills	1115	96	75°38'39"S 159°01'42"E
Mt Billing	1300	44	75°42'12"S 160°54'28"E
Trio Nunatak site 2	1400	84.5	75°28'59"S 159°35'21"E
Thern Promontory	1500	29	74°33'S 162°04'E
Bobby Rocks	1680	91	75°48'35"S 159°11'15"E
Mt Bowen	1874	39.5	75°45'24"S 161°03'46"E
Richard Nunatak	2000	71.6	75°56'53"S 159°42'57"E
Stewart Heights	2670	74	73°29'26"S 163°54'44"E
Timber Peak	2800	49.5	74°10'13"S 162°25'31"E
Mt New Zealand	2888	47	74°10'46"S 162°31'01"E
Shafer Peak site 1	3100	59	74°02'19"S 162°37'16"E
Shafer Peak site 2	3300	48	74°02'19"S 162°37'16"E

3.2.2 DNA extraction and sequencing

Two rock samples from each site were crushed under sterile conditions; DNA was extracted from 0.3 g using MOBIO Power Soil DNA Extraction kit (MOBIO Laboratories, Carlsbad, CA, USA), according to the manufacturer's protocol. DNA was extracted in technical duplicate. ITS1 rRNA region was amplified. PCR reactions were carried out with a total volume of 25 µl, containing 1 µl of each primer, 12.5 µl of Taq DNA Polymerase (Thermo Fischer Scientific Inc., Waltham, MA, USA), 9.5 µl of

nuclease-free water (Sigma–Aldrich, UK) and 5 ng of DNA. We amplified the ITS1 region using ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) primers developed for short read length (White *et al*., 1990; Smith and Peay, 2014). PCRs were then subjected to an initial denaturation at 93°C for 3 min, 35 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 1 min, extension at 72°C for 90 s, followed by a final extension at 72°C for 10 min in an automated thermal cycler (BioRad, Hercules, CA). Amplicons were then purified with Qiagen PCR CleanUp kit (Macherey-Nagel, Hoerdt, France) and normalized after quantifying with the Qubit dsDNA HS Assay Kit (Life Technologies, USA). Equimolar pooling of the differentially barcoded amplicons and paired-end sequencing (2×300 bp) were carried out on an Illumina MiSeq sequencer at the Institute for Integrative Genome Biology, University of California, Riverside.

The two sandstone sequencing data and technical replicates had the replicate merged within their respective samples to increase the amount of valuable sequence information.

3.2.3 Amplicon sequencing data

The ITS1 datasets were processed with the AMPtk: Amplicon ToolKit for NGS data (formally UFITS) 0.9.3v. (Palmer *et al*., 2017) (https://github.com/nextgenusfs/amptk). Barcodes and primers were removed from the amplicons sequencing data and reads were demultiplexed with split_ libraries.py (QIIME v 1.9.1 Caporaso *et al*., 2010). Reads were subjected to quality trimming and chimera removal in AMPtk utilizing USEARCH using default parameters (v. 9.1.13) (Edgar, 2010). The cleaned individual sample sequence files were merged into a single file clustered to identify molecular Operational Taxonomic Units (OTUs) with a 97% identity threshold using the VSEARCH (v 2.3.2) (Rognes *et al.*, 2016) algorithm. Taxonomic identification was performed with SINTAX/UTAX (Edgar, 2010) classificators. In addition, we mapped the relative abundances of compositional 'core' OTUs, defined as being present in at least 75% of the analyzed samples. The matrix display function in PRIMER-E was used to illustrate and compare the relative abundance of the community 'core' members on a heat-map using log-transformed taxonomic counts, while a UPGMA clustering method was implemented to reveal similarities in the community composition among the sampled sites, calculating Bray-Curtis index.

All raw sequence data are submitted to the GenBank databases under BioProject accession number PRJNA379160.

3.2.4 Biodiversity and statistical analysis

Biodiversity indices were estimated on rarefied and averaged data using Primer-E software (version 6, PRIMER-E Ltd. Plymouth, UK) to investigate species richness and evenness of the fungal community. Following Morris *et al.* (2014), our analyses included (i) species richness (S), estimated as a count of the total number of species found in each sample, (ii) the Shannon index (H'), a phylotype-based approach constructed using OTU groupings (Shannon and Weaver, 1949; Ludwig, 1988), (iii) the Simpson's Index of Dominance (1-D), calculated to measure the probability that two individuals randomly selected from a sample will belong to the same species (or some category other than species) (Simpson, 1949), and (iv) the Pielou's equitability index (J') (Pielou, 1969). The non-parametric Spearman's correlation coefficients were calculated and graphically represented to explore relationships between the biodiversity indices and sampled localities (Spearman, 1904).

The variability in species composition of the communities in 14 sites was calculated (β diversity), (Whittaker, 1960; Anderson *et al*., 2006). The relationship between the considered environmental variables (altitude and sea distance) and β diversity was tested by multiple linear regression on distance matrices (MRM) (Legendre *et al.*, 1994; Lichstein, 2006; Ptacnik *et al*., 2010) implemented in the 'ecodist' package (Goslee and Urban, 2007) of R version 3.4.2 (R Development Core Team, 2010). In this analysis, the environmental distance matrices between sampling sites were regressed against the species composition dissimilarity, to verify the effect of altitude (m a.s.l.) and sea distance (km) on β diversity. Environmental distances were quantified by means of the Euclidean distance between each pair of sites, while the pattern in community similarity was calculated with the Jaccard index, starting from the species occurrence matrix. The robustness of results was estimated performing 1000 permutations on the original dataset, and *P*-values for MRM models were obtained by comparing each observed regression coefficient with the distribution of 1000 permuted values. All statistical tests were considered significant at $P < 0.05$.

To further show the evenness of these communities, Lorenz distribution curves were set up based on the meta-barcoding profiles to analyze the functional organization index (F_o). In this study, the Lorenz curves were also evaluated based on the Pareto principle (Pareto, 1897). The theoretical perfect uniformity, represented by a line with a slope of 45° (F_o= 25%) means that all species in the community have the same number of individuals. F_o value of 45% indicates a community where few species are dominant and better adapted to environmental conditions while higher values represent a highly specialized community where a small amount of species is dominant and all others are present with few representatives (Marzorati, 2008).

3.3 Results

3.3.1 DNA sequencing

A total of 1090171 fungal ITS rRNA gene reads passed the quality filtering step and the samples averaged 77869 reads, with a minimum of 10813 to a maximum of 297482 reads. DNA was obtained from all samples, including those collected at highest elevations, such as Mt New Zealand (2888 m a.s.l.) and Shafer Peak site 1 (3100 m a.s.l.) in Northern Victoria Land, the latter with no visible colonization. Clustering of Operational Taxonomic Units (OTUs) was performed at 97% identity threshold, obtaining a total of 362 OTUs.

3.3.2 Fungal community description

Data were rarefied to 1000 reads per sample and the sequences were assigned to OTUs after singleton removal. About 25% of the total OTUs retrieved were unidentified at Phylum or sub-Phylum level. The relative abundance of Ascomycota and Basidiomycota ITS sequences varied among locations. Indeed, the majority of the identified fungal sequences recovered among all samples belonged to the Ascomycota (ranging from 55 to 70% of relative abundances), followed by Basidiomycota (from 4 to 12%), and Mucoromycota and Zoopagomycota (present at 5% only in three sites: Battleship Promontory, Ricker Hills and Stewart Heights) (Fig. 3.3).

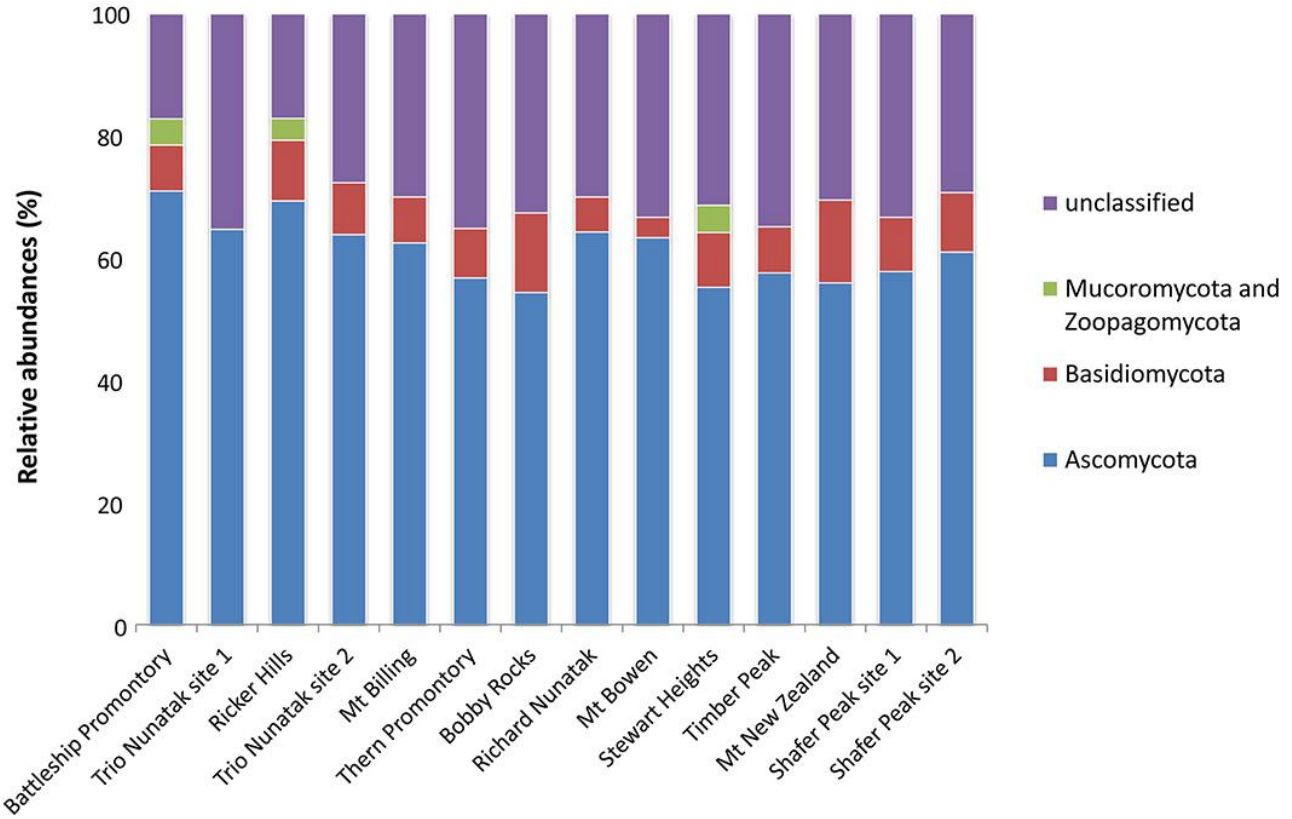


Figure 3.3 Relative abundances of the dominant fungal OTUs in the cryptoendolithic communities in Victoria Land, Antarctica. All relative abundances are based upon the proportional frequency of sequences that could be identified at the Phylum level.

Also OTUs distribution at the Class level varied among sites (Fig. 3.4). The lichenized fungi in the Lecanoromycetes (Ascomycota) were the most abundant *taxa* and occurred in all analyzed samples (relative abundance ranging from 23% to 60%), followed by ascomycete classes Dothideomycetes (10 to 30%) and Eurotiomycetes (10 to 20%). The Tremellomycetes (Basidiomycota) were present at 10% of relative abundance in most of sites and totally absent in Trio Nunatak site 1; Agaricomycetes, Saccharomycetes and Taphrinomycetes were the rarest members in these communities, detected only in few sites (e.g. Agaricomycetes at Thern Promontory, Mt Bowen, Stewart Heights, and Shafer Peak site 2).

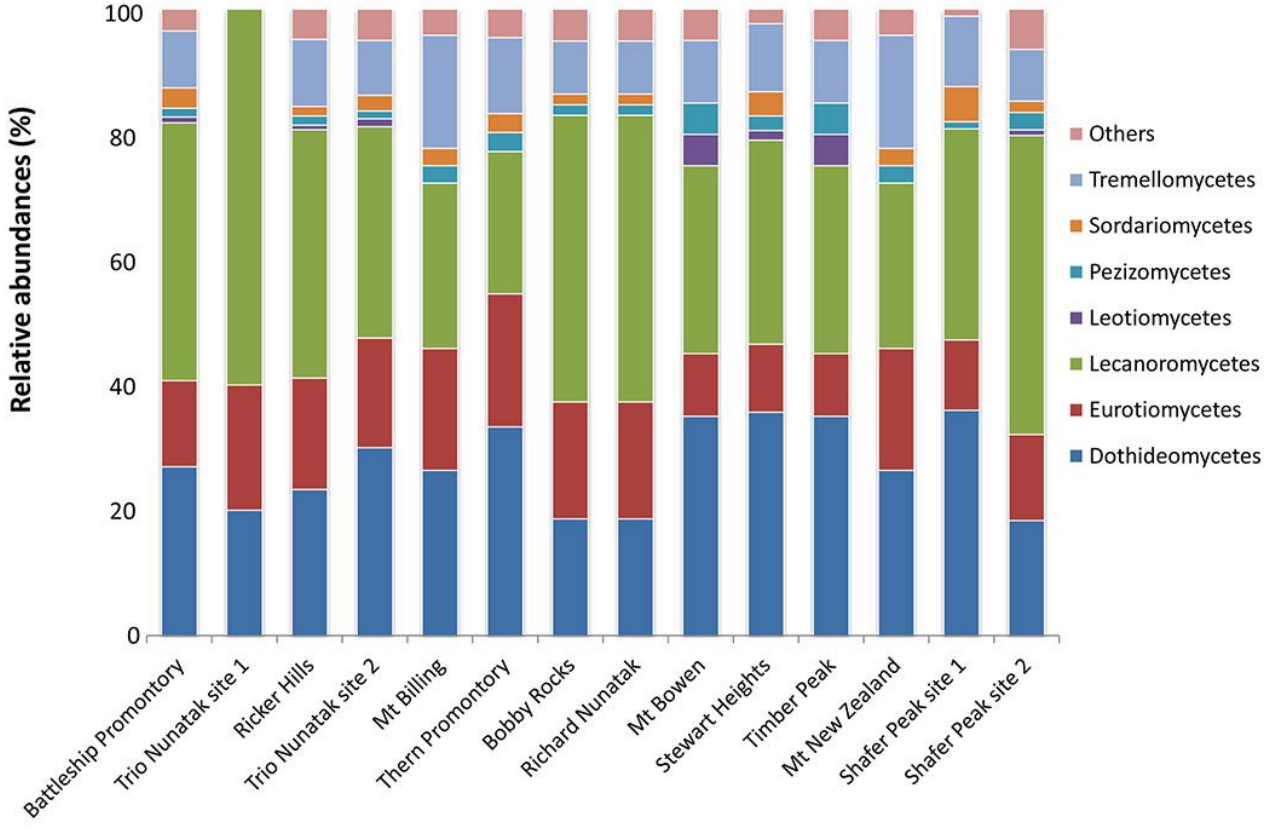


Figure 3.4 Relative abundances of the dominant fungal OTUs in the cryptoendolithic communities in Victoria Land, Antarctica**.** All relative abundances are based upon the proportional frequency of sequences that could be identified at the class level. 'Others' includes classes Agaricomycetes, Microbotryomycetes, Saccharomycetes, Taphrinomycetes and Cystobasidiomycetes, with relative abundances ≤ 5%.

Additionally, to identify the fungal 'core' community (i.e. OTUs present in at least 75% of the samples), a total of 47 OTUs has been retained (Table 3.2). Twelve OTUs were unclassified beyond the Kingdom level, while most OTU 'core' components belonged to the phylum Ascomycota and only three OTUs were assigned to the phylum Basidiomycota, including *Cryptococcus* sp. and *Solicoccozyma aeria.* Among Ascomycota, most *taxa* belonged to the Classes Lecanoromycetes and Dothideomycetes, followed by lower percentages of Sordariomycetes, Pezizomycetes and Eurotiomycetes.

Table 3.2 Fungal 'core' composition (OTUs present in ≥75 samples) 97% of identity.

	Taxonomic assignment	
OTU id	**Phylum**	**Identification (confidence >0.97)**
OTU1	Ascomycota	Genus *Buellia*
OTU10	Ascomycota	*Friedmanniomyces endolithicus*
OTU104	Ascomycota	Genus *Alternaria*
OTU106	Ascomycota	Family Acarosporaceae
OTU109	Ascomycota	*Lecidea laboriosa*
OTU11	Basidiomycota	Genus *Cryptococcus*
OTU110	Ascomycota	Order Dothideales
OTU117	Unclassified	-
OTU118	Unclassified	-
OTU122	Ascomycota	Order Pezizales
OTU126	Ascomycota	-
OTU127	Unclassified	-
OTU13	Basidiomycota	-
OTU130	Unclassified	-
OTU133	Ascomycota	Class Dothideomycetes
OTU137	Unclassified	-
OTU14	Ascomycota	Genus *Acarospora*
OTU146	Ascomycota	*Sarcinomyces crustaceus*
OTU151	Ascomycota	Genus *Verrucari*a
OTU158	Ascomycota	Genus *Acarospora*
OTU161	Basidiomycota	*Solicoccozyma aeria*
OTU169	Unclassified	-
OTU17	Ascomycota	Genus *Acarospora*
OTU178	Ascomycota	OrderPleosporales
OTU18	Ascomycota	Family Acarosporaceae

OTU186	Unclassified	-
OTU119	Ascomycota	*Saitoella coloradoensis*
OTU199	Ascomycota	Family Sporormiaceae
OTU2	Ascomycota	*Lecidea cancriformis*
OTU203	Ascomycota	Order Pleosporales
OTU205	Ascomycota	Order Pleosporales
OTU207	Unclassified	-
OTU7	Ascomycota	*Cryomyces antarcticus*
OTU211	Ascomycota	Class Eurotiomycetes
OTU67	Ascomycota	Genus *Aspergillus*
OTU217	Unclassified	-
OTU227	Unclassified	-
OTU23	Ascomycota	*Fusarium proliferatum*
OTU26	Ascomycota	Family Sporormiaceae
OTU28	Unclassified	-
OTU281	Ascomycota	Family Acarosporaceae
OTU29	Ascomycota	*Aureobasidium pullulans*
OTU345	Unclassified	-
OTU353	Ascomycota	Family Parmeliaceae
OTU351	Ascomycota	Order Lecanorales
OTU37	Ascomycota	Genus *Penicillium*
OTU4	Ascomycota	Order Lecanorales

We further examined the distribution of the fungal *taxa* from the 'core' community. Several phylotypes, as *Solicoccozyma aeria* (OTU 161), unidentified Lecanorales (OTU 351), the unclassified *taxa* OTUs 345-117-227, unidentified Acarosporaceae (OTU 281), *Cryomyces antarcticus* (OTU 7), *Friedmanniomyces endolithicus* (OTU 10), unidentified Basidiomycota (OTU 13), *Buellia* sp. (OTU 1), unidentified Sporormiaceae (OTU 26), *Fusarium proliferatum* (OTU 23), *Penicillium* sp. (OTU 37) and unidentified Pleosporales (OTUs 2-250) were present in all sites. Abundance had a mostly uniform distribution across all the altitudes. Other 'core' *taxa*, such as unidentified Dothideomycetes (OTU 133), the unclassified *taxa* OTUs 137-217-186, unidentified Dothideales (OTU 110) and unidentified Pleosporales (OTUs 203-205), were present only intermittently among sites. Sampled sites were also hierarchically clustered by OTU abundance to examine patterns of similarities in community composition but did not exhibit any clustering by sampled localities (Fig. 3.5).

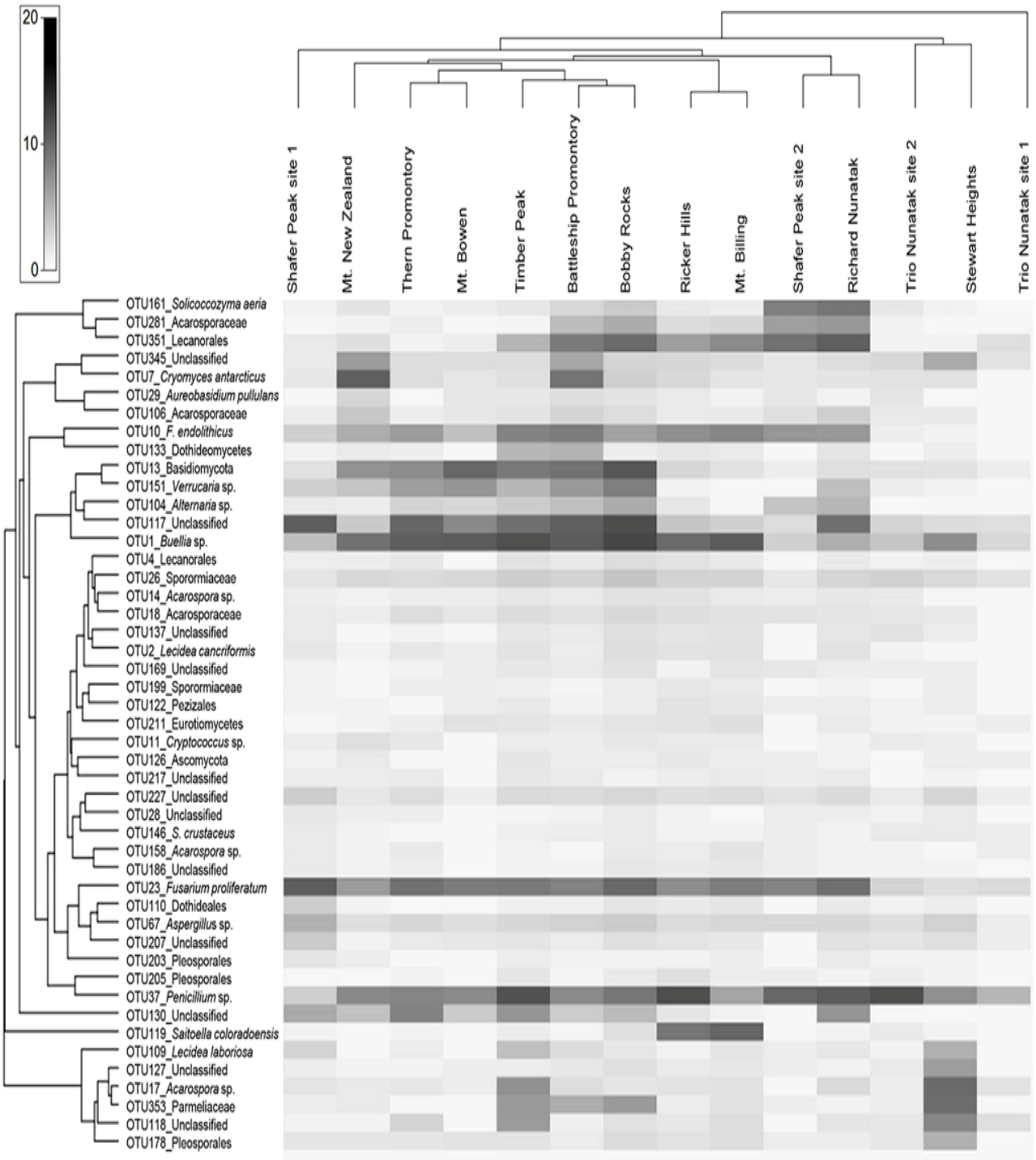


Figure 3.5 Heat map of the 'core' *taxa* relative frequency and UPGMA hierarchical clustering of samples. Values are scaled (log transformed) by *taxon* relative frequency across all samples. Frequencies are indicated by the color intensity: dark grey indicates a *taxon* with high relative frequency; white indicates absence; light grey represents lower relative frequency. Both the 'core' OTUs and sites were clustered using a Bray-Curtis index.

3.3.3 Diversity analysis

Species richness (S), Shannon (H'), Simpson (1-D), and Pielou (J') indices for each site are reported in Table 3.3. The highest observed fungal richness (111 OTUs) was recorded at Ricker Hills (1400 m a.s.l.). In contrast, the Trio Nunatak site 1 (1000 m a.s.l.) exhibited low fungal richness with value of 17 OTUs. Battleship Promontory, Richard Nunatak and Stewart Heights showed the highest values for the other biodiversity indices. Among all sites, H' ranged from 0.55 to 2.47, 1-D from 0.57 to 0.84 and J' from 0.21 to 0.44 (Table 3.3).

Table 3.3 Diversity metrics for fungal ITS rRNA gene sequencing for each site. Analysis included: number of reads, species richness (S), Shannon index (H'), Simpson's Index of Dominance (1-D), and Pielou's equitability index (J').

Sites	Reads	S	H'	1-D	J'
Battleship Promontory	80426	93	1.92	0.75	0.39
Trio Nunatak site 1	18013	17	0.55	0.77	0.21
Ricker Hills	139839	111	2.4	0.64	0.44
Trio Nunatak site 2	75956	47	1.19	0.57	0.30
Mt Billing	68369	40	1.59	0.66	0.38
Thern Promontory	57090	42	1.49	0.69	0.36
Bobby Rocks	297482	55	1.56	0.69	0.33
Richard Nunatak	74913	70	1.89	0.85	0.42
Mt Bowen	54834	30	1.12	0.58	0.32
Stewart Heights	41391	67	2.47	0.84	0.55
Timber Peak	60549	51	1.30	0.69	0.30
Mt New Zealand	40160	66	1.42	0.59	0.32
Shafer Peak site 1	57635	48	0.77	0.75	0.29
Shafer Peak site 2	30714	42	1.62	0.69	0.30

3.3.4 Statistical analysis

Spearman's correlation ρ values represented correlation between biodiversity indices and sampled sites. In all cases we found no significant correlation between diversity indices and locations, even among sites located at similar altitudes (P values > 0.1) (Fig. 3.6). Similar trend was observed correlating biodiversity indices and sea distance values (data not shown).

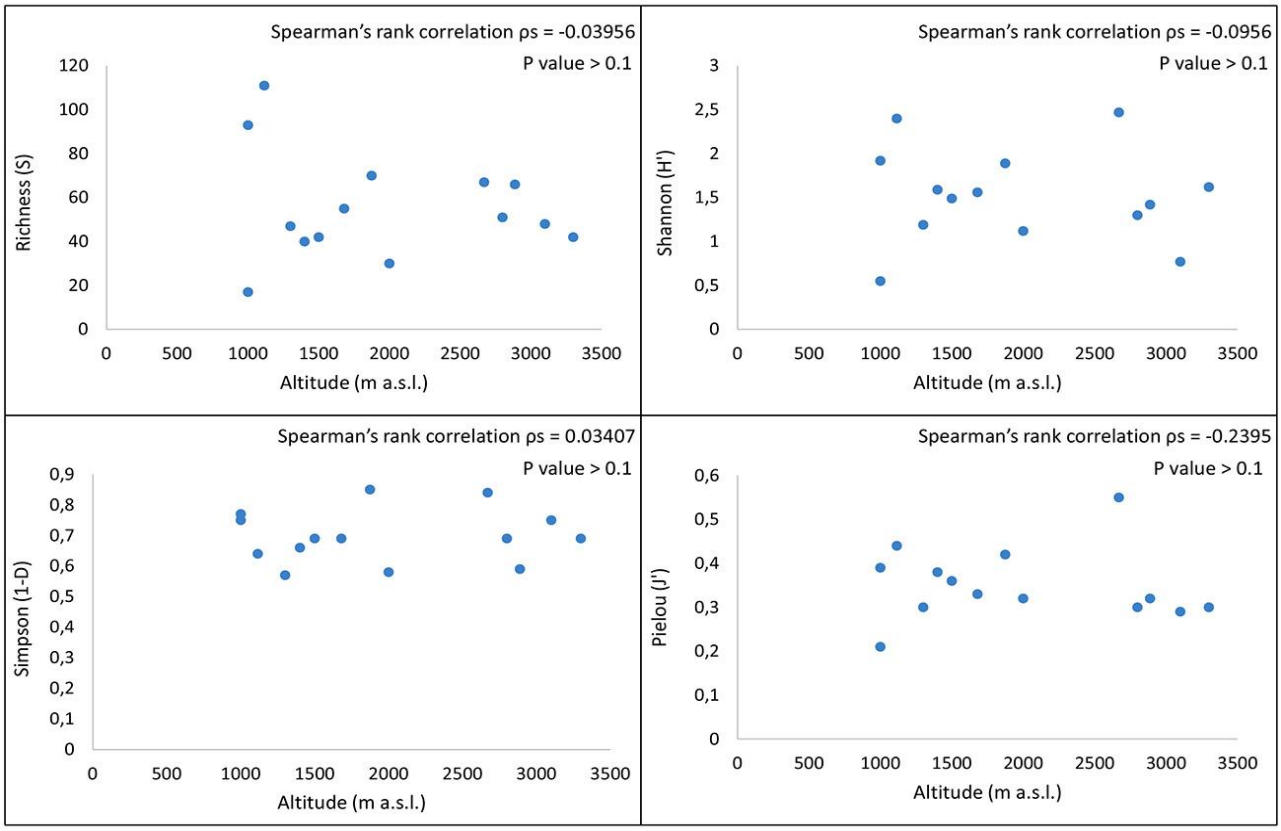


Figure 3.6 Spearman's correlation coefficients between the fungal diversity indices (Species richness, Shannon, Simpson, and Pielou indices) calculated on 14 endolithic communities, and altitudinal gradient.

The variability in species composition of these communities among 14 sites was measured with β diversity and to estimate the contribution of different geographic parameters (altitude: m a.s.l.; sea distance: km) between sampling sites, an MRM analysis was implemented.

Considering the community composition calculated on incidence-based Jaccard index, β diversity varied among all locations. Highest similarity values occurred between Timber Peak (2800 m a.s.l., 49.5 km) and Bobby Rocks (1680 m a.s.l., 91 km) (80% of similarity) and between Timber Peak, Mt. Billing (1300 m a.s.l., 44 km) and Ricker Hills (1115 m a.s.l., 96 km) (70 % of similarity). The highest values of dissimilarity were obtained between Mt Bowen (1874 m a.s.l., 39.5) and Battleship Promontory (1000 m a.s.l., 33.5) (only 30% of similarity). Even sites visited in the same locations (i.e Trio Nunatak site 1 and 2, Shafer Peak site 1 and 2) showed low percentages of similarity.

In addition, there was no significant correlation between fungal communities and both altitude (m a.s.l.) and sea distance (km) among all sites (P value > 0.05). MRM analysis clearly indicated that fungal community composition is not significantly affected by the environmental parameters considered.

3.3.5 Pareto Lorenz curves

Pareto-Lorenz curves distribution patterns of the meta-barcoding profiles were plotted based on the numbers of OTUs and their frequencies. F_o values were high in all sampled sites, ranging from 86 to 100%. These results indicate these fungal endolithic communities were dominated by few, abundant, and specialized species and some other rare groups.

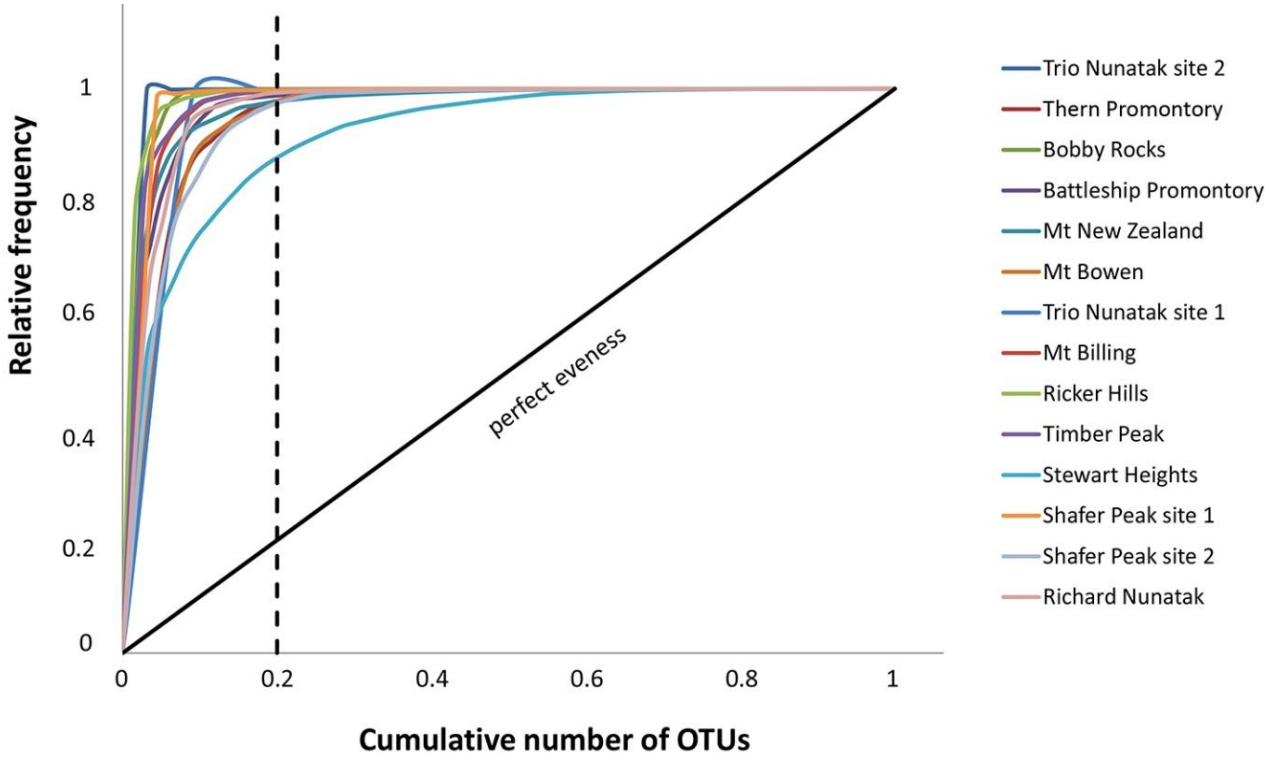


Fig. 3.7 Pareto-Lorenz curves. The analysis was based on number of OTUS and their frequencies in the meta-barcoding profiles. Theoretical perfect evenness (perfect evenness line) is characterized by a curve close to the 45°C diagonal.

3.4 Discussion

Endolithic microbial communities occur globally and play an important role in biogeochemical processes, including rock and mineral transformations, bio-weathering, and biomineral formation (Gadd *et al*., 2012), particularly in border ecosystems (Nienow and Friedmann, 1993). Nevertheless, little is known about the composition, diversity and distribution of these communities, especially in continental Antarctica, where they represent the predominant life form (Nienow and Friedmann, 1993).

Sequence-based fungal communities have been retrieved from samples in all sampled locations, including the highest altitude sites, such as Shafer Peak (3300 m a.s.l.) and Mt New Zealand (3100 m a.s.l.) in Northern Victoria Land, previously reported as colonized by fungal populations (Zucconi *et al*., 2016).

Most of the OTUs were identified as Ascomycota, as already reported in previous molecular surveys in soil Antarctic communities (Cox *et al.*, 2016; Ji *et al*., 2016; Wei *et al.*, 2016), chasmoendolithic communities in Miers Valley, McMurdo Dry Valleys (Yung *et al*., 2014), and in association with mosses in ice-free coastal outcrops (Hirose *et al*., 2016), supporting that most fungi in the Antarctic mycobiome are ascomycetes. The majority of Ascomycota were identified as members of the lichen-forming Class Lecanoromycetes; the second most abundant was Dothideomycetes, the widest and most diversified Class in the Ascomycota. Our observations are consistent with 20 years of culture-based isolation and identification which recover Dothideomycetes as the most frequently isolated fungi from these communities (Selbmann *et al*., 2005, 2008; Egidi *et al*., 2014). In contrast, Lecanoromycetes are infrequently detected with standard isolation procedures, maybe due to the trouble and difficulties in cultivating fungi with obligate symbiotic life-style. The widespread presence of Lecanoromycetes detected with molecular approaches is not surprising, as lichen-forming fungi are known to dominate cryptoendolithic communities colonizing sandstone rocks in Victoria Land (Friedmann *et al*., 1988; Cockell *et al*., 2003; Zucconi *et al*., 2016). Similarly, lichens predominate in many other continental Antarctic localities, where cold-adapted mycobionts have been previously recorded (e.g. Ruprecht *et al*., 2010; 2012). In this study, *Lecidea* sp. (Lecideaceae) and *Buellia* sp. (Physciaceae) were recorded in almost all the analyzed samples. The Genus *Buellia* encompasses species considered endemic to Antarctica, such as *Buellia frigida*, a crustose lichen which grows on rock surfaces in ice-free areas of Antarctica and has been widely found in both coastal and mountain locations across the continent (Jones *et al*., 2015). Similarly, *Lecidea* species live endolithically in granite rocks of continental Antarctica (de Los Rios *et al*., 2005). Lichens are considered exceptionally well adapted to the lithic lifestyle, thanks to their low mineral nutrient demand, high freezing tolerance, and ability to be photosynthetically active at suboptimal temperatures (Kappen, 2000). Consistent with our findings, a recent study on lithic colonization patterns from an additional locality in the McMurdo Dry Valleys, University Valley, Southern Victoria Land, documented a lichen mycobiont prevalence in sandstone communities (Archer *et al*., 2016).

In addition to the lichen-forming *taxa Buellia* and *Lecidea*, *Friedmanniomyces endolithicus* is a 'core' member of the sandstone cryptoendolithic community as it was found in the majority of the analyzed samples, confirming the widespread presence of *Friedmanniomyces* spp. in Northern Victoria Land. The Genus *Friedmanniomyces* (Dothideomycetes) includes two described species of rock-inhabiting meristematic black fungi, *F. simplex* and *F. endolithicus* (Onofri *et al*., 1999; Selbmann *et al*., 2005); it is strictly endemic to Victoria Land and the most-frequently isolated non-lichenized fungus from rock substrata in this area (Selbmann *et al.*, 2005, 2015a; Ruisi *et al*., 2007). *Friedmanniomyces* spp., well studied for their phylogenetic position, taxonomic classification, and physiological characteristics (Egidi

et al., 2014; Selbmann *et al*., 2005), possess stress-tolerant adaptations which allow them to inhabit inert surfaces and survive long time under drought conditions (Onofri *et al.*, 2004). We also retrieved *Cryomyces antarcticus* (Dothideomycetes) as 'core' member, although in relatively lower abundance as compared to the dominant *taxa*. The Genus *Cryomyces* encompasses four species of cold-adapted rock-inhabiting black yeasts; *C. montanus* and *C. funiculosus* have been isolated from Alpine rocks collected above 3000 m a.s.l. (Selbmann *et al*., 2014b), while the two Antarctic species, *C. antarcticus* and *C. minteri* have been isolated primarily from the McMurdo Dry Valleys in Southern Victoria Land (Selbmann *et al.*, 2005), and rarely in Northern Victoria Land (Cecchini C., PhD thesis). Our results suggest an even wider distribution for *C. antarcticus* in the continent than previously recorded, highlighting the higher power of meta-barcoding approaches compared to the culture-dependent approach in recovering low abundant, even if cultivable, slow growing fungi. Additional members of the Ascomycota were found in the cryptoendolithic fungal communities including one *Aspergillus* sp. (Trichocomaceae, Eurotiomycetes) and several unidentified *taxa* belonging to the Order Pleosporales (Dothideomycetes). Several members of the Pleosporales have been previously associated with rock formations in both cold and hot areas (Ruibal *et al.*, 2009; Egidi *et al.*, 2014). Filamentous fungi such as *Aspergillus* spp. in Antarctica have been isolated and described from oligotrophic soils (Godinho *et al*. 2015), but never from rocks. Indeed, the environmental pressure typically associated with the exposed polar rocks requires a high degree of specialization (Selbmann *et al*., 2005, 2013; Onofri *et al*., 2007), making this substratum not suitable for fast-growing, cosmopolitan *taxa*, such as members of the Genus *Aspergillus*. Therefore, although a long-range aerial spore dispersal cannot be completely excluded (see Pearce *et al*., 2009), we hypothesize that the occurrence of *Aspergillus* spp. in our dataset is more likely the result of a post-sampling contamination, rather than the reflection of a true component of the Antarctic rock mycobiome. Basidiomycota represents a rare fraction of the 'core' community. Only three Basidiomycota phylotypes were recovered, two identified as *Cryptococcus* sp. and one as *Solicoccozyma aeria* (former *C. aerius*) as recurring communities members. Members of the Genus *Cryptococcus* have been isolated globally, including Antarctica, both from rock and soil communities (Vishniac *et al*., 1985; Vishniac and Kurtzman, 1992; Montes *et al*., 1999; Scorzetti *et al*., 2000). Although their association with rock substrates from cold sites worldwide has been previously reported by Selbmann *et al*. (2014c), overall Basidiomycota represent an infrequent group in the cryptoendolithic community, making up at most 3% or less of fungal sequences on the sub-Antarctic, low maritime and high maritime Antarctic (Cox *et al*., 2016). The Class Taphrinomycetes has been repeatedly isolated and described as new and endemic Antarctic species (Selbmann *et al*., 2014d), but rarely retained in this dataset. Interestingly, we have been able to retrieve members of Saccharomycetes, even if with scarce incidence, although these *taxa* have never been isolated from Antarctic cryptoendolithic communities. Overall, the low biodiversity

indices values obtained in this study are consistent with what recently observed in Antarctic lithic communities in Victoria Land on different rock typology (Selbmann *et al*., 2017). Compared with indices recorded even in soil microbial communities of Antarctic Peninsula they are very low (Chong *et al*., 2009), indicating a strict predominance of a restricted number of specialized species. The fungal community composition did not appear influenced by elevation: indeed, samples collected on the top of Mt New Zealand, Stewart Heights at 2670 m a.s.l., and Richard Nunatak at 2000 m a.s.l. had biodiversity richness values similar to those from communities of samples from lower altitude e.g. Trio Nunatak (1400 m a.s.l.), Ricker Hills (1115 m a.s.l.), Battleship Promontory (1000 m a.s.l.), and Mt Billing (1300 m a.s.l.).

There is a remarkable variability in the occurrence of lichen-forming and meristematic fungi, regardless the altitude, suggesting that this parameter alone is not a determining driving factor for fungal community composition. The black yeast *Cryomyces antarcticus* was consistently present among localities, suggesting that this 'core' *taxon* is remarkably resistant to ecological stresses, even at high altitudes. The species of the *Cryomyces* Genus are among the most resistant species know to date: they are able to resist extreme temperatures and UV radiations (Onofri *et al*., 2007; Selbmann *et al*., 2011; Pacelli *et al*., 2017a). In particular, *C. antarcticus* was able to withstand ground simulated space and Mars conditions (Pacelli *et al*., 2017a) as well as 18 months of real Space exposition and Mars-simulated exposure outside the International Space Station (Onofri *et al*., 2012, 2015; unpublished); *C. antarcticus* is considered among the best eukaryotic models for astrobiological studies.

The functionality (F_o) of these communities was represented by the Pareto-Lorenz curves, showing all values mostly around 100%. The theoretical perfect uniformity (e.g. a slope of 45°, F_o= 25%) means all species in the community have the same number of individuals and none is dominant. Values of F_o near 100% indicate highly specialized community where few species are dominant and all others are present as few representatives (Marzorati *et al*., 2008). This result clearly indicated that all the communities here studied have a high degree of specialization and adaptation; conversely, they are scarcely resilient and prone to external perturbations, as reported by Selbmann *et al*. (2017).

Statistical analysis performed, calculating Spearman's coefficient correlation, confirms that fungal biodiversity (i.e. number of OTUs retrieved and biodiversity indices) was not related to sampled sites and environmental parameters (altitude, sea distance). These data were further supported by MRM analysis, which did not show any correlation between considered parameters and community composition, highlighting also a high level of dissimilarity in samples collected in same locations.

These results lead to the conclusions that the environmental variables here considered (altitude and sea distance) did not play a role on fungal diversity and composition in these peculiar ecosystems; it can be expected that, in a borderline ecosystem, additional parameters that can influence local variations (e.g.

water availability, rock temperature and sun exposition) could be crucial. Therefore, a constant monitoring of temperature and water availability, both of the ambient and in the airspaces of rocks, considering also sun exposure, is auspicable. For this reason, sensors have already been installed in some sites of both northern and southern Victoria Land and the widening of this monitoring is in progress. Combined data on the climatic and environmental conditions, and their daily and seasonal variation, will be of help to elucidate the processes influencing biodiversity variations, community composition and species extinction. This information, estimated on a longer period, may allow to be predictive on possible consequences of Climate Change on terrestrial biota in Antarctica (Nienow *et al*., 1988; Friedmann *et al*., 1987; McKay *et al*., 1993).

This study is the largest attempt to comprehensively identify patterns of fungal diversity in rocks in Antarctica over high altitudes and along a sea distance gradient. With the advent of NGS technologies, our understanding of Antarctic microbial biodiversity and evolution is improving, but further investigations are required to elucidate how microbiota responds to environmental pressure and, in the long run, how future environmental changes will impact these unique communities (Tilman, 1996; Van Horn *et al*., 2014). The high degree of specialization and the low taxonomic richness found in this study alert on the high potential susceptibility of Antarctic endolithic communities to environmental changes (Tilman, 1996; Neilson *et al*., 2012; Van Horn *et al*., 2014). Data obtained in this study are of importance to set a proper experimental plan in the future, taking into consideration additional environmental parameters and organizing a more targeted sampling to be studied with NGS-based approaches to provide a better evaluation of the potential consequences of environmental changes on these unique ecosystems.

CHAPTER 4

BACTERIAL DIVERSITY AND COMPOSITION IN CRYPTOENDOLITHIC LICHEN-DOMINATED COMMUNITIES IN VICTORIA LAND, ANTARCTICA.

Abstract

The endolithic lifestyle represents a borderline adaptation to extreme environmental stressors and is the predominant life-form in the ice-free regions of Victoria Land, Antarctica. Microbial diversity of the endolithic communities in this area is scarcely investigated, and studies of the last decade mainly focused on prokaryotic biodiversity of soils. In this chapter, diversity and composition of bacterial endolithic communities of Victoria Land, Antarctica, were investigated using a high-throughput meta-barcoding approach to amplify V4 region of 16S rDNA. Colonized rock samples were collected from 14 different sites along an altitudinal gradient ranging from 1000 to 3300 m a.s.l. altitude, over a latitudinal transect of about 3° and from 29 to 96 km sea distance. Biodiversity indices and Pareto Lorenz curves were implemented to describe the richness and evenness of these communities; in addition, statistical analyses were performed to investigate the variation of bacterial diversity in relation to the environmental parameters of altitude and sea distance. Results revealed a predominance of Actinobacteria and Proteobacteria among all samples and defined a 'core' group of bacterial *taxa* across all sites. Also, the detection of a substantial number of unidentified uncultured bacterial phylotypes (about 20% of relative abundance) indicates Antarctic rocks as a possible reservoir of novel bacterial *taxa*.

Keywords: Antarctica, Meta-barcoding, Endolithic communities, 16S, Extremophiles

4.1 Introduction

Victoria Land region (Antarctica) ranges from the west side of the Ross Sea southward from 70°30'S to 78°00'S and westward from the coastline to the edge of the polar plateau. It is divided into two regions: Northern Victoria Land, that encompasses Terra Nova Bay, Edmonson Point and Cape Hallett, and Southern Victoria Land, that includes the McMurdo Dry Valleys and nearby coastal regions.

Most of the studies on microbial diversity of Antarctic soils concern prokaryotes and are focused on East Antarctica as, for instance, Bratina Island and Windmill Islands (Smith *et al.*, 2006; Chong *et al.*, 2009), South Shetland Archipelago (Ganzert *et al*., 2011), but even Victoria Land (Niederberger *et al*., 2008). In this last work, soils from Luther Vale, located close to the north border of Victoria Land, were analysed. Results from all these studies indicated an unexpected bacterial diversity. Soils of the McMurdo Dry Valleys are, instead, highly oligotrophic and support relatively low biomass (Cowan *et al*., 2002; Pointing *et al*., 2009; Rao *et al*., 2011; Lee *et al*., 2012). In this area, soil communities are dominated by Actinobacteria and other cosmopolitan soil bacteria (Aislabie *et al.*, 2006; Smith *et al*., 2006; Niederberger *et al.*, 2008. 2012; Stomeo *et al*., 2012), while soil-associated hypolithic communities (Chan *et al*., 2012; Wei *et al*., 2016) are dominated by cyanobacteria-dominated biofilms (de los Rìos *et al.*, 2014).

Differently, rocks of both the ice-free areas of the McMurdo Dry Valleys as well as mountain tops hanging from the Polar Plateau along the Victoria Land, support the highest standing biomass in the Antarctic ice-free areas; thus, stones supply the main substratum for life (Cowan and Tow, 2004; Cary *et al*., 2010; Cowan *et al*., 2014). There, the heavy injuries of low temperatures, wide thermal fluctuations, high irradiation incidence, low relative humidity, and scarce liquid water availability force life, almost exclusively represented by specialized microbes (Nienow and Friedmann, 1993; Vincent, 2000; Zucconi *et al*., 2016), to withdraw inside the rock substratum to find more permissive conditions (Friedmann, 1982).

The endolithic habit represents a stunning adaptation to extreme environmental stressors and the predominant life-form in the Antarctic ice-free inner regions of Victoria Land (Nienow and Friedmann, 1993; Cary *et al*., 2010; Cowan *et al*., 2014). Cryptoendolithic communities are complex assemblage of microorganisms, including bacteria, cyanobacteria, chlorophyte algae and ascomycetous free-living fungi or in lichen symbioses (Friedmann, 1982; de la Torre *et al.*, 2003; Yung *et al.*, 2014).

Studies on microbial diversity in these communities, almost invariably focused on the prokaryotic compartment, are just at the beginning. Maybe due to the difficulty of sampling, data available are patching and based on a single or few rock samples from different locations or on few samples from a single site (de La Torre *et al*., 2003; Pointing *et al*., 2009; Yung *et al*., 2014; Archer *et al*., 2016).

Moreover, nothing is known about the distribution and composition of bacterial endolithic ecosystems and the role of environmental factors as driving forces shaping patterns of diversity.

The development of culture-independent molecular methods such as meta-barcoding are powerful approaches to achieve a deeply detailed description of microbial diversity and understand ecology of microbial communities (Ji *et al*., 2013). This study takes advantage of this approach to investigate the bacterial component, targeting the V4 region of 16S on a large sampling of endolithic lichen-dominated communities from 12 localities and 14 sites of Victoria Land along an altitudinal and sea distance gradient, already studied for fungal assemblage, as reported in chapter 3. The aims of this study were to i) assess the bacterial diversity, structure and composition in endolithic communities in Victoria Land; ii) identify a possible 'core' group among prokaryotes; iii) relate bacterial diversity and composition with the considered environmental parameters.

4.2 Materials and methods

4.2.1 Study area

Samples analyzed in this section were the same listed and described in chapter 3 and are reported in Table 3.1. Sandstone samples were collected along a latitudinal transect ranging from 73°29'26"S (Stewart Heights, Northern Victoria Land) to 76°54'36''S (Battleship Promontory, McMurdo Dry Valleys, Southern Victoria Land). Rocks were collected during the XXVI Italian Antarctic Expedition (2010-2011).

4.2.2 DNA extraction and sequencing

Total DNA was extracted from rocks using MOBIO Power Soil DNA Extraction kit (MOBIO Laboratories, Carlsbad, CA, USA), according to the manufacturer's protocol. V4 region of 16S rDNA region was amplified. PCR reactions were carried out with a total volume of 25 µl, containing 1 µl of each primer, 12.5 µl of Taq DNA Polymerase (Thermo Fischer Scientific Inc., Waltham, MA, USA), 9.5 µl of nuclease-free water (Sigma–Aldrich, UK) and 5 ng of DNA. We amplified the V4 region using the new developed barcoded primers F515/R806 (515F: GTGYCAGCMGCCGCGGTAA; R806: GGACTACNVGGGTWTCTAAT), modified from the original primers F515/R806 (Caporaso *et al.*, 2010). PCRs were then subjected to an initial denaturation at 93°C for 3 min, 35 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 1 min, extension at 72°C for 90 s, followed by a final extension at 72°C for 10 min in an automated thermal cycler (BioRad, Hercules, CA). Amplicons have been purified and normalized after quantifying with the Qubit dsDNA HS Assay Kit (Life Technologies, USA) and

then pooled. Sequencing was performed on Illumina MiSeq platform at the Institute for Integrative Genome Biology, University of California, Riverside.
Raw sequence data were submitted to the GenBank databases under BioProject accession number PRJNA379160.

4.2.3 Bioinformatics analysis

The V4 datasets were processed similarly to ITS dataset described in chapter 3 with the AMPtk: Amplicon ToolKit for NGS data (formally UFITS) 0.9.3 v. (https://github.com/nextgenusfs/amptk) (Palmer *et al*., 2017). Briefly, barcodes and primers were first removed from the amplicons sequencing data, reads were demultiplexed with split_libraries.py (QIIME v 1.9.1 Caporaso *et al*., 2010) and then processed utilizing USEARCH (v.9.1.13) (Edgar, 2010) as reported in chapter 3.2.3. Bacterial sequences were clustered into Operational Taxonomic Units (OTUs) using the VSEARCH (v 2.3.2) (Rognes *et al.,* 2016) algorithm, and taxonomic assignment was performed by using SINTAX/UTAX and UCHIME_REF (Edgar, 2010). Additionally, we calculated and mapped the OTU 'core', defined as the OTUs present in at least 75% of the samples. PRIMER-E software v7 (PRIMER-E Ltd. Plymouth, UK) was utilized to map the relative abundance of the community 'core' members on a heat-map using log-transformed taxonomic counts and UPGMA clustering approach was implemented to highlight similarities in the bacterial community composition among the sampled sites.

4.2.4 Diversity analysis

Biodiversity indices were calculated to investigate species richness, evenness and composition of the bacterial community utilizing rarefied data. As described in the paragraph 3.2.4, (i) the species richness (S) index, (ii) the Shannon index (H') (Shannon and Weaver, 1963), (iii) the Simpson's Index of Dominance (1-D) (Simpson, 1949), and (iv) the Pielou's equitability index (J') (Pielou, 1969) were calculated.

4.2.5 Statistical analysis

In order to evaluate any relationship between the environmental parameters (altitude: m a.s.l. and sea distance: km) of 14 sampled sites and biodiversity indices considered (S, H', 1-D and J'), Spearman's rank correlation was implemented.
In addition, to establish correlation between the community composition (considering presence/absence data) and sampled locations, we calculated the β diversity, using the incidence-based Jaccard index.

Multiple Regression on distance Matrices (MRM) (Legendre *et al*., 1994; Lichstein, 2007; Ptacnik *et al*., 2010) was computed by using 'MRM' function implemented in ecodist package (Goslee and Urban, 2007) in R (R Development Core Team, 2010) version 3.4.2. Environmental distances matrices (quantified by means of the Euclidean distance pairwise) between sampling sites were regressed against the species composition dissimilarity. To validate the significance, we performed 10000 permutations on the original dataset, and *P*-values for MRM models were obtained by comparing each observed regression coefficient with the distribution of 10000 permuted values. All statistical tests were considered significant at $p<0.05$.

4.2.6 Pareto Lorenz curves

The evenness of these communities was graphically represented with Lorenz distribution curves (Lorenz, 1905). For each site, the cumulative normalized number of the OTUs was used as x-axis, and their respective cumulative normalized frequencies represented the y-axis. An increasing deviation of Lorenz distribution curve from the theoretical perfect evenness line (i.e., the 45° diagonal), means that a lower evenness can be observed in the structure of the studied communities. Curves were also evaluated based on the Pareto principle, where the cumulative y axis value (F_o value) corresponding to the 20% level on the x-axis is evaluated (Possemiers *et al.*, 2004; Al-Mutairi, 2009).

4.3 Results

4.3.1 Sequencing data analysis

A total of 864425 bacterial V4 region rRNA gene reads passed the quality filtering step and the reads averaged 61744 reads, with a minimum of 32481 to a maximum of 75174 reads (Table 4.1). Reads were retrieved from all samples, including those collected at highest elevations, such as Mt New Zealand (2888 m a.s.l.) and Shafer Peak site 1 (3100 m a.s.l.) in Northern Victoria Land. Sequences were clustered into Operational Taxonomic Units (OTUs), obtaining a total of 560 OTUs.

Table 4.1 Diversity metrics for bacterial ITS rRNA gene sequencing for each site. Analysis included: number of reads, species richness index (S), Shannon index (H'), Simpson's Index of Dominance (1-D), and Pielou's equitability index (J').

Sites	Reads	Richness (S)	Shannon (H')	Simpson (1-D)	Pielou (J')
Battleship Promontory	66567	287	3.12	0.89	0.55
Trio Nunatak site 1	64152	57	2.59	0.88	0.64
Ricker Hills	64744	69	2.89	0.91	0.69
Mt Billing	59433	156	2.15	0.70	0.42
Trio Nunatak site 2	32481	101	2.24	0.82	0.48
Thern Promontory	54279	57	1.36	0.65	0.35
Bobby Rocks	61406	73	2.64	0.88	0.62
Mt Bowen	58002	157	2.64	0.86	0.53
Richard Nunatak	70826	86	2.55	0.87	0.58
Stewart Heights	75174	218	4.24	0.98	0.79
Timber Peak	64023	201	1.89	0.70	0.40
Mt New Zealand	61739	144	2.79	0.89	0.56
Shafer Peak site 1	66326	50	1.1	0.61	0.28
Shafer Peak site 2	65273	156	3.46	0.91	0.70

4.3.2 Bacterial communities description

The majority of the bacterial sequences retrieved among all samples belonged to the Actinobacteria (ranging from 25 to 50%) and Proteobacteria (from 10 to 30%) phyla, followed in lower relative abundances by Acidobacteria (from 0 to 10%), Cyanobacteria (from 3 to 8%), Bacteroidetes (about 10%) and Planctomycetes (from 1 to 10%). Acidobacteria were retained in all sites with the exception of Shafer Peak site 1, while phylotypes belonging to Cyanobacteria in all sites, except Shafer Peak site 1 and Mt Bowen. *Taxa* belonging to *Deinococcus-Thermus*, Fusobacteria and Verrucomicrobia were retrieved at lowest percentage and in only few sites. On the contrary, Proteobacteria and Actinobacteria were recorded in all sites (i.e. *taxa* belonging to the Phylum Actinobacteria were present with highest relative abundance values at Shafer Peak site 1, Thern Promontory and Mt Billing). Generally, from 5% (Shafer Peack site 2 and Battleship Promontory) to 25% (Shafer Peak site 1) unclassified OTUs were recorded (Fig.4.1)

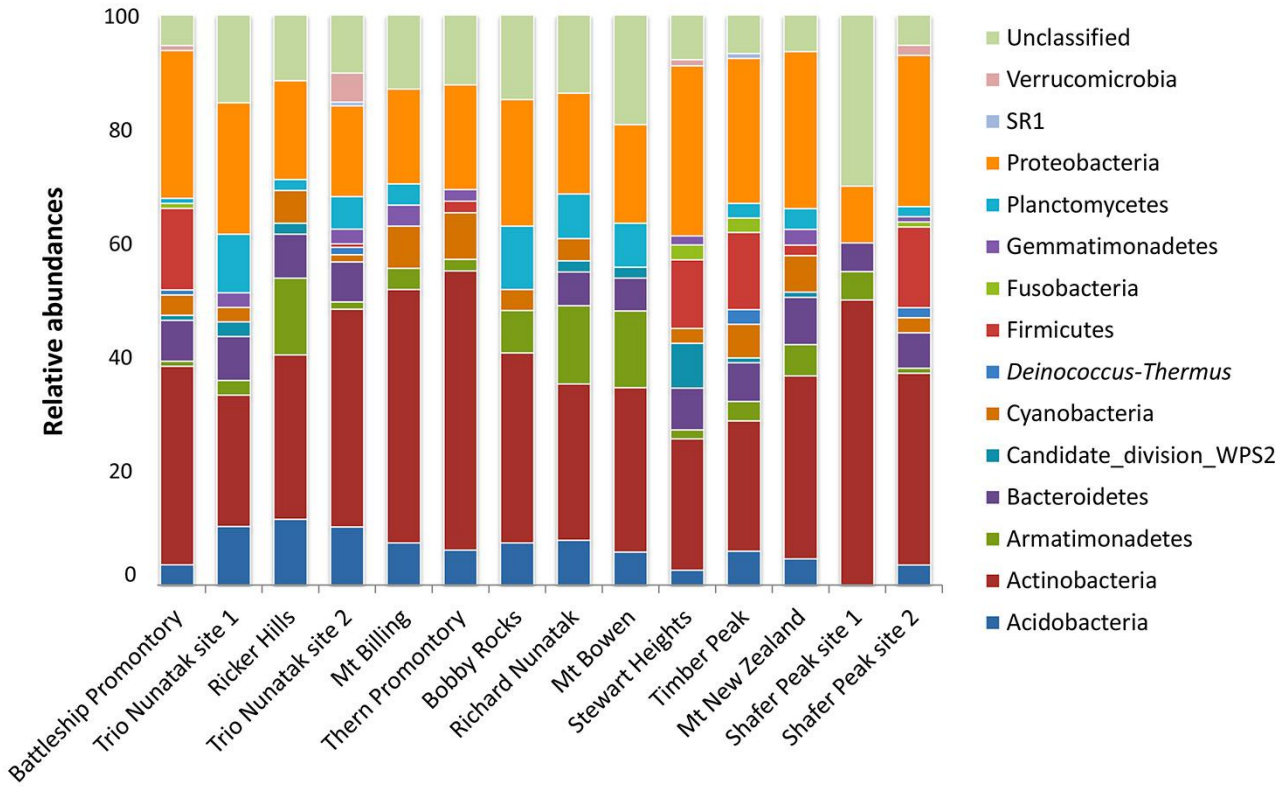


Figure 4.1 Relative abundances of the dominant bacterial OTUs in the cryptoendolithic communities in Victoria Land, Antarctica. All abundances are based upon sequences identified at the Phylum level. Samples are displayed according to the altidunal gradient as reported in Table 3.1.

Additionally, even the OTUs distribution at the Order level varied among sampled site as showed in Fig. 4.2. OTUs belonging to the Orders Actinobacteriales were the most relative abundant and recorded in almost all samples, except at Trio Nunatak site 2, where *taxa* belonging to Rhodospirillales were not detected. Other Orders were present in lowest percentage (i.e Armatimonadales, Bacillales, Planctomycetales and Sphingobacteriales) (Fig. 4.2).

A bacterial 'core' community (i.e. OTUs present in at least 75% of the samples), encompassing 48 OTUs (only 9% of the total 560 OTUs) was identified. Most OTUs 'core' members belonged to the Family Acetobacteracae, few *taxa* to Family Conexibacteraceae and only one OTU to Family Comomonadaceae. Phylotypes identified at Genus level belonged to *Granulicella* sp., *Acidisoma* sp., *Frankineae* sp. and *Mucilaginibacter* sp. Others, identified at Order or sub-Order levels belonged to Corynebacterineae (sub-Order), Micrococcineae (sub-Order), Rhizobiales (Order) and Actinomycetales (Order). Few *taxa* wew unclassified (Table 4.2).

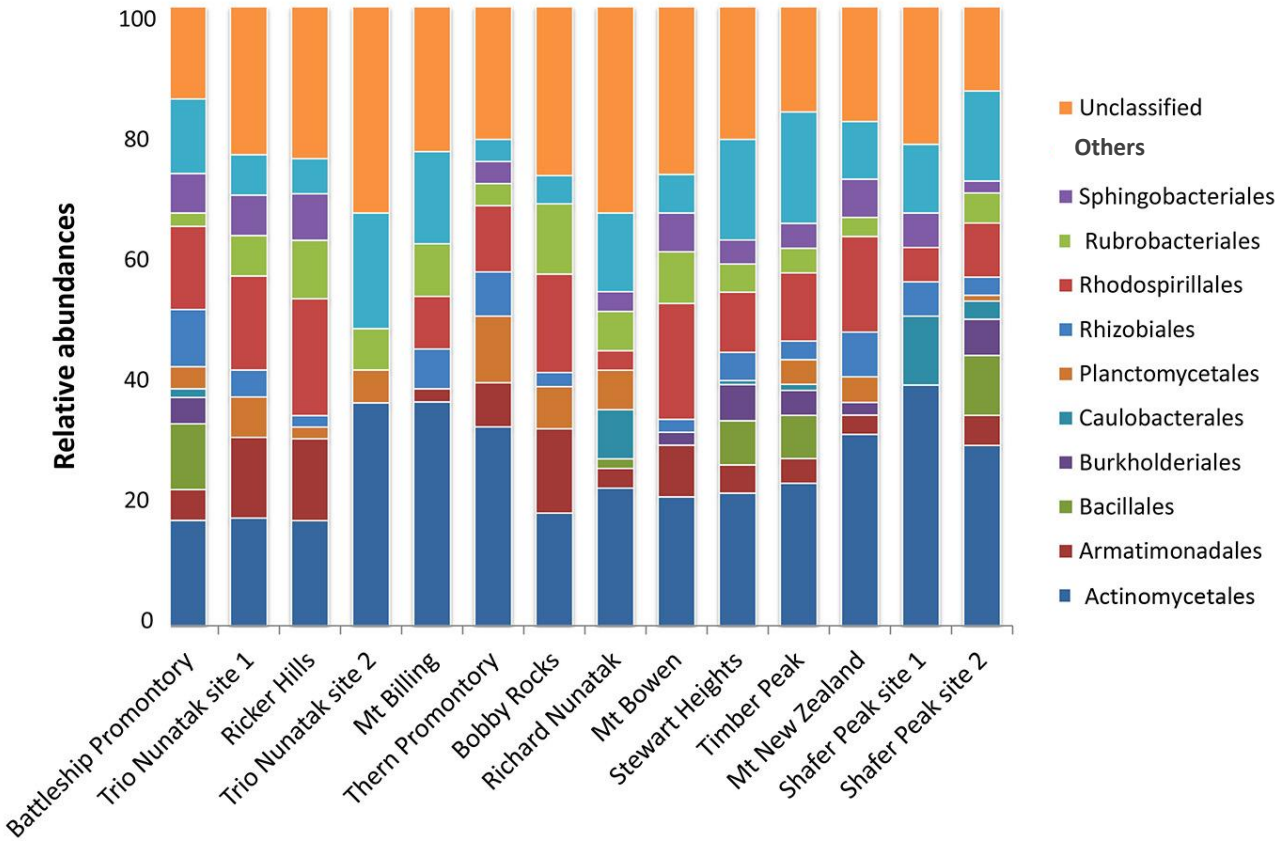


Figure 4.2 Relative abundances of the dominant bacterial OTUs in the cryptoendolithic communities in Victoria Land, Antarctica. All abundances are based upon sequences identified at the Order level.

'Others' included *taxa* recorded at ≤ 1%: Acidimicrobidae, Bacteroidales, Clostridiales, Cytophagales, Flavobacteriales, Fusobacteriales, Gemmatimonadales, Gp4, Gp6, Gp16, Granulicella, Nitrososphaeraceae, Sneathiellales, Sphingomonadales, *Terriglobus* and Verrucomicrobiales.

Table 4.2 List of the 48 members of the 'core' community (*taxa* present in ≥75 samples) with 97% identity threshold.

	Taxonomic assignment	
OTU id	**Phylum (confidence >1)**	**Identification (confidence >0.97)**
OTU1	Cyanobacteria	-
OTU4	Actinobacteria	Suborder Corynebacterineae
OTU7	Bacteroidetes	Family Sphingobacteriaceae
OTU8	Proteobacteria	Genus *Acidisoma*
OTU10	Proteobacteria	Order Rhizobiales
OTU11	Armatimonadetes	-
OTU12	Proteobacteria	Family Acetobacteraceae
OTU16	Proteobacteria	Family Acetobacteraceae
OTU17	Acidobacteria	Genus *Granulicella*
OTU18	Actinobacteria	Genus *Frankineae*
OTU20	Acidobacteria	Genus *Granulicella*
OTU21	Proteobacteria	Family Acetobacteraceae
OTU23	Actinobacteria	Order Actinomycetales
OTU24	Proteobacteria	Family Acetobacteraceae
OTU25	Proteobacteria	Family Acetobacteraceae
OTU27	Unclassified	-
OTU31	Proteobacteria	Family Acetobacteraceae
OTU32	Unclassified	-
OTU36	Actinobacteria	Suborder Corynebacterineae
OTU37	Armatimonadetes	-
OTU39	Actinobacteria	Family Conexibacteraceae
OTU40	Actinobacteria	Suborder Micrococcineae
OTU43	Acidobacteria	Subgroup Acidobacteria Gp1
OTU49	Bacteroidetes	Genus *Mucilaginibacter*
OTU58	Planctomycetes	Family Planctomycetaceae
OTU70	Bacteroidetes	-
OTU80	Proteobacteria	Family Acetobacteraceae
OTU81	Actinobacteria	Phylum Actinobacteria
OTU87	Armatimonades	-
OTU98	Actinobacteria	Order Actinomycetales
OTU115	Actinobacteria	Suborder Pseudonocardineae
OTU129	Unclassified	-
OTU150	Actinobacteria	Family Micromonosporineae
OTU164	Actinobacteria	Family Conexibacteraceae

OTU 170	Planctomycetes	Family Planctomycetaceae
OTU191	Proteobacteria	Order Rhizobiales
OTU195	Actinobacteria	Suborder Micrococcineae
OTU217	Actinobacteria	Phylum Actinobacteria
OTU233	Actinobacteria	Family Conexibacteraceae
OTU245	Unclassified	-
OTU249	Proteobacteria	Family Acetobacteraceae
OTU281	Unclassified	-
OTU322	Actinobacteria	Order Actinomycetales
OTU334	Unclassified	-
OTU412	Actinobacteria	Genus *Frankineae*
OTU587	Actinobacteria	Order Actinomycetales
OTU614	Acidobacteria	Subgroup Acidobacteria Gp1
OTU621	Proteobacteria	Family Comamonadaceae

Distribution of 'core' *taxa* was examined and graphically represented to identify association between *taxa* and locations. All the 48 most informative *taxa* were present in all sites, with exception for the phylotypes belonging to the Suborder Corynebacterineae (OTU 4) and the Order Actinomycetales (OTU 98), the former totally absent at Trio Nunatak site 2 and Ricker Hills and the latter at Stewart Heights and Trio Nunatak site 2.

Species belonging to the Conexibacteraceae, Actinomycetales, Comamonadaceae, Rhizobiales, Acetobacteraceae, Micromonosporineae, Frankineae and Micrococcineae had an abundance distribution mostly uniform across all sites.

Acetobacteraceae (i.e. OTUs 16-25-249) were particularly present in sites at low altitude (Trio Nunatak, 1000 m a.s.l.; Battleship Promontory, 1000 m a.s.l.; Ricker Hills, 1115 m a.s.l. and Bobby Rocks, 1680 m a.s.l.), even if recorded at higher altitude.

Visited sites were also hierarchically clustered by OTUs abundance to identify patterns of similarities in community composition but did not exhibit a huge clustering of locations by geography (Fig.4.3).

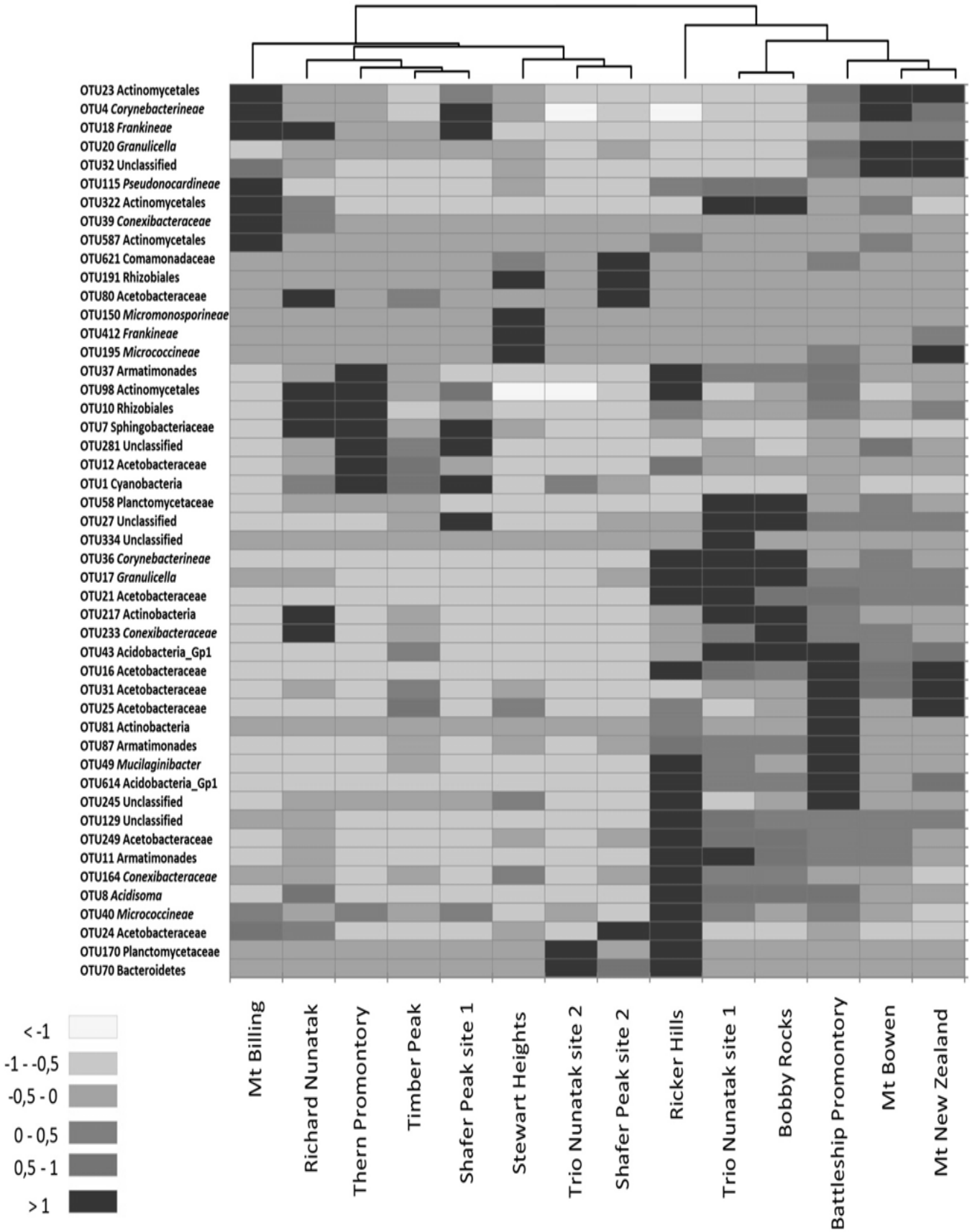


Figure 4.3 Heat map of the 'core' *taxa* relative abundance and UPGMA hierarchical clustering of samples. Values are scaled (log transformed) by OTUs relative abundances across all sites. Abundances are indicated by the color

intensity: black-dark grey colors indicate high relative abundance; light grey-white indicate low relative abundance or absence. Both the 'core' OTUs and sites were clustered using a Bray-Curtis index.

4.3.3 Biodiversity analysis

To describe bacterial diversity, we calculated species richness (S), Shannon (H'), Simpson (1-D), and Pielou (J') indices for each locality, as reported in Table 4.1. Bacterial species richness values ranged from 50 OTUs (Shafer Peak site 1) to 287 OTUs (Battleship Promontory) with an average of 130 OTUs. H' values varied among all sites, ranging from 1.1 detected at Shafer Peak site 1 to 4.24 at Stewart Heights, where 218 OTUs were retained. The lowest 1-D values were recorded at Shafer Peak site 1 (0.61) and Thern Promontory (0.65), the highest values at Stewart Heights (0.98). Shafer Peak site 2 exhibited also the highest value for J' (0.70) and Shafer Peak site 1 the lowest value (0.28).

4.3.4 Statistical analysis

Spearman's correlation ranks analysis indicated that all diversity indices were not correlated with the altitude. All rank analyses were not statistically significant with P values > 0.01 (Fig. 4.4). Same result was obtained when sea distance was considered (data not shown).

The distance matrix computed, based on community composition, showed highly variable values. The highest similarity on bacterial composition was between Trio Nunatak site 1 (altitude: 1000 m a.s.l. and sea distance: 82 km) and Bobby Rocks (altitude: 1680 m a.s.l. and sea distance: 91 km) samples, with 78.5% of similarity; both sites are located at high sea distance and low altitude (Table 3.1). Instead, the lowest similarity was recorded between Trio Nunatak (site 1) and Stewart Heights (altitude: 2670 m a.s.l. and sea distance: 74 km) sites, with 15,2% of similarity. Interestingly, the two visited sites at Trio Nunatak showed only 18% of similarity; similar trend was obtained for sample sites at Shafer Peak (25.4% of similarity).

Multiple regression coefficients on distance matrices are not statistically significant ($p>0.1$), considering both environmental variables (data not shown), highlighting that these geographical parameters did not drive bacterial composition and diversity.

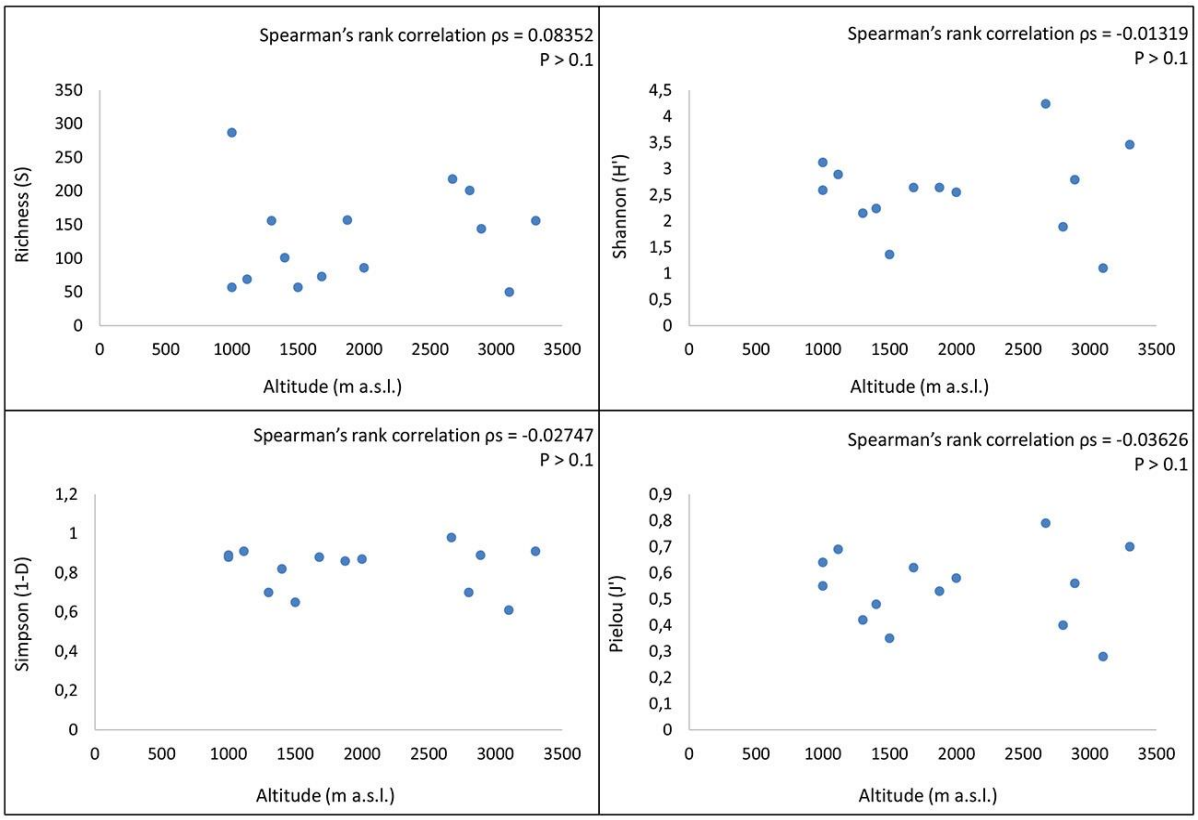


Figure 4.4 Spearman's correlation coefficients between the bacterial diversity indices (S, H', 1-D and J') and the altitudinal gradient.

4.3.5 Pareto Lorenz curves

To evaluate the interspecies frequencies ratios of bacterial component, Lorenz distribution curves were plotted based on the number of OTUs and their frequencies. Fig. 4.5 showed F_o values ranging from 77 to 99.8%, with average at 89%. These data indicated that very few specialized *taxa* were numerically dominant while most of the species were rare (Fig. 4.5).

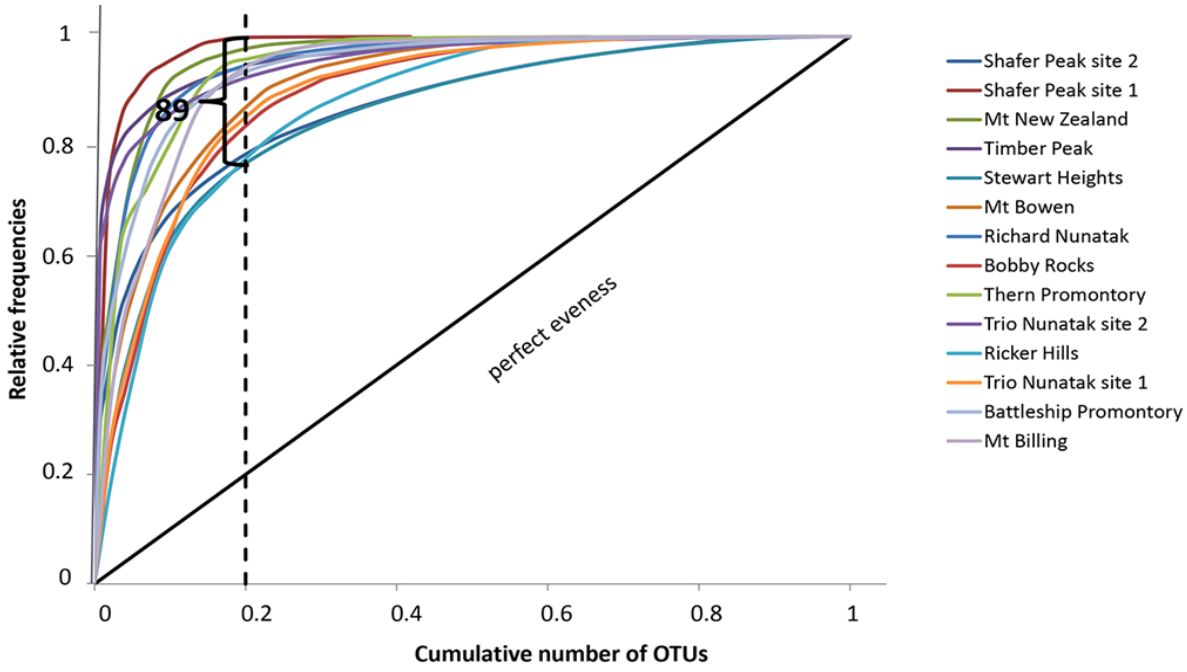


Figure 4.5 Pareto-Lorenz distribution curves based on the number of OTUs and their frequencies. The dashed vertical line at the 0.2 x-axis level is plotted to evaluate the range of the Pareto values.

4.4 Discussion

Despite the increasing interest in investigating the microbial components in the extremely-adapted endolithic microbial communities in Antarctica, our knowledge on microbial distribution and composition is still scant (Yung *et al*., 2014; Archer *et al.*, 2016).

To providing new insights into microbial diversity, we surveyed the bacterial diversity of selected cryptoendolithic lichen-dominated communities (detailed in chapter 3) in Victoria Land by using a meta-barcoding approach targeting the V4 of 16S rDNA region. Reads were retrieved from all samples, including those collected at highest elevations, such as Mt New Zealand (2888 m a.s.l.) and Shafer Peak site 1 (3100 m a.s.l.) in Northern Victoria Land, confirming data reported on the fungal component in chapter 3. Over 550 OTUs were overall retained, suggesting that the bacterial component may play an important role in the endolithic communities. Antarctic endolithic communities harbor a bacterial species richness much lower than temperate soil biotopes, which typically have values of richness between 6 and 7 (Dunbar *et al*., 2000), and relatively low microbial species diversity (Archer *et al*., 2016; Selbmann *et al*., 2017).

A major contribution was from Proteobacteria and Actinobacteria, according with molecular-based studies of Antarctic microbial habitats including soil biotopes (Saul *et al*., 2005; Aislabie *et al*., 2006), cryoconite holes (Christner *et al*., 2003) and cryptoendolithic communities (de la Torre *et al*., 2003). Also

dry mineral soils dominated by members of Actinobacteria have been reported from Signy Island (maritime Antarctic), Alexander Island (maritime/continental transition zone) and the McMurdo Dry Valleys (continental Antarctic) (Smith *et al*., 2006; Pointing *et al*., 2009; Chong *et al*., 2010, 2012), confirming the culture-dependent studies in the McMurdo Dry Valleys (Cameron *et al.*, 1972). The high frequency of actinobacterial phylotypes suggests that this group of Gram-positive heterotrophic bacteria plays an important role in rock communities.

On the contrary, bacteria belonging to Acidobacteria, Verrucomicrobia, and Bacteroidetes, reported in classic culture-dependent studies, have been rarely obtained (Smith *et al*., 2006). Likewise, Cyanobacteria have been here detected in few sampled sites and with low relative abundance. In Victoria Land, Cyanobacteria were often isolated in aquatic environments showing a great diversity (Vincent, 1988; Cavacini and Fumanti, 2005), but only few *taxa* with a high resistance to extreme conditions were retained from endolithic communities in the Dry Valleys (Southern Victoria Land) (Friedmann and Ocampo, 1976; Friedmann *et al*., 1988). Adaptation to extreme environmental factors is advantageous to organisms living in the desiccating environment both of hot and cold deserts; for example, the cyanobacterial Genus *Chroococcidiopsis*, isolated from Antarctic endolithic communities, showed an extreme resistance to desiccation, ionizing and UVC radiation and even to simulated space and Martian conditions (i.e. the accumulation of carotenoids seems to contribute to UVC resistance by providing protection against oxidative stress) (Billi *et al*., 2000, 2011; Baqué *et al.*, 2013), confirming its relevance in understanding the limits of life and potential habitability of the solar system and beyond (Baqué *et al*., 2013).

Several *Deinococcus*-like organisms were largely obtained both from sequencing and culture-dependent approaches from endolithic communities (de la Torre *et al*., 2003; Hirsch *et al*., 1988; Siebert and Hirsch, 1988) as well as from lichen thallus of *Umbilicaria decussata* collected in Kay Island, Antarctica (Selbmann *et al*., 2010), while were only occasionally detected in our study. They grow easily in laboratory conditions and can be isolated despite their low frequency. This group is known for its ability to withstand large amounts of both UV and ionizing radiation limiting damage to their DNA (Mattimore and Battista, 1996). Due to the high solar irradiation at the South Pole, it is not surprising the occurrence of these resistant *taxa* in our samples.

The present study revealed also the occurrence and abundance of several still undescribed organisms (about 15%), some of which are part of the 'core' members; being particularly frequent and abundant, they are surmised to play important roles in Antarctic cryptoendolithic communities.

Bacterial diversity varied among the considered 14 sites such as communities composition did. This study highlighted the presence of a relatively high level of diversity regardless altitudinal, sea distance or even latitudinal gradients (Chong *et al*., 2012). The absence of any correlation between biodiversity indexes

and the environmental parameters here considered clearly indicates that altitude and sea distance do not influence the establishment and development of the endolithic communities, despite the expected increase of environmental pressure. Richness was, in fact, highest at Battleship Promontory (287), at about 1000 m a.s.l. and 33 km sea distance, and Stewart Heights (218), at about 2670 m a.s.l. and 74 km sea distance.

The Pareto Lorentz curves clearly indicate a high degree of specialization of these communities; *Fo* average was 89%, indicating the dominance of a very small number of highly specialized species, while all the others occur at very low frequency. This high degree of functional organization renders these communities very resistant and very well adapted, but prone to external perturbations: recovering remains very difficult if not impossible (Marzorati *et al*., 2008). The same specialization was recently found in the fungal compartment of these communities too (Selbmann *et al*., 2017).

It is highly probable that different environmental parameters may play a crucial role (e.g. water availability, rock temperature and sun exposition) and must be taken into account in future studies. A monitoring of these parameters is actually ongoing in selected localities; coming data are expected to be of help for understanding the relationship between biodiversity variation and environmental parameters, in order to be predictive on the effects of climate change on Antarctic endolithic communities (Nienow *et al*., 1988; Friedmann *et al*., 1987; Van Horn *et al.*, 2014). Moreover, these data may be of help in defining the boundaries of habitability in extraterrestrial analogues on Earth and supply valuable information for searching life beyond Earth (Cockell *et al.* 2016).

CHAPTER 5.

BLACK FUNGI WHOLE-GENOME SEQUENCING

5.1 Introduction

In 1996 the genome of *Saccharomyces cerevisiae* was published and marked the beginning of a new era in fungal biology (Goffeau *et al*., 1966). The advancement in high throughput sequencing technology have been rapidly progressing and leading to sequencing of species that can be incorporated into genome scale phylogenies, as evidenced by MycoCosm, with more than 800 fungal genomes (http://genome.jgi.doe.gov/fungi/).

In the last decades, the sequencing of fungal genomes has become routine and straightforward enabling these data as the starting point for an increasing number and types of researches (Spatafora *et al*., 2017). Studies on the evolution of biological diversity seek to understand the amplitude of variation that can be expected among closely or related genomes. Sequencing of large numbers of fungal genomes, in particular of extremophilic fungi, will allow to understand the diversity of genes encoding enzymes, and pathways that produce several novel compounds (Mohanta *et al*., 2015; Teixeira *et al*., 2017). With the rapid accumulation of sequenced fungal genomes, the observed diversity of genes encoding organic acids, antibiotics, enzymes, pigments and their pathways-as melanin, known to play an important role in fungal protection against different types of stress preserving cells, for instance from both UV and desiccation damages (Butler and Day, 1998; Gorbushina, 2003; Gorbushina *et al*., 2003), has increased exponentially.

Fungi are a large group of eukaryotic organisms ranging from yeast to mushrooms. They have a worldwide distribution due to their small size and their cryptic lifestyle in soil, and decomposing matter, as symbionts with algae, bryophyte, plants and animals (Das *et al*., 2007; Anderson *et al*., 2003; Spatafora *et al*., 2017; Richards *et al*., 2017). These organisms are found in every biome including polar, temperate and tropical environments (Lau *et al*., 2009; Ma *et al*., 1999). Within the fungal Kingdom, black yeasts are a polyphyletic morpho-ecological group within the ascomycetes that is characterized by melanized cells and eventual transient yeast-like growth (Sterflinger *et al*., 2006; Blasi *et al*., 2015). All black yeasts share a number of characters, such as strong melanization, thick and even multi-layered cell walls and exo-polysaccharide production, resulting in an extraordinary ability to tolerate chemical and physical stresses such as extreme pH, high and low temperature, desiccation, UV ionizing radiation and even alpha particles (Dadachova and Casedevall, 2008; Onofri *et al*., 2008, 2012, 2015; Selbmann *et al*., 2011, 2017; Pacelli *et al*., 2017a,b,c). Indeed, they are known for their ability to survive in extreme habitats, which

range from hot and cold deserts, rock surfaces and glaciers, and even real and simulated space and Mars conditions (Adams *et al*., 2006; Abdel-Hafez *et al*., 1994; Friedmann, 1982; Friedmann *et al*., 1987; Gunde-Cimerman *et al*., 2003; Sterflinger *et al.,* 2012; Selbmann *et al*., 2015a). Some black yeasts usually colonize human environments like dishwashers and steam bath or sauna facilities, some have been isolated from a silicone seal in a hospital and in tap water (Matos *et al*., 2002; Blasi *et al*., 2015; Listemann *et al*., 1996; Gümral *et al*., 2016; Seyedmousavi *et al.*, 2014). Some of them are also involved in a broad range of diseases (Vicente *et al*., 2008), while others, for their ability to degrade pollutants, are good candidates for bioremediation (Prenafeta-Boldù *et al*., 2012; Badali *et al*., 2011).

The diverse ecological dominance and importance of black yeasts make them important from an evolutionary point of view. Phylogenetic, ecological and molecular studies contributed to understand their evolutionary history and the processes that enable their survival in extreme environments. Black fungi isolated from the McMurdo Dry Valleys in the Antarctic continent, the most similar to Mars environment on Earth (Selbmann *et al*., 2005), have been tested in astrobiological experiments (Gorbushina, 2003; Onofri *et al*., 2007; Pacelli *et al*., 2017a). In this respect, molecular, phylogenetic, and physiological studies have been undertaken, in order to explain the origin, evolution and adaptation of these microorganisms in the most extreme terrestrial ice-free environment on Earth (Onofri *et al*., 2007).

Systematic integration of physiological properties, ecological context with comparative genomic and genome-derived phylogenies will give clues to better understand the distribution of black fungi and fungal metabolic traits across ecosystems and ecological niches. To support these comparative genomics studies, we started genome sequencing of multiple species of black fungi. Here, the assembly and annotation of genomes of seven Antarctic black yeast fungal species isolated from Antarctic endolithic communities are reported: *Rachicladosporium antarcticum*, *Rachicladosporium* sp., *Hortaea thailandica*, *Friedmanniomyces endolithicus*, *Friedmanniomyces simplex* and *Cryomyces minteri* belong to the Class Dothideomycetes, while *Exophiala mesophila* to the Class Chaetothyriomycetes (Fig. 5.1, Table 5.1).

Dothideomycetes constitute the largest Class of ascomycetes with approximately 19000 species, 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*., 2009). Early studies have shown that a large part of the non-lichenized, slow-growing melanized fungi isolated from rock surfaces 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, 2009; Selbmann *et al.,* 2005, 2008).

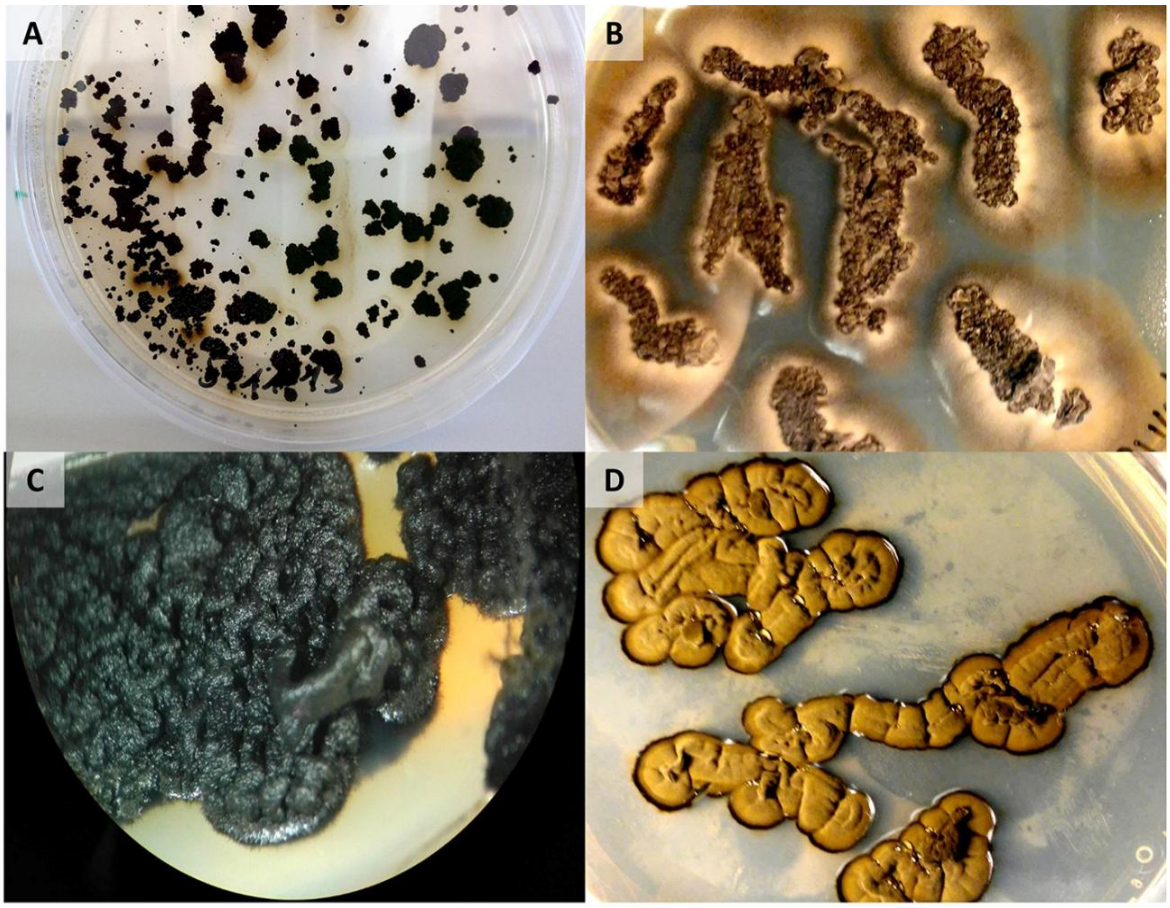


Figure 5.1 A) *Cryomyces minteri*; **B)** *Exophiala mesophila*; **C)** *Hortaea thailandica*; **D)** *Rachicladosporium antarcticum.*

Table 5.1 Metadata of genomes

Strains	CCFEE N°	Site collection	Coordinates	Date collection
Rachicladosporium. antarcticum	5527	Antarctic Peninsula	64.35S 61.583W	Jan 2004
Rachicladosporium sp.	5018	Battleship Promontory	76.9107S 160.9278E	Dec 1996
E. mesophila	6314	Mt Billing	75.6442S 159.0283E	Dec 2010
H. thailandica	6315	Ricker Hills	75.7033S 160.9077E	Dec 2010
F. endolithicus	5311	Ford Peak	75.6906S 160.4403E	Dec 1996
F. simplex	5184	Battleship Promontory	76.9107S 160.9278E	Dec 1996
C. minteri	5187	Battleship Promontory	76.9107S 160.9278E	Dec 1996

5.2 Materials and Methods

Isolates of the black fungi *Rachicladosporium antarcticum* CCFEE 5527, *Rachicladosporium* sp. CCFEE 5018, *Exophiala mesophila* CCFFE 6314, *Hortaea thailandica* CCFEE 6315, *Friedmanniomyces endolithicus* CCFEE 5534, *Friedmanniomyces simplex* CCFEE 5184, and *Cryomyces minteri* CCFEE 5187 were kindly supplied by the Culture Collection of Fungi from Extreme Environments (Viterbo, Italy) (Selbmann *et al*., 2015b). These strains were previously isolated from Antarctic rock samples.

Metadata of sequenced genomes are reported in Table 6.1.

Isolation procedure has been performed following Selbmann *et al.*, 2005. After approximately 3-4 weeks of growth, DNA was extracted from the cultures using a cetyltrimethylammonium bromide (CTAB)-based protocol (Fulton *et al*., 1995). Additional phenol-chloroform purification steps were performed to eliminate melanin that is highly abundant in most black fungi. DNA concentration was estimated with the QuBit dsDNA HS Assay Kit (Life Technologies, USA). The total genomic DNA was sheared with a Covaris S220 ultrasonicator. Sequencing libraries were constructed using NeoPrep TruSeq Nano DNA sample prep (Illumina, Inc., San Diego, CA). Libraries were normalized, pooled, and sequenced on an Illumina MiSeq 2x300 paired-end reads format where the target reads per library was 1-2 Million. DNA concentration, shearing, and library preparation were performed in the Genomics Core facility in the Institute for Integrative Genome Biology at the University of California, Riverside. Genome assembly was performed with MaSuRCA version 2.3.2 (Zimin *et al*., 2013). Assemblies were also performed with SPAdes. The assemblies for each strain with the best statistics based on N50 and longest scaffolds as estimated by QUAST (Gurevich *et al*., 2013) were selected. The selected assembly was further filtered for vector sequence within Sequin (https://www.ncbi.nlm.nih.gov/Sequin/). Redundant contigs which aligned by MUMMer (Kurtz *et al*., 2004) at 95% across their entire length were removed as part of the funannote clean step (http://github.com/nextgenufs/funannotate). The protein coding and tRNA genes were annotated in each genome with funannotate utilizing Augustus (Stanke *et al*., 2013). GeneMark.hmm-ES (Ter-Hovhannisyan *et al*., 2008), followed by a consensus gene calling with EVM (Haas *et al*., 2008). The genome annotations were prepared for GenBank with Genome Annotation Generator (Hall *et al*., 2014) and finalized with Sequin and tbl2asn. Gene function predictions were assigned by matches to the Pfam (Finn *et al*., 2014). MEROPS (Rawling *et al*., 2014), CAZy (Lombard *et al*., 2013). InterProScan (Jones *et al*., 2014), and Swiss-Prot databases (Boutet *et al*., 2016). Product descriptions were transferred from homologs with 60% similar alignments across 60% of the protein length. Sequences genomes, assembly and annotations were deposited at DDBJ/ENA/GenBank under BioProject ID PRJNA342238.

5.3 *Rachicladosporium* sp. CCFEE 5018 and *Rachicladosporium antarcticum* CCFEE 5527

Abstract

The draft genome sequences of *Rachicladosporium antarcticum* CCFEE 5527 and *Rachicladosporium* sp. CCFEE 5018 are the first sequenced genomes from this Genus, which comprises rock-inhabiting fungi. These endolithic strains were isolated from inside rocks collected from the Antarctic Peninsula and Battleship Promontory (McMurdo Dry Valleys), Antarctica, respectively.

Coleine. C., Masonjones. S., Selbmann. L., Zucconi. L., Onofri. S., Pacelli. C., and Stajich. J.E. (2017). Draft Genome Sequences of the Antarctic Endolithic Fungi *Rachicladosporium antarcticum* CCFEE 5527 and *Rachicladosporium* sp. CCFEE 5018, *Genome announcements* 5(27), e00397-17.

The past decade has revealed an unexpected fungal diversity associated with rocks, which serves as a primary substrate colonized by microorganisms in extreme dry and cold or hot environments. Under these harsh conditions active growth is rare on exposed surfaces, and endolithism is a necessary ecological adaptation for survival (Zucconi *et al*., 2016). Black meristematic fungi are a morpho-ecological group of ascomycetes with a peculiar tendency to the extremes and are characterized by melanin pigmentation. They are typical and abundant members of Antarctic cryptoendolithic communities (Egidi *et al*., 2014). These fungi are equally named black yeasts, microcolonial fungi or rock inhabitant fungi when found growing within rocks (Sterflinger *et al*., 1998; Selbmann *et al*., 2004, 2005; Ruibal *et al*., 2009; Selbmann *et al*., 2014a). We produced draft genome sequences of the Antarctic fungi *Rachicladosporium antarcticum* CCFEE 5527 Onofri & Egidi (Egidi *et al*., 2014) and *Rachicladosporium* sp. strain CCFEE 5018 to provide genome resources to study fungal adaptation to extreme environments and endolithic lifestyles. These genomic resources may give clues for studying the evolution of extremophiles and stress adaptation in these enigmatic fungi. Species designation of *Rachicladosporium* sp. strain CCFEE 5018 is still being determined, and the internal transcribed spacer sequence is 98.6% identical to *Rachicladosporium monterosium* strain CBS 137178 Isola & Zucconi (Egidi *et al*., 2014).

The *R. antarcticum* genome was sequenced to a depth of 41x and *Rachicladosporium* sp. CCFEE 5018 to 175x. The deeper coverage was in an attempt improve assembly quality. The *R. antarcticum* genome assembly was 47.4 Mb (number of contigs, 267; *N*50, 896 kb; *L*50, 20). The initial *Rachicladosporium* sp. CCFEE 5018 assembly was fragmented (2099 contigs) but was scaffolded by synteny to *R. antarcticum* with Satsuma2 (Grabherr *et al*., 2010) and Mercator (Dewey *et al*., 2006) into 233 scaffolds (*N*50, 1.35 Mb; *L*50, 12). A total of 18781 protein-coding genes were predicted in *R. antarcticum* and 18892 in *Rachicladosporium* sp. CCFEE 5018.

Accession numbers. These whole-genome shotgun projects has been deposited at DDBJ/ENA/GenBank under the accession numbers NAJO00000000 and NAEU00000000.

5.4 *Exophiala mesophila* CCFEE 6314

Within the black yeasts, the Genus *Exophiala* is an evolutionary hot spot, with a high diversification and emerging adaptation toward animals and human environments. Indeed, the Genus *Exophiala* contains a number of species recognized as pathogens of vertebrates (Porteous *et al*., 2003), is often isolated from oligotrophic water sources, such as sinks, drainpipes, swimming pools, bathing facilities and drinking water (Matos *et al*., 2002; Nishimura *et al*., 1987; Göttlich *et al*., 2002).

Also, recently, *Exophiala* species have begun to be considered for its bioremediation potential, having been isolated from different polluted sources such as industrial spills, car gasoline tanks, railway sleepers and air biofilters (Isola *et al*., 2013; Zhang *et al*., 2012; Estévez *et al*., 2005; Blasi *et al*., 2015; Seyedmousavi *et al*., 2011). Interestingly, despite species of this Genus shows high prevalence in the human environment, it has also been isolated from glaciers (Branda *et al*., 2010) as well as in the Arctic and Antarctic environments (Vaz *et al*., 2011).

In this thesis, an Antarctic strain of *E. mesophila* has been sequenced; its genome sequences were assembled and annotated, and they will be compared with those of other *Exophiala* species (e.g. *E. aquamarina*, involved in clod-blooded animal infection and closest species) as well as with its related *E. mesophila* CBS 40295 (Fig. 6.3).

The *E. mesophila* genome assembly was 30.43 Mb (number of contigs, 207; *N*50, 522 kb; *L*50, 20).

A total of 10355 protein-encoding genes were predicted.

Accession number. These whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number NAJM00000000.

5.5 *Hortaea thailandica* CCFEE 6315

Genus *Hortaea* includes three described species: i) *H. acidophila*, an acid-tolerant black yeast able to grow under extremely acidic conditions (Hölker *et al*., 2004) at a pH as low as 0.6, and to produce functional laccases that are involved in melanin synthesis (Tetsch *et al*., 2005, 2006); ii) *H. werneckii* is an halophilic species, worldwide isolated from natural hypersaline environments and recognized as model organism in eukaryotes in exploring conditions of extremotolerance (e.g. oxidative stress and osmotic adaptation) (Vaupotič and Plemenita, 2007; Gunde-Cimerman *et al*., 2000; Sinha *et al*., 2017); it is a rare examples of black yeasts in the Class Dothideomycetes reported as human opportunist, causing a disease in immunocompetent individuals known as *Tinea nigra* (Bonifaz *et al*., 2008); iii) *H. thailandica*, a rare halotolerant species firstly described from plant material in Thailand (Crous *et al*., 2009), but frequently isolated from stone in monumental sites and artistic tiles (Isola *et al*., 2016; Coutinho *et al*., 2012).

Genome sequences are already available for *H. acidophila* and *H. werneckii* on GenBank database.

We sequenced a strain of *H. thailandica* isolated from Antarctic sandstone. Genome is being compared with its close relative *H. werneckii*, focusing on evolution following whole genome duplication (*H. werneckii* genome assembly: ~50 MB and contains 15974 genes, 7698 of which, corresponding to 90%, are duplicates).

The *H. thailandica* genome assembly was 23.88 Mb (number of contigs, 163; *N*50, 331 kb; *L*50, 23).

A total of 8778 protein coding genes were predicted.

Accession number. These whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number NAJL00000000.

5.6 *Friedmanniomyces endolithicus* CCFEE 5534 and *F. simplex* CCFEE 5184

The Genus *Friedmanniomyces* (Dothideomycetes) contains two species of rock-inhabiting meristematic black fungi, *F. simplex* and *F. endolithicus* (Onofri *et al*., 1999; Selbmann *et al*., 2005). *F. endolithicus* is strictly endemic to Victoria Land and is one of the most-frequently isolated non-lichenized fungus from rock (Selbmann *et al.*, 2005; Ruisi *et al*., 2007; Selbmann *et al*., 2015); the large geographic distribution of this species in Victoria Land was confirmed by its presence as a 'core' member of cryptoendolitic lichen communities and as one of the most retrieved *taxon* from culture-independent survey (see chapter 3). *F. simplex* also occurs in endolithic communities of Victoria Land; it is represented by a single isolate to date, strain CCFEE 5184.

Friedmanniomyces spp. are black yeasts that have been well studied for their phylogenetic position, taxonomic classification, and physiological characteristics (Selbmann *et al*., 2005; Egidi *et al*., 2014), and possess stress-tolerant adaptations which allow them to inhabit inert surfaces and survive under long drought conditions (Onofri *et al.*, 2004).

F. endolithicus genome assembly was 46.75 Mb (number of contigs, 427; *N*50, 383 kb; *L*50, 39) and *F. simplex* genome assembly was 37.75 Mb (number of contigs, 2988; *N*50, 30 kb; *L*50, 345).

A total of 18027 protein coding genes were predicted in *F. endolithicus* and 13766 in *F. simplex*.

Accession numbers. These whole-genome shotgun projects have been deposited at DDBJ/ENA/GenBank under the accession numbers NAJP00000000 and NAJQ00000000.

5.7 *Cryomyces minteri* CCFEE 5187

The Genus *Cryomyces*, belonging to the Class Dothideomycetes, is still considered as *incertae sedis*. as no close relationship is still identified for this enigmatic Antarctic Genus (Selbmann *et al.*, 2005). This Genus encompasses four species of cold-adapted rock-inhabiting black yeasts: *C. montanus* and *C. funiculosus* have been isolated from Alpine rocks collected above 3000 m a.s.l. (Selbmann *et al*., 2014b), while the two Antarctic endemic species, *C. antarcticus* and *C. minteri* have been isolated primarily from the McMurdo Dry Valleys in Southern Victoria Land (Selbmann *et al.*, 2005), and more rarely in Northern Victoria Land (unpublished data). Recently, meta-barconding analysis confirms this Genus as one of the most recorded in Victoria Land, suggesting an even wider distribution. Astrobiological experiments have been performed using *C. minteri* and *C. antarcticus* as well. *C. antarcticus* in particular represents one of the best models to study the possibility of extraterrestrial life; it has been exposed for 18 months on the International Space Station to real space conditions (Onofri *et al*., 2008; 2012) and to simulated space and Martian conditions (Pacelli *et al*., 2017a).

Genome sequences of *C. antarcticus* have been obtained from Sterflinger *et al*., (2014) reporting a genome size of 24 Mb.

The *C. minteri* genome assembly was 44.1 Mb (number of contigs, 4506; *N*50, 17; *L*50, 806).

A total of 13960 protein coding genes were predicted.

Accession number. These whole-genome shotgun projects have been deposited at DDBJ/ENA/GenBank under the accession number NAJN00000000.

CHAPTER 6. SYNTHESIS AND CONCLUSIONS

Endolithic growth represent one of the most spectacular extreme-adaptation and allow microbial communities to withstand environmental stressors such as extreme aridity, thermal fluctuations and high UV irradiation (Friedmann and Ocampo-Friedmann, 1984; Nienow, 2009; Wierzchos *et al*., 2012).

This thesis aimed to contribute on the knowledge, still limited to date, of the biodiversity of microbial components of Antarctic lichen-dominated endolithic communities and to elucidate how these peculiar ecosystems respond to increasing environmental pressure.

In chapter 1, we introduced the endolithism that made possible the microbial life in extreme and highly vulnerable terrestrial environments, where endolithic niches are considered to be environmental refuges, even the only visible life-form (Golubic *et al*., 1981; Friedmann, 1982; Walker and Pace, 2007; Pointing and Belnap, 2012; Wierzchos *et al*., 2012). Indeed, the rock substratum provides protection from incident UV, excessive solar radiation, and freeze-thaw events while providing physical stability, and enhanced moisture availability (Walker and Pace, 2007; Chan *et al*., 2012) both in hot and cold deserts (Cowan and Ah Tow, 2004).

In chapter 2, we utilized a DGGE fingerprinting approach to analyze the fungal assemblage on a wide sampling (46 localities) of different typologies of rocks (e.g. sandstone, granite, dolerite and quartz) collected during the XXVI Italian Antarctic Campaign (2010-11) in Victoria Land, along a latitudinal transect ranging from 73°24’46’’S to 76°54’36’’S, and an altitudinal and sea distance gradients (sea level to 3600 m a.s.l. and 0 to ~100 km, respectively). The study indicated that rock porosity has a protective role for endolithic growth (Zucconi *et al*., 2016) and a high degree of specialization of the fungal communities, thus reflecting a specialized adaptations but low resilience, suggesting that these vulnerable ecosystems may be tragically affected by any external variations (Selbmann *et al*., 2017).

Based on these findings, in chapters 3 and 4, the research was deepened on a selection of sandstone samples, the best substratum for cryptoendolithic colonization as confirmed in chapter 2, collected from 14 localities along an altitudinal transect ranging from 1000 m a.s.l. to 3300 m a.s.l. and from 29 km to 96 km sea distance. A more exhaustive molecular approach was performed focusing on fungal (chapter 3) and bacterial compartment (chapter 4), using ITS and 16S meta-barcoding, respectively. Both in fungal and bacterial assemblages, sequences were obtained from each site, even increasing the environmental pressure (i.e. Mt New Zealand, 3100 m a.s.l. and Shafer Peak, 3300 m a.s.l.) and in those samples were colonization was not visible.

In chapter 3, we described the fungal communities, composed mostly by OTUs identified as Ascomycota, already reported as dominant in soil Antarctic communities (Cox *et al*., 2016; Ji *et al*., 2016; Wei *et al.*, 2016), supporting that in the Antarctic mycobiome, ascomycetes are predominant. Lichen-forming fungi

in the Lecanoromycetes Class and *taxa* belonging to the Class of Dothideomycetes, were the most representative phylotypes among the Ascomycota along the sampled area. The widespread frequency of Lecanoromycetes confirmed that the lichen-forming fungi are dominant in cryptoendolithic communities as previously reported (Friedmann *et al*., 1988; Cockell *et al*., 2003; Zucconi *et al*., 2016; Archer *et al*., 2016). Dothideomycetes encompasses meristematic black fungi with particular stress-tolerance and representing optimal eukaryotic models for astrobiological studies (Onofri *et al*., 2012, 2015; Pacelli *et al*., 2017a). The high frequency of this group is consistent with the traditional cultivation tests (Selbmann *et al*., 2005, 2008; Egidi *et al*., 2014).

In chapter 4, we provided new insights into the bacterial assemblage, dominated mostly by Proteobacteria and Actinobacteria phyla, according with previous reports from Antarctic soils (Saul *et al*., 2005; Aislabie *et al*., 2006) and cryptoendolithic communities (de la Torre *et al*., 2003). In particular, Actinobacteria were already reported as predominant members in soils communities in continental Antarctic, but even in other regions as maritime Antarctic (Smith *et al*., 2006; Chong *et al*., 2010, 2012; Pointing *et al*., 2009), suggesting that this groups play a crucial role in Antarctic microbiome.

Results showed also the occurrence of several 'core' *taxa*, present in almost all localities, highlighting that they may play a crucial in Antarctic cryptoendolithic communities; also unidentified OTUs were retained from this study, suggesting that Antarctic endolithic communities harbor a pool of novel *taxa*. It was observed a variation in diversity and composition along all localities, even in samples collected at similar altitude and sea distance; besides, these parameters were not related in this study with microbial diversity and composition, while additional parameters (e.g. water availability, rock temperature and sun exposition) could be crucial and must be considered in future studies. We have also demonstrated a high degree of specialization of these communities, dominated by very few highly specialized species while all the others occur at very low frequency. On the other hands, these communities are prone to any external perturbations (Selbmann *et al*., 2017).

In chapter 5 we listed the whole-genome sequences we have obtained from seven endolithic black yeast, known for their ability to survive in extreme habitats such as hot and cold deserts, rock surfaces, glaciers, and even real and simulated space and Mars conditions (Adams *et al*., 2006; Abdel-Hafez *et al*., 1994; Friedmann, 1982; Friedmann *et al*., 1987; Gunde-Cimerman *et al*., 2003; Sterflinger *et al*., 2012; Selbmann *et al*., 2015). The genome sequences were assembled and functionally annotated to further integrate physiological, ecological and phylogenetics knowledge with the genomics and functional data. In addition, the genome sequences will be also compared with genomes sequenced from same or related species, isolated from other extreme environments to find correspondence with potential characteristics of adaptation to cold environments.

The main findings of this work were: i) sandstone resulted the best substratum for microbial colonization; ii) knowledge of fungal and bacterial diversity has improved considerable and was shown an high diversity variation even within the sampled locality, suggesting that other studies are necessary to estimate the minimal sampling amplitude for a proper biodiversity description; iii) no correlation was found between fungal and bacterial diversity and composition and the here considered environmental parameters (altitude and sea distance).

Future researches are needed i) to decipher how these peculiar ecosystems respond to environmental pressure, recording other environmental variables, such as sun exposition, water availability, Photosynthetic Active Radiation (PAR) and rock temperature; ii) to give insights on how Antarctic endolithic communities may be affected by Climate Change and iii) to define the habitability in extraterrestrial analogues on Earth and to increase knowledge for searching life beyond Earth (Cockell *et al.*, 2016).

REFERENCES

Abdel-Hafez, S.I.I. (1994) Studies on soil mycoflora of desert soils in Saudi Arabia. Mycopathologia 80: 3e8.

Adams, B.J., Bardgett, R.D., Ayres, E., Wall, D.H., Aislabie, J., Bamforth, S., *et al*. (2006) Diversity and distribution of Victoria Land biota. Soil Biol Biochem 38: 3003-3018.

Aislabie, J.M., Chhour, K.L., Saul, D.J., Miyauchi, S., Ayton, J., Paetzold, R.F., and Balks, M.R. (2006) Dominant bacteria in soils of Marble point and Wright Valley, Victoria Land, Antarctica. Soil Biol Biochem 38(10): 3041-3056.

Al-Mutairi, N.Z., 2009. Variable distributional characteristics of substrate utilization patterns in activated sludge plants in Kuwait. Bioresource Technol 100: 1524-1532.

Anderson, M.J., Ellingsen, K.E., and McArdle, B.H. (2006) Multivariate dispersion as a measure of beta diversity. Ecol Lett 9(6): 683-693.

Archer, S.D., de los Ríos, A., Lee, K.C., Niederberger, T.S., Cary, S.C., Coyne, K.J., *et al.* (2016) Endolithic microbial diversity in sandstone and granite from the McMurdo Dry Valleys, Antarctica. Polar Biol 40 (5), 997-1006.

Arenz, B.E., Held, B.W., Jurgens, J.A., Farrell, R.L., and Blanchette, R.A. (2006) Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. Soil Biol Biochem 38: 3057-3064.

Badali H., Carvalho, V.O., Vicente, V., Attili-Angelis, D., Kwiatkowski, I.B., Gerrits Van Den Ende, A.H., *et al*. (2009) *Cladophialophora saturnica* sp. nov. a new opportunistic species of Chaetothyriales revealed using molecular data. Med Mycol 47: 51-66.

Baqué, M., de Vera, J.P., Rettberg, P., and Billi, D. (2013) The BOSS and BIOMEX space experiments on the EXPOSE-R2 mission: Endurance of the desert cyanobacterium *Chroococcidiopsis* under simulated space vacuum, Martian atmosphere, UVC radiation and temperature extremes. Acta Astronaut 91: 180-186.

Bargagli, R. (2008) Environmental contamination in Antarctic ecosystems. Sci Total Environ 400(1): 212-226.

Barrett, J.E., Virginia, R.A., Hopkins, D.W., Aislabie, J., Bargagli, R., Bockheim, J.G., *et al.* (2006a) Terrestrial ecosystem processes of Victoria Land, Antarctica. Soil Biol Biochem 38(10): 3019-3034.

Barrett, J.E., Virginia, R.A., Wall, D.H., Cary, S.C., Adams, B.J., Hacker, A.L., *et al.* (2006b) Co-variation in soil biodiversity and biogeochemistry in northern and southern Victoria Land, Antarctica. Antarctic Sci 18: 535-548.

Bell, R.A. (1993) Cryptoendolithic algae of hot semiarid lands and deserts. J Phycol 29: 133-139.

Bik, H.M., Porazinska, D.L., Creer, S., Caporaso, J.G., Knight, R., and Thomas, W.K.. (2012) Sequencing our way towards understanding global eukaryotic biodiversity. Trends Ecol Evol 27(4): 233-243.

Billi, D., Friedmann, E.I., Hofer, K.G., Caiola, M.G., and Ocampo-Friedmann, R. (2000) Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. Appl Environ Microbiol 66(4): 1489-1492.

Billi, D., Viaggiu, E., Cockell, C.S., Rabbow, E., Horneck, G., and Onofri, S. (2011) Damage escape and repair in dried *Chroococcidiopsis* spp. from hot and cold deserts exposed to simulated space and Martian conditions. Astrobiology 11(1): 65-73.

Blasi, B., Tafer, H., Tesei, D., and Sterflinger, K. (2015) From glacier to sauna: Rna-seq of the human pathogen black fungus *Exophiala dermatitidis* under varying temperature conditions exhibits common and novel fungal response. PloS one 10: e0127103.

Bockheim, J.G., and McLeod, M. (2008) Soil distribution in the McMurdo Dry Valleys, Antarctica. Geoderma 144(1): 43-49.

Bomblies, A., McKnight, D.M., and Andrews, E.D. (2001) Retrospective simulation of lake-level rise in Lake Bonney based on recent 21-yr record: indication of recent climate change in the McMurdo Dry Valleys. Antarctica. J Paleolimnol 25: 477-492.

Bonifaz, A., Badali, H., De Hoog, G.S., Cruz, M., Araiza, J., Cruz, M.A., *et al*. (2008) *Tinea nigra* by *Hortaea werneckii*, a report of 22 cases from Mexico. Stud Mycol 61: 77-82.

Boutet, E., Lieberherr, D., Tognolli, M., Schneider, M., Bansal, P., Bridge, A.J., *et al*. (2016) UniProtKB/Swiss-Prot. the Manually Annotated Section of the UniProt KnowledgeBase: How to Use the Entry View. Methods Mol Biol 1374: 23-54.

Branda, E., Turchetti, B., Diolaiuti, G., Pecci, M., Smiraglia, C., and Buzzini, P. (2010) Yeast and yeast-like diversity in the southernmost glacier of Europe (Calderone Glacier, Apennines, Italy). FEMS Microbiol Ecol 72(3): 354-369.

Broady, P.A. (1981) The ecology of chasmolithic algae at coastal locations of Antarctica. Phycologia 20(3): 259-272.

Büdel, B., Schulz, B., Reichenberger, H., Bicker, F., and Green, T.G. (2009) Cryptoendolithic cyanobacteria from calcite marble rock ridges, Taylor Valley, Antarctica. Algol Stud 129(1): 61-69.

Butler, M.J., and Day, A.W. (1998) Fungal melanins: a review. Can J Microbiol 44(12): 1115-1136.

Buzzini, P., Branda, E., Goretti, M., and Turchetti, B. (2012) Psychrophilic yeasts from worldwide glacial habitats: diversity, adaptation strategies and biotechnological potential. FEMS Microbiol Ecol 82: 217-241.

Buzzini, P., Turk, M., Perini, L., Turchetti, B., and Gunde-Cimerman, N. (2017) Yeasts in polar and sub-polar habitats. In: Buzzini, P., Lachance, M.A., and Yurkov, A.M. (eds) Yeasts in Natural Ecosystems: Diversity. Springer International Publishing. pp 331-365.

Camara, B., Suzuki, S., Nealson, K.H., Wierzchos, J., Ascaso, C., Artieda, O., *et al.* (2014) Ignimbrite textural properties as determinants of endolithic colonization patterns from hyper-arid Atacama Desert. Int Microbiol 17: 235-247.

Cameron, R., Morelli, F.A., and Johnson, R.M. (1972) Bacterial species in soil and air of the Antarctic continent. Antarct J 7: 187-189

Cannone, N., and Seppelt, R. (2008) A preliminary floristic classification of southern and northern Victoria Land vegetation, Continental Antarctica. Antarctic Sci 20: 553-562.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7: 335-336.

Cary, S.C., McDonald, I.R., Barrett, J.E., and Cowan, D.A. (2010) On the rocks: the microbiology of Antarctic Dry Valley soils. Nat Rev Microbiol 8: 129-138.

Cavacini, P., and Fumanti, B. (2005) Cyanobacterial and algal biodiversity at Edmonson Point (Northern Victoria Land, Antarctica). In Proceedings of the Fifth PNRA Meeting on Antarctic Biology. Polarnet Tech Rep Vol. 1, No. 2005, pp: 19-22.

Chan, Y., Lacap, D.C., Lau, M.C., Ha, K.Y., Warren-Rhodes, K.A., Cockell, C.S., *et al.* (2012) Hypolithic microbial communities: between a rock and a hard place. Environ Microbiol 14(9): 2272-2282.

Chong, C.W., Tan, G.A., Wong, R.C., Riddle, M.J., and Tan, I.K.P. (2009) DGGE fingerprinting of bacteria in soils from eight ecologically different sites around Casey Station, Antarctica. Polar Biol 32: 853-860.

Chong, C.W., Pearce, D.A., Convey, P., Tan, G.A., Wong, R.C., and Tan, I.K.P. (2010) High levels of spatial heterogeneity in the biodiversity of soil prokaryotes on Signy Island, Antarctica. Soil Biol Biochem 42(4): 601-610.

Chong, C.W., Pearce, D.A., Convey, P., Yew, W.C., and Tan, I.K.P. (2012) Patterns in the distribution of soil bacterial 16S rRNA gene sequences from different regions of Antarctica. Geoderma 181: 45-55.

Christner, B.C., Kvitko, B.H., and Reeve, J.N. (2003) Molecular identification of bacteria and eukarya inhabiting an Antarctic cryoconite hole. Extremophiles 7(3): 177-183.

Clarke, K.R. (1993) Non-parametric multivariate analysis of changes in community structure. Aust J Ecol 18: 117-143.

Cockell, C.S., McKay, C.P., and Omelon, C. (2002) Polar endoliths-an anti-correlation of climatic extremes and microbial biodiversity. Int J Astrobiol 1(4): 305-310.

Cockell, C.S,. McKay, C.P., and Omelon, C. (2003) Polar endoliths - an anti-correlation of climatic extremes and microbial biodiversity. Int J Astrobiol 1: 305-310.

Cockell, C.S., Bush, T., Bryce, C., Direito, S., Fox-Powell, M., Harrison, J.P., *et al.* (2016) Habitability: a review. Astrobiology 16: 89-117.

Coleine, C., Selbmann, L., Ventura, S., D'Acqui, L.P., Onofri, S., and Zucconi, L. (2015) Fungal Biodiversity in the Alpine Tarfala Valley. Microorganisms 3: 612-624.

Connell, L.B., Redman, R., Craig, S., Scorzetti, G., Iszard, M., and Rodriguez, R. (2008) Diversity of soil yeasts isolated from South Victoria Land, Antarctica. Microbial Ecol 56: 448-459.

Connell, L.B., Rodriguez, R.R., Redman, R.S., and Dalluge, J.J. (2014) Cold-adapted yeasts in Antarctic deserts. In: Buzzini, P., and Margesin, R. (eds) Cold-Adapted Yeasts. Springer. Berlin. pp 75-98.

Convey, P., Gibson, J.A., Hillenbrand, C.D., Hodgson, D.A., Pugh, P.J., Smellie, J.L., and Stevens, M.I. (2008) Antarctic terrestrial life–challenging the history of the frozen continent? Biological Rev 83(2): 103-117.

Cowan, D. Makhalanyane, T.P., Dennis, P.G., and Hopkins, D.W. (2014) Microbial ecology and biogeochemistry of continental Antarctic soils. Front Microbiol 5: 154, doi: 10.3389/fmicb.2014.00154.

Cowan, D.A., Russell, N.J., Mamais, A., and Sheppard, D.M. (2002) Antarctic Dry Valley mineral soils contain unexpectedly high levels of microbial biomass. Extremophiles 6(5): 431-436.

Cowan, D., and Tow, L. (2004) Endangered Antarctic environments. Annu Rev Microbiol 58: 649-690.

Cox, F., Newsham, K.K., Bol, R., Dungait, J.A., and Robinson, C.H. (2016) Not poles apart: Antarctic soil fungal communities show similarities to those of the distant Arctic. Ecol Lett 19: 528-536.

Coutinho, M.L., Miller, A.Z., Gutierrez-Patricio, S., Hernandez-Marine, M., Gomez-Bolea, A., Rogerio-Candelera, M. A., *et al.* (2012) nd Microbial communities on deteriorated artistic tiles from Pena National Palace (Sintra, Portugal). Int Biodeterior Biodegradation 30: 1e11.

Creer, S., Fonseca, V.G., Porazinska, D.L., Giblin-Davis, R.M., Sung, W., Power, D.M., *et al.* (2010) Ultrasequencing of the meiofaunal biosphere: practice, pitfalls and promises. Mol Ecol 19(s1): 4-20.

Crous, P.W., Schoch, C.L., Hyde, K.D., Wood, A.R., Gueidan, C., De Hoog, G.S., and Groenewald, J.Z. (2009) Phylogenetic lineages in the Capnodiales. Stud Mycol 64: 17-47.

Dadachova, E., and Casadevall, A. (2008) Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. Curr Opin Microbiol 11(6): 525-531.

Das, M., Royer, T.V., and Leff, L.G. (2007) Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream. Appl Environ Microbiol 73: 756-767.

de Hoog, G.S., Vicente, V.A., Najafzadeh, M.J., Harrak, M.J., Badali, H., and Seyedmousavi, S. (2011) Waterborne *Exophiala* species causing disease in cold-blooded animals. Persoonia 27: 46-72.

de la Torre, J.R., Goebel, B.M., Friedmann, E., and Pace, N.R. (2003) Microbial diversity of cryptoendolithic communities from the McMurdo Dry Valleys. Antarctica. Appl Environ Microbiol 69: 3858-3867.

de los Ríos, A., Sancho, L.G., Grube, M., Wierzchos, J., and Ascaso, C. (2005) Endolithic growth of two *Lecidea* lichens in granite from continental Antarctica detected by molecular and microscopy techniques. New Phytol 165: 181-190.

de los Ríos, A., Wierzchos, J., and Ascaso, C. (2014) The lithic microbial ecosystems of Antarctica's McMurdo Dry Valleys. Antarctic Sci 26(5): 459-477.

Dewey, C. (2007) Aligning multiple whole genomes with Mercator and MAVID. In N. Bergman, editor, Comparative Genomics, volume 395 of Methods in Molecular Biology. Humana Press.

Domaschke, S., Fernandez-Mendoza, F., García, M., Martín, M., and Printzen, C. (2012) Low genetic diversity in Antarctic populations of the lichen-forming ascomycete Cetraria aculeata and its photobiont. Polar Research 31(1): 17353.

Doran, P.T., McKay, C.P., Clow, G.D., Dana, G.L., Fountain, A.G., Nylen, T., and Lyons, W.B. (2002) Valley floor climate observations from the McMurdo Dry Valleys, Antarctica, 1986–2000. J Geophys Res Atmosph 107 (D24).

Dunbar, J., Ticknor, L.O., and Kuske, C.R. (2000) Assessment of microbial diversity in four southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis. Appl Environ Microbiol 66(7): 2943-2950.

Edgar, R.C. (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26: 2460-2461.

Egidi, E., de Hoog, G.S., Isola, D., Onofri, S., Quadvlieg, W. De Vries, M., Verkley, G.J.M., *et al*. (2014) Phylogeny and taxonomy of meristematic rock-inhabiting black fungi in the Dothideomycetes based on multi-locus phylogenies. Fungal Divers 65: 127-165.

Estévez, E., Veiga, M.C., and Kennes, C. (2005) Biofiltration of waste gases with the fungi *Exophiala oligosperma* and *Paecilomyces variotii*. Appl Environ Microbiol 67(4): 563-568.

Faizutdinova, R.N., Suzina, N.E., Duda, V.I., Petrovskaya, L.E., and Gilichinsky, D.A. (2005) Yeasts isolated from ancient permafrost. In: Castello, J.D., and Rogers, S.O. (eds) Life in Ancient Ice. Princeton University Press. Princeton. 118-126.

Farrell, R.L., Arenz, B.E., Duncan, S.M., Held, B.W., Jurgens, J.A., and Blanchette, R.A. (2011) Introduced and indigenous fungi of the Ross Island historic huts and pristine areas of Antarctica. Polar Biol 34: 1669-1677.

Finn, R.D., Bateman, A., Clements, J., Coggill, P., Eberhardt, R.Y., Eddy, S.R. *et al.* (2014) Pfam: the protein families database. Nucleic Acids Res 42: D222-30.

Fox, A. J., Paul, A., and Cooper, R. (1994) Measured properties of the Antarctic ice sheet derived from the SCAR Antarctic digital database. Polar Record 30(174): 201-206.

Frenot, Y., Chown, S.L., Whinam, J., Selkirk, P.M., Convey, P., Skotnicki, M, *et al.* (2005) Biological invasions in the Antarctic: extent. impacts and implications. Biol Rev Camb Philos Soc. 80: 45-72.

Friedmann, E.I., and Ocampo, R. (1976) Endolithic blue-green algae in dry valleys-primary producers in Antarctic desert ecosystem. Science 193: 1247-1249.

Friedmann, E.I. (1982) Endolithic microorganisms in the Antarctic cold desert. Science 215: 1045-1053.

Friedmann, E.I., and Ocampo-Friedmann, R. (1984) The Antarctic cryptoendolithic ecosystem: relevance to exobiology. Orig Life Evol Biosph 14(1): 771-776.

Friedmann, E.I., McKay, C.P., and Nienow, J.A. (1987) The cryptoendolithic microbial environment in the Ross Desert of Antarctica: satellite-transmitted continuous nanoclimate data, 1984 to 1986. Polar Biol 7: 273-287.

Friedmann, E.I., Hua, M., and Ocampo-Friedmann, R. (1988) Cryptoendolithic lichen and cyanobacterial communities of the Ross Desert, Antarctica. Polarforschung 58: 251-259.

Fulton, T.M., Chunwongse, J., and Tanksley, S.D. (1995) Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol Biol Rep 13: 207-209.

Gadd, G.M., Rhee Y.J., Stephenson, K., and Wei, Z. *(*2012) Geomycology: metals, actinides and biominerals. Environ Microbiol Rep 4*:* 270-296*.*

Ganzert, L., Lipski, A., Hubberten, H.W., and Wagner, D. (2011) The impact of different soil parameters on the community structure of dominant bacteria from nine different soils located on Livingston Island, South Shetland Archipelago, Antarctica. FEMS Microbiol Ecol 76(3): 476-491.

Glenn, T.C. (2011) Field guide to next-generation DNA sequencers. Mol Ecol Resour 11(5): 759-769.

Godinho, V.M., Gonçalves, V.N., Santiago, I.F., Figueredo, H.M., Vitoreli, G.A., Schaefer, C.E., *et al*. (2015) Diversity and bioprospection of fungal community present in oligotrophic soil of continental Antarctica. Extremophiles 19: 585-596.

Goffeau, A., Barrell, B.G., Bussey, H., Davis, R.W., Dujon, B., Feldmann, H., *et al*. (1996) Life with 6000 genes. Science 274(5287): 546-567.

Golubic, S., Friedmann, I., and Schneider, J. (1981) The lithobiontic ecological niche, with special reference to microorganisms. J Sediment Res 51(2).

Gorbushina, A. (2003) Microcolonial fungi: survival potential of terrestrial vegetative structures. Astrobiology 3(3): 543-554.

Gorbushina, A.A., Whitehead, K., Dornieden, T., Niesse, A., Schulte, A., and Hedges, J.I. (2003) Black fungal colonies as units of survival: hyphal mycosporines synthesized by rock-dwelling microcolonial fungi. Can J Bot 81(2): 131-138.

Goslee, S.C., and Urban, D.L. (2007) The ecodist package for dissimilarity-based analysis of ecological data. J Stat Softw 22(7): 1-19.

Göttlich, E., van der Lubbe, W., Lange, B., Fiedler, S., Melchert, I., Reifenrath, M., *et al*. (2002) Fungal flora in groundwater-derived public drinking water. Int J Hyg Environ Health 205: 260-279.

Grabherr, M.G., Russell, P., Meyer, M., Mauceli, E., Alföldi, J., Di Palma, F., *et al.* (2010) Genome-wide synteny through highly sensitive sequence alignment: Satsuma. Bioinformatics 26: 1145-1151.

Gümral, R., Özhak-Baysan, B., Tümgör, A., Saraçlı, M.A., Yıldıran, Ş.T., Ilkit, M., *et al.* (2016) Dishwashers provide a selective extreme environment for human-opportunistic yeast-like fungi. Fungal Divers 76(1): 1-9.

Gunde-Cimerman, N., Zalar, P., de Hoog, S., and Plemenitaš, A. (2000) Hypersaline waters in salterns–natural ecological niches for halophilic black yeasts. FEMS Microbiol Ecol 32(3): 235-240.

Gunde-Cimerman, N., Sonjak, S., Zalar, P., Frisvad, J.C., Diderichsen, B., and Plemenitaš, A. (2003) Extremophilic fungi in arctic ice: a relationship between adaptation to low temperature and water activity. Phys Chem Earth Parts A/B/C 28(28): 1273-1278.

Gurevich, A., Saveliev, V., Vyahhi, N., and Tesler, G. (2013) QUAST: quality assessment tool for genome assemblies. Bioinformatics 29(8): 1072-1075.

Haas, B.J., Salzberg, S.L., Zhu, W., Pertea, M., Allen, J,E,, Orvis, J., *et al.* (2008) Automated eukaryotic gene structure annotation using EVidenceModeler and the Program to Assemble Spliced Alignments. Genome Biol 9: R7.

Hall, B., DeRego, T., and Geib, S. (2014) GAG: the Genome Annotation Generator. https://genomeannotation.github.io/GAG/.

Hammer, O., Harper, D.A.T., and Ryan, P.D. (2001) PAST: Paleontological statistics software package for education and data analysis. Palaeontol Electron 4: 9 pp.

Hirose, D., Hobara, S., Matsuoka, S., Kato, K., Tanabe, Y., Uchida, M., *et al.* (2016) Diversity and community assembly of moss-associated fungi in ice-free coastal outcrops of Continental Antarctica. Fungal Ecol 24: 94-101.

Hirsch, P., Hoffmann, B., Gallikowski, C.C., Mevs, U., Siebert, J., and Sittig, M. (1988) Diversity and Identification of Heterotrophs from Antarctic Rocks of the McMurdo Dry Valleys (Ross Desert). Polarforschung 58(2/3): 261-269.

Hogg, I.D., and Wall, D.H. (2011) Global change and Antarctic terrestrial biodiversity. Polar Biol 34: 1625-1627.

Hölker, U., Höfer, M., and Lenz, J. (2004) Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. Appl Microbiol Biotechnol 64(2): 175-186.

Isola, D., Selbmann, L., de Hoog G.S., Fenice, M., Onofri, S., Prenafeta-Boldú, F.X., *et al.* (2013) Isolation and screening of black fungi as degraders of volatile aromatic hydrocarbons. Mycopathologia 175: 369-379.

Isola, D., Zucconi, L., Onofri, S., Caneva, G., De Hoog, G. S., and Selbmann, L. (2016) Extremotolerant rock inhabiting black fungi from Italian monumental sites. Fungal Divers 76(1): 75-96.

Ji, Y., Ashton, L., Pedley, S.M., Edwards, D.P., Tang, Y., Nakamura, A., *et al.* (2013) Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. Ecol Lett 16: 1245-1257.

Jones, P., Binns, D., Chang, H.Y., Fraser, M., Li, W., McAnulla, C, *et al.* (2014) InterProScan 5: genome-scale protein function classification. Bioinformatics 30: 1236-1240.

Kappen, L. (2000) Some aspects of the great success of lichens in Antarctica. Antarct Sci 12: 314-324.

Kurtz, S., Phillippy, A., Delcher, A.L., Smoot, M., Shumway, M., Antonescu, C., and Salzberg, S.L. (2004) Versatile and open software for comparing large genomes. Genome Biol 5: R12.

Lee, C.K., Barbier, B.A., Bottos, E.M., McDonald, I.R., and Cary, S.C. (2012) The inter-valley soil comparative survey: the ecology of Dry Valley edaphic microbial communities. ISME J 6: 1046-1057.

Legendre, P., Borcard, D., and Peres-Neto, P.R. (2005). Analyzing beta diversity: partitioning the spatial variation of community composition data. Ecol Monog 75(4): 435-450.

Lichstein, J.W. (2007) Multiple regression on distance matrices: a multivariate spatial analysis tool. Plant Ecol 188: 117-131.

Listemann, H., and Freiesleben, H. (1996) *Exophiala mesophila* spec. nov. Mycoses 39: 1-2 1-3.

Liu, X.Z., Wang, Q.M., Goker, M., Groenewald, M., Kachalkin, A.V., Lumbsch, H.T., *et al.* (2015) Towards an integrated phylogenetic classification of the Tremellomycetes. Stud Mycol 81: 85-147.

Logares, R., Haverkamp, T.H., Kumar, S., Lanzén, A., Nederbragt, A.J., Quince, C., *et al*. (2012) Environmental microbiology through the lens of high-throughput DNA sequencing: synopsis of current platforms and bioinformatics approaches. J Microbial Methods 91(1): 106-113.

Lombard, V., Golaconda R.H., Drula, E., Coutinho, P.M., and Henrissat, B. (2013) The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42: D490-5.

Lorenz, M.O. (1905) Methods of measuring the concentration of wealth. Publications of the American statistical association 9(70): 209-219.

Ludwig, J.A. (1988) Statistical Ecology: A Primer in Methods and Computing. New York, NY: John Wiley & Sons.

Marzorati, M., Wittebolle, L., Boon, N., Daffonchio, D., and Verstraete, W. (2008) How to get more out of molecular fingerprints: practical tools for microbial ecology. Environ Microbiol 10: 1571-1581.

Matos, T., de Hoog, G.S., de Boer, A.G, De Crom, I., and Haase, G. (2002) High prevalence of the neurotrope *Exophiala dermatitidis* and related oligotrophic black yeasts in sauna facilities. Mycoses 45: 373-377.

Mattimore, V., and Battista, J. R. (1996) Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. J Bacteriol 178(3): 633-637.

McKay, C.P., Nienow, J.A., Meyer, M.A., and Friedmann, E.I. (1993) Continuous nanoclimate data (1985-1988) from the Ross Desert (McMurdo Dry Valleys) cryptoendolithic microbial ecosystem. In Antarctic Meteorology and Climatology: Studies Based on Automatic Weather Stations. Bromwich, D.H., and Stearns C.R. (eds). Washington D.C.: American Geophysical Union, pp. 201-207.

Mohanta, T.K., and Bae, H. (2015) Functional Genomics and Signaling Events in Mycorrhizal Symbiosis. J Plant Interact. 10: 21-40.

Montes, M.J., Belloch, C., Galiana, M., Garcia, M.D., Andrés, C., Ferrer, S., *et al*. (1999) Polyphasic taxonomy of a novel yeast isolated from antarctic environment; description of *Cryptococcus victoriae* sp. nov. Syst Appl Microbiol 22: 97-105.

Moorhead, D.L., Doran, P.T., Fountain, A.G., Lyons, W.B., McKnight, D.M., Priscu, J.C., *et al.* (1999) Ecological legacies: impacts on ecosystems of the McMurdo Dry Valleys. Bioscience 49(12): 1009-1019.

Morris, E.K., Caruso, T., Buscot, F., Fischer, M., Hancock, C., Maier, T.S., *et al.* (2014) Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories. Ecol Evol 4: 3514-3524.

Muggia, L., Pérez-Ortega, S., Kopun, T., Zellnig, G., and Grube, M. (2014) Photobiont selectivity leads to ecological tolerance and evolutionary divergence in a polymorphic complex of lichenized fungi. Ann Bot 114(3): 463-475.

Muyzer, G., de Waal, E.C., and Uitterlinden, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Appl Environ Microbiol 59: 695-700.

National Research Council (2007) Committee on Metagenomics: Challenges and Functional Applications. *The new science of metagenomics: revealing the secrets of our microbial planet*.

Niederberger, T.D., McDonald, I.R., Hacker, A.L., Soo, R.M., Barrett, J.E., Tirindelli, J., *et al.* (2008) Microbial community composition in soils of Northern Victoria Land, Antarctica. Environ Microbiol 10(7): 1713-1724.

Niederberger, T.D., Sohm, J.A., Tirindelli, J., Gunderson, T., Capone, D.G., Carpenter, E.J., *et al*. (2012) Diverse and highly active diazotrophic assemblages inhabit ephemerally wetted soils of the Antarctic Dry Valleys. FEMS Microbiol Ecol 82(2): 376-390.

Nielsen, U.N., Wall, D.H., Adams, B.J., Virginia, R.A., Ball, B.A., Gooseff, M.N., and McKnight, D.M. (2012) The ecology of pulse events: insights from an extreme climatic event in a polar desert ecosystem. Ecosphere 3: 1-15.

Nienow, J.A., McKay, C.P., and Friedmann, E.I. (1988) The cryptoendolithic microbial environment in the Ross Desert of Antarctica: light in the photosynthetically active region. Microb Ecol 16: 271-289.

Nienow, J.A., and Friedmann, E.I. (1993) Terrestrial lithophytic (rock) communities. In: Friedmann, E.I. (ed) Antarctic Microbiology. Wiley-Liss. New York. pp 343-412.

Nienow, J. A., Friedmann, E. I., and Ocampo-Friedmann, R. (2003). Endolithic microorganisms in arid regions. Encyclopedia of Environmental Microbiology.

Nishimura, K., Miyaji, M., Taguchi, H., and Tanaka, R. (1987) Fungi in bathwater and sludge of bathroom drainpipes. Frequent isolation of *Exophiala* species. Mycopathol 97: 17-23.

Olech, M., and Chwedorzewska, K.J. (2011) Short Note. The first appearance and establishment of an alien vascular plant in natural habitats on the forefield of a retreating glacier in Antarctica. Antarctic Sci 23: 153-154.

Onofri, S., Pagano, S., Zucconi, L., and Tosi, S. (1999) *Friedmanniomyces endolithicus* (Fungi, Hyphomycetes), anam-gen and sp nov, from continental Antarctica. Nova Hedwigia 68(1): 175-182.

Onofri, S., Selbmann, L., Zucconi, L., and Pagano, S. (2004) Antarctic microfungi as models for exobiology. Planet Space Sci 52: 229-237.

Onofri, S., Zucconi, L., Selbmann, L., de Hoog, S., de los Rios, A., Ruisi, S. *et al.* (2007) Fungal associations at the cold edge of life. In: Seckbach, J. (ed) Algae and cyanobacteria in extreme environments. Springer. Netherlands. pp 735-757.

Onofri, S., Barreca, D., Selbmann, L., Isola, D., Rabbow, E., Horneck, G., *et al.* (2008) Resistance of Antarctic black fungi and cryptoendolithic communities to simulated space and Martian conditions. Stud Mycol 61: 99-109.

Onofri, S., de la Torre, R., de Vera, J.P., Ott, S., Zucconi, L., Selbmann, L., *et al.* (2012) Survival of rock-colonizing organisms after 1.5 Years in outer space. Astrobiology 12: 508-516.

Onofri, S., de Vera, J.P., Zucconi, L., Selbmann, L., Scalzi, G., Venkateswaran, K.J., *et al.* (2015) Survival of Antarctic cryptoendolithic fungi in simulated Martian conditions on-board the International Space Station. Astrobiology 15: 1052-1059.

Ovstedal, D., and Lewis Smith, R.I. (2001) Lichens of Antarctica and South Georgia. In: Ovstedal, D.O., Lewis Smith, R.I. (eds) A Guide to their Identification and Ecology. Studies in Polar Research. Cambridge University. Cambridge. pp 4-5.

Pacelli, C., Selbmann, L., Zucconi, L., de Vera, J.P., Rabbow, E., Horneck, G., *et al.* (2017a) BIOMEX experiment: ultrastructural alterations. molecular damage and survival of the fungus *Cryomyces antarcticus* after the Experiment Verification Tests. Orig Life Evol Biosph 47: 187-202.

Pacelli, C., Selbmann, L., Moeller, R., Zucconi, L., Fujimori, A., and Onofri, S. (2017b) Cryptoendolithic Antarctic black fungus *Cryomyces antarcticus* irradiated with accelerated helium ions: survival and metabolic activity, DNA and ultrastructural damage. Front Microbiol 8.

Pacelli, C., Selbmann, L., Zucconi, L., Raguse, M., Moeller, R., Shuryak, I., *et al.* (2017c) Survival. DNA integrity, and ultrastructural damage in antarctic cryptoendolithic eukaryotic microorganisms exposed to ionizing radiation. Astrobiology 17: 126-135.

Palmer, J.M., Jusino, M.A., Banik, M.T., and Lindner, D.L. (2017) Non-biological synthetic spike-in controls and the AMPtk software pipeline improve fungal high throughput amplicon sequencing data. BioRxiv, 213470.

Pareto, V. (1897) Cours d'economie politique. Macmillan. London.

Pearce, D.A., Bridge, P.D., Hughes, K.A., Sattler, B., Psenner, R., and Russell, N.J. (2009) Microorganisms in the atmosphere over Antarctica. FEMS Microbiol Ecol 69: 143-157.

Pielou, E. (1966) The measurement of diversity in different types of biological collections. J Theoret Biol 13: 131-144.

Pointing, S.B., Chan, Y., Lacap, D.C., Lau, M.C., and Jurgens, J.A. (2009) Highly specialized microbial diversity in hyper arid polar desert. Proc Natl Acad Sci 106: 19964-19969.

Pointing, S.B., and Belnap, J. (2012) Microbial colonization and controls in dryland systems. Nat Rev Microbiol 10: 551-562.

Pointing, S.B., Bollard-Breen, B., and Gillman, L.N. (2014). Diverse cryptic refuges for life during glaciation. Proc Natl Acad Sci 111(15): 5452-5453.

Pointing, S.B., Buedel, B., Convey, P., Gillman, L.N., Koerner, C., Leuzinger, S., *et al*. (2015) Biogeography of photoautotrophs in the high polar biome. Front Plant Sci 6: 692

Porteous, N.B., Grooters, A.M., Redding, S.W., Thompson, E.H., Rinaldi, M.G., de Hoog, G.S., *et al*. (2003) Identification of *Exophiala mesophila* isolated from treated dental unit waterlines. J Clin Microbiol 41: 3885-3889.

Possemiers, S., Verthé, K., Uyttendaele, S., and Verstraete, W. (2004) PCR-DGGE-based quantification of stability of the microbial community in a simulator of the human intestinal microbial ecosystem. FEMS Microbiol Ecol 49(3): 495-507.

Prenafeta-Boldu, F.X., Summerbell, R., and de Hoog, G.S. (2006) Fungi growing on aromatic hydrocarbons: biotechnology's unexpected encounter with biohazard? FEMS Microbiol Rev 30: 109-130.

Prenafeta-Boldú, F.X., Guivernau, M., Gallastegui, G., Viñas, M., de Hoog, G.S., and Elías, A. (2012) Fungal/bacterial interactions during the biodegradation of TEX hydrocarbons (toluene, ethylbenzene and p-xylene) in gas biofilters operated under xerophilic conditions. FEMS Microbiol Ecol 80(3): 722-734.

Ptacnik, R., Andersen, T., Brettum, P., Lepistö, L., and Willén, E. (2010) Regional species pools control community saturation in lake phytoplankton. Pr R Soc London B: Biol Sci 277(1701): 3755-3764.

Rao, S., Chan, Y., Lacap, D.C., Hyde, K.D., Pointing, S.B., and Farrell, R.L. (2011) Low-diversity fungal assemblage in an Antarctic Dry Valleys soil. Polar Biol 35: 567-574.

Rawlings, N.D., Barrett, A.J., and Bateman, A. (2014) Using the MEROPS Database for Proteolytic Enzymes and Their Inhibitors and Substrates. Curr Protoc Bioinformatics 48: 1.25.1-1.25.33.

Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016) VSEARCH: a versatile open source tool for metagenomics. Peer J 4, e2584.

Ruibal, C., Gueidan, C., Selbmann, L., Gorbushina, A.A., Crous, P.W., Groenewald, J.Z., *et al.* (2009) Phylogeny of rock-inhabiting fungi related to Dothideomycetes. Stud Mycol 64: 123-133.

Ruisi, S., Barreca, D., Selbmann, L., Zucconi, L., and Onofri, S. (2007) Fungi in Antarctica. Rev Environ Sci Bio 6: 127-141.

Ruprecht, U., Lumbsch, H.T., Brunauer, G., Green, T.A., and Türk, R. (2010) Diversity of *Lecidea* (Lecideaceae, Ascomycota) species revealed by molecular data and morphological characters. Antarctic Sci 22: 727-741.

Ruprecht, U., Brunauer, G., and Printzen, C. (2012) Genetic diversity of photobionts in Antarctic lecideoid lichens from an ecological view point. The Lichenologist 44: 661-678.

Saul, D.J., Aislabie, J.M., Brown, C.E., Harris, L., and Foght, J.M. (2005) Hydrocarbon contamination changes the bacterial diversity of soil from around Scott Base, Antarctica. FEMS Microbiol Ecol 53(1): 141-155.

Scorzetti, G., Petrescu, I., Yarrow, D., and Fell, J.W. (2000) *Cryptococcus adeliensis* sp. nov., a xylanase producing basidiomycetous yeast from Antarctica. A van Leeuw J Microb 77: 153-157.

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 deserts. Stud Mycol 51: 1-32.

Selbmann, L., de Hoog, G.S., Zucconi, L., Isola, D., Ruisi, S., Gerrits van den Ende, A.H.G., *et al.* (2008) Drought meets acid: three new genera in a dothidealean clade of extremotolerant fungi. Stud Mycol 61: 1-20.

Selbmann, L., Zucconi, L., Ruisi, S., Grube, M., Cardinale, M., and Onofri, S. (2010) Culturable bacteria associated with Antarctic lichens: affiliation and psychrotolerance. Polar Biol 33: 71–83.

Selbmann, L., Isola, D., Zucconi, L., and Onofri, S. (2011) Resistance to UV-B induced DNA damage in extreme-tolerant cryptoendolithic Antarctic fungi: detection by PCR assays. Fungal Biol 115: 937-944.

Selbmann, L., Isola, D., Fenice, F., Zucconi, L., Sterflinger, K., and Onofri, S. (2012) Potential extinction of Antarctic endemic fungal species as a consequence of global warming. Sci Tot Environ 438: 127-134.

Selbmann, L., Grube, M., Onofri, S., Isola, D., and Zucconi, L. (2013) Antarctic epilithic lichens as niches for black meristematic fungi. Biology 2: 784-797.

Selbmann, L., de Hoog, G.S., Zucconi, L., Isola, D., and Onofri, S. (2014a) Black yeasts in cold habitats. pp173-189. In Buzzini, P., and Margesin, R. (ed). Cold-adapted yeasts. Springer. Berlin. Germany.

Selbmann, L., Isola, D., Egidi, E., Zucconi, L., Gueidan, C., de Hoog, G. S., *et al.* (2014b) Mountain tips as reservoirs for new rock-fungal entities: *Saxomyces* gen. nov. and four new species from the Alps. Fungal Divers 65: 167-182.

Selbmann, L., Zucconi, L., Onofri, S., Cecchini, C., Isola, D., Turchetti, B., *et al*. (2014c) Taxonomic and phenotypic characterization of yeasts isolated from worldwide cold rock-associated habitats. Fungal Biol 118: 61-71.

Selbmann, L., Turchetti, B., Yurkov, A., Cecchini, C., Zucconi, L., Isola, D., *et al.* (2014d) Description of *Taphrina antarctica* fa sp. nov., a new anamorphic ascomycetous yeast species associated with Antarctic endolithic microbial communities and transfer of four *Lalaria* species in the genus *Taphrina*. Extremophiles 18: 707-721.

Selbmann, L., Zucconi, L., Isola, D., and Onofri, S. (2015a) Rock black fungi: excellence in the extremes. From the Antarctic to space. Curr Genet 61: 335-345.

Selbmann, L., Onofri, S., Zucconi, L., Isola, D., Rottigni, M., Ghiglione, C., *et al.* (2015b) 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). MycoKeys 10: 57-71.

Selbmann, L., Onofri, S., Coleine, C., Buzzini, P., Canini, F., and Zucconi L. (2017) Effect of environmental parameters on biodiversity of the fungal component in the lithic Antarctic communities. Extremophiles 1-12.

Seyedmousavi, S., Badali, H., Chlebicki, A., Zhao, J., Prenafeta-Boldu, F.X., and de Hoog, G.S. (2011) *Exophiala sideris*, a novel black yeast isolated from environments polluted with toxic alkyl benzenes and arsenic. Fungal Biol 115(10): 1030-1037.

Shade, A., and Handelsman, J. (2011) Beyond the Venn diagram: the hunt for a core microbiomeemi_2585.

Shannon, C.E., and Weaver, W. (1963) The mathematical theory of communication. University of Illinois Press. Urbana.

Shendure, J., and Ji, H. (2008) Next-generation DNA sequencing. Nat Biotechnol 26(10): 1135-1145.

Siebert, J., and Hirsch, P. (1988) Characterization of 15 selected coccal bacteria isolated from Antarctic rock and soil samples from the McMurdo-Dry Valleys (South-Victoria Land). Polar Biol 9(1): 37-44.

Simpson, E.H. (1949) Measurement of diversity. Nature 163: 688.

Sinha, S., Flibotte, S., Niera, M., Formby, S., Plemenitaš, A., Cimerman, N.G., *et al.* (2017) Insight into the Recent Genome Duplication of the Halophilic Yeast *Hortaea werneckii*: Combining an Improved Genome with Gene Expression and Chromatin Structure. G3: Genes, Genomes, Genetics, g3-117.

Smith, J.J., Tow, L.A., Stafford, W., Cary, C., and Cowan, D. (2006) Bacterial diversity in three different Antarctic cold desert mineral soils. Microb Ecol 51(4): 413-421.

Smith, D.P., and Peay, K.G. (2014) Sequence Depth, Not PCR Replication, Improves Ecological Inference from Next Generation DNA Sequencing. PLoS ONE 9: e90234.

Spatafora, J.W., Aime, M.C., Grigoriev, I.V., Martin, F., Stajich, J.E., and Blackwell, M. (2017) The Fungal Tree of Life: from Molecular Systematics to Genome-Scale Phylogenies. Microbiol Spectr 5(5).

Spearman, C. (1904) The proof and measurement of association between two things. A J Psychol 15(1): 72-101.

Staley, J.T., Palmer, F., and Adams, J.B. (1982) Microcolonial fungi: common inhabitants on desert rocks? Science 215(4536): 1093-1095.

Stanke, M., Keller, O., Gunduz, I., Hayes, A., Waack, S., and Morgenstern, B. (2006) AUGUSTUS: ab initio prediction of alternative transcripts. Nucleic Acids Res 34: W435-9.

Steig, E.J., Schneider, D.P., Rutherford, S.D., Mann, M.E., Comiso, J.C., and Shindell D.T. (2009) Warming of the Antarctic ice-sheet surface since the 1957 International Geophysical Year. Nature 457: 459-462.

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 Biodiversity and ecophysiology of yeasts (pp. 501-514). Springer Berlin Heidelberg.

Sterflinger, K., Tesei, D., and Zakharova, K. (2012) Fungi in hot and cold deserts with particular reference to microcolonial fungi. Fungal Ecol 5(4): 453-462.

Sterflinger, K., Lopandic, K., Pandey, R.V., Blasi, B., and Kriegner, A. (2014) Nothing special in the specialist? Draft genome sequence of *Cryomyces antarcticus*, the most extremophilic fungus from Antarctica. PloS one 9(10): e109908.

Stomeo, F., Makhalanyane, T.P., Valverde, A., Pointing, S.B., Stevens, M.I., Cary, C.S., *et al.* (2012) Abiotic factors influence microbial diversity in permanently cold soil horizons of a maritime-associated Antarctic Dry Valley. FEMS Microbiol Ecol 82(2): 326-340.

Tao, G., Liu, Z.Y., Hyde, K.D., Lui, X.Z., and Yu, Z.N, (2008) Whole rDNA analysis reveals novel and endophytic fungi in *Bletilla ochracea* (Orchidaceae). Fungal Divers 33: 101-122.

Tedersoo, L., Anslan, S., Bahram, M., Polme, S., Riit, T., Liiv, I., *et al.* (2015) Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. MycoKeys 10: 1-43.

Teixeira, M.D.M., Moreno, L.F., Stielow, B.J., Muszewska, A., Hainaut, M., Gonzaga, L., *et al.* (2017) Exploring the genomic diversity of black yeasts and relatives (Chaetothyriales, Ascomycota). Stud Mycol 86: 1-28.

Ter-Hovhannisyan, V., Lomsadze, A., Chernoff, Y.O., and Borodovsky, M. (2008) Gene prediction in novel fungal genomes using an ab initio algorithm with unsupervised training. Genome Res 18:1979-1990.

Tetsch, L., Bend, J., and Hölker, U. (2006). Molecular and enzymatic characterisation of extra-and intracellular laccases from the acidophilic ascomycete *Hortaea acidophila*. Antonie van Leeuwenhoek 90(2): 183-194.

Tilman, D. (1996) Biodiversity: population versus ecosystem stability. Ecology 77: 350-363.

Turchetti, B., Goretti, M., Branda, E., Diolaiuti, G., D'Agata, C., Smiraglia, C., *et al.* (2013) Influence of abiotic variables on culturable yeast diversity in two distinct Alpine glaciers. FEMS Microbiol Ecol 86: 327-340.

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F.C., *et al.* (2016) Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Mol Ecol 25: 929-942.

Van Horn, D.J., Okie, J.G., Buelow, H.N., Gooseff, M.N., Barrett, J.E., and Takacs-Vesbach, C.D. (2014) Soil microbial responses to increased moisture and organic resources along a salinity gradient in a polar desert. Appl Environ Microbiol 80: 3034-3043.

Vaupotič, T., and Plemenitaš, A. (2007) Differential gene expression and Hog1 interaction with osmoresponsive genes in the extremely halotolerant black yeast *Hortaea werneckii*. BMC genomics 8(1): 280.

Vicente, V.A., Attili-Angelis, D., Pie, M.R., Queiroz-Telles, F., Cruz, L.M., Najafzadeh, M.J., *et al.* (2008) Environmental isolation of black yeast-like fungi involved in human infection. Stud Mycol 61: 137-144.

Vincent, W.F. (1988) Microbial Ecosystems of Antarctica (Studies in Polar Research). Cambridge University Press, Cambridge.

Vincent, W. (2000) Evolutionary origins of Antarctic mycobiota: invasion. selection and endemism. Antarct Sci 12: 374-385.

Vishniac, H.S. (1985) *Cryptococcus friedmannii*. a new species of yeast from the Antarctic. Mycologia 77: 149-153.

Vishniac, H.S., and Kurtzman, C.P. (1992) *Cryptococcus antarcticus* sp. nov. and *Cryptococcus albidosimilis* sp. nov., basidioblastomycetes from Antarctic soils. Int J Syst Evol Microbiol 42: 547-553.

Vishniac, H.S. (2006a) A multivariate analysis of soil yeasts isolated from a latitudinal gradient. Microb Ecol 52: 90-103.

Vishniac, H.S. (2006b) Yeasts biodiversity in the Antarctic. In: Rosa, C.A., and Peter, G. (eds) Biodiversity and ecophysiology of yeasts. Springer. Berlin. pp 420-440.

Walker, J.J., Spear, J.R., and Pace, N.R. (2005) Geobiology of a microbial endolithic community in the Yellowstone geothermal environment. Nature 434: 1011-1014.

Walker, J.J., and Pace, N.R. (2007) Endolithic microbial ecosystems. Annu Rev Microbiol 61: 331-347.

Wang, Q.M., Yurkov, A.M., Goker, M., Lumbsch, H.T., Leavitt, S.D., Groenewald, M., *et al.* (2015) Phylogenetic classification of yeasts and related *taxa* within Pucciniomycotina. Stud Mycol 81: 149-189.

Wei, S.T., Lacap-Bugler, D.C., Lau, M.C., Caruso, T., Rao, S., de Los Rios, A., *et al.* (2016) Taxonomic and functional diversity of soil and hypolithic microbial communities in Miers Valley, McMurdo Dry Valleys, Antarctica. Front Microbiol 7 Article 1642.

White, T.J., Bruns, T., Lee, S., and Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., and White, T.J (eds) PCR Protocols: a Guide to Methods and Applications, pp. 315-322. Academic Press, New York.

Whittaker, R.H. (1960) Vegetation of the Siskiyou Mountains, Oregon and California. Ecolol Monogr 30: 280-338.

Wierzchos, J., Ascaso, C., and McKay, C.P. (2006) Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. Astrobiology 6(3): 415-422.

Wierzchos, J., de los Ríos, A., and Ascaso, C. (2012) Microorganisms in desert rocks: the edge of life on Earth. Int Microbiol 15: 173-183.

Wynn-Williams, D.D. (1990) Ecological aspects of Antarctic microbiology. In. Marshall, K.C. (ed) Advances in microbial ecology, pp. 71-146. Springer, US.

Wynn-Williams, D.D., and Edwards, H.G.M. (2000) Proximal analysis of regolith habitats and protective biomolecules in situ by laser Raman spectroscopy: overview of terrestrial Antarctic habitats and Mars analogs. Icarus 144(2): 486-503.

Yung, C.C.M., Chan Y., Lacap D.C., Pérez-Ortega, S., de los Rios-Murillo, A., Lee, C.K., *et al.* (2014) Characterization of chasmoendolithic community in Miers Valley, McMurdo Dry Valleys, Antarctica. Microb Ecol 68: 351-359.

Zalar, P., and Gunde-Cimerman, N. (2014) Cold-adapted yeasts in Arctic habitats. In: Buzzini, P., and Margesin, R. (eds) Cold-Adapted Yeasts, pp. 49-74. Springer, Berlin.

Zhang, Y.J., Li, H.C., and Zhao, Z.W. (2012) Tolerance and Accumulation of Heavy Metals by *Exophiala pisciphila* Strain Isolated From Plant Roots Growing in Metal Polluted Soils. Soils 3: 017.

Zucconi, L., Onofri, S., Cecchini, C., Isola, D., Ripa, C., Fenice, M., *et al*. (2016) Mapping the lithic colonization at the boundaries of life in Northern Victoria Land, Antarctica. Polar Biol 39(1): 91-102.

AKNOWLEDGEMENTS

In everyone's life, a time when wishes do come true seems to be there. I am almost at the end of my PhD journey and there are no rights words to express the feelings when a dream is turning to a reality. It was a true pleasure to look back and to express gratitude to all those whom without their help, encouragements and support, this thesis have never been possible. All of these people really deserve an acknowledgement and now it is time for do that.

First of all, I would like to express my special appreciation to one of my supervisor Prof. Laura Selbmann for giving me the chance to work on this scientific field and for being an enthusiastic and engaging supervisor. Sometimes in life you are lucky enough to come across people that are kind, generous and genuine plus talented and this is exactly what you are.
It was an enormous pleasure work with you in the past years and thank you for giving me the opportunity to continue my research even today and for believing in me anytime, your advices on research as well as on my career and future are priceless. Thank you also for your knowledgeable and flexible supervision, but especially for encouraging me and instilling me the passion, determination and enthusiasm for this job, even in hardest days and thank you also for allowing me to grow as a research scientist. Your guidance, endless-patience and support always motivated me, from start till end, to do my best.

I'm extremely grateful to my other supervisor, Prof. Laura Zucconi. Thanks Laura for always helping and supporting me, from scientifically and non-point of view; thanks for all useful discussions, never-ending encouragement and motivation throughout this journey. Your guidance for experimental design and manuscript writing were really outstanding.

I would like to express my sincere esteem to Prof. Onofri for creating this great research group and gratitude for welcoming me in, thank you for taking care of all of us anytime.

My extreme admiration and gratitude also goes to Prof. Jason E. Stajich and his excellent research group and for accepting and hosting me in his lab. at University of California, Riverside for eight months and for all fruitful discussion throughout my stay. That was a lifetime experience and I really want to thank you for warmly and generously welcoming me in your team, for letting me feel at home and for believing in me and in my research project, demonstrating always your esteem. I am grateful for your expert support towards bioinformatics and data analysis, for your incredible help and in-depth discussion about results whenever I was in Riverside, but even after my comeback. Your work on genome sequences assembly, annotation and SRA submission was inestimable and your contribution on this thesis was priceless.

I truly thank my thesis examiners for having accepted to review it: Prof. Benedetta Turchetti (University of Perugia, Italy) and Dr. Lucia Muggia (University of Trieste, Italy). Your insightful comments greatly strengthened the quality of this thesis.

I wish to thank the Italian National Program for Antarctic Researches (PNRA) for funding Antarctic sampling expeditions and my PhD research and the Italian Antarctic National Museum (MNA) for supporting the Mycological section on the MNA for preserving rock samples I analyzed and fungal strains I sequenced in this study, stored in the CCFEE (Culture Collection of Fungi From Extreme Environments).

Aldo a good support system is important for surviving during the PhD journey, and I was lucky to have support from all of my PhD students colleagues (Clarissa, Eleonora E., Eleonora M., Claudia, Fabiana and Federica); thank you guys for your companionship and good times during past years and for making them comfortable by various ways.
I owe a special thanks to Claudia, we have spent the last several years inspiring one another and holding each other up as we navigate our lives as researches and people. We've laughed till we've cried and we've cried till we've laughed, and our lives have been enriched beyond measure by one another. Let's remember that we can accomplish so much more together than we can apart.

I am so thankful to all undergraduates who joined our lab, I was lucky to be surrounded by such wonderful people such as Valeria, Alessia, Ilaria, Valentina, Federica, Flavio and Daniele who made these years and PhD everyday life very pleasant, even after working hours. I am sure you will achieve great things in your future.

Thanks Caterina and Daniela for helping me during different stage of this PhD journey and for your encouragement during my surges.

My thankfulness goes also to Bruno Bellisario; I really appreciated your patience on my statistical question. Your feedback certainly helped me.

I would like to thank Prof. Daniele Canestrelli and Prof. Roberta Cimmaruta for their guidance and for carefully coordinating the XXX PhD course.

My sincere esteem and gratitude goes to Prof. Katja Sterflinger (University of Natural Resources and Life Science, Austria) for kindly hosting me one month in her lab. to introduce me to the Next-Generation Sequencing approach. I would like thank all her staff, in particular Dr. Guadalupe Pinar for carefully taking care of me and for all help and suggestions during and after my stage; Ksenija Lopandic to show

me the experimental design of Whole-Genome Sequencing by Ion Torrent; Christian Voitl for teaching me the best protocol to get high-quality DNA; also thanks to Dr. Barbara Blasi, Donatella Tesei and Justyna Adamiak for the time spent together during those weeks.

I really want to thank the Institute for Integrative Genome Biology (University of California, Riverside), where all sequencing analysis have been performed. In particular thanks to the Director Glenn Hicks for all your suggestions, patience and for planning and optimize the best approach for Illumina Miseq; another big thanks goes to the staff, in particular to Holly Eckelhoefer for being extremely patient during the experimental design and for letting me assist to all steps.

I am grateful to the Alfred P. Sloan Foundation Built Environment Program for developing and providing me the 16S and ITS primers and to the High-Performance Computing Cluster at the University of California-Riverside to support all data analyses.

I also would like to acknowledge Prof. Rob Knight (University of California-San Diego, USA) and Prof. Henrik Nilsson (University of Gothenburg, Sweden) for kindly inviting me at QIIME/QIITA and UNITE workshops. Attending to these courses was very helpful during bioinformatics analyses.

Thanks to my American Lab: Marco, Nat, Derreck, Sawyer, Jinfeng, George and Jericho for the time spent together and all celebrations, I am grateful for your help both at professional and personal levels. Thank you Sawyer for your bioinformatics skilled assistance and competence before and after genomes sequencing. Thank you Nat for being "my angel", your absolute patience and generosity, for helping me anytime, I also really enjoyed your company during the study and throughout our joint sampling trips.
I am extremely grateful to the people I have met during the American experience, for making that a lifetime one and for becoming my family during those months. Thanks Rick, Angelo, David, and Lorena for our trips, holidays, excursions, adventures, cheers, dinners and for everything we made together. I owe a unique acknowledgment to Deborah Pagliaccia and Georgios Vidalakis for welcoming me with open arms to your home to celebrate the Easter Italian-style.

At the last but not the least, my sincere gratitude goes to my friends and my family; I would not have made it, without your encouragement, your endless love and boost. I truly thank all of you for standing by me, even when it was really impossible. A special thanks go to my little niece Anita, when I looked at your smile, I always felt high-spirited.