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Introduced and indigenous fungi of the Ross Island historic huts and pristine areas of Antarctica

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Abstract This review summarizes research concerning Antarctic fungi at the century-old historic huts of the Heroic Period of exploration in the Ross Dependency 1898–1917 and fungi in pristine terrestrial locations. The motivation of the research was initially to identify potential fungal causes of degradation of the historic huts and artifacts. The research was extended to study fungal presence at pristine sites for comparison purposes and to consider the role of fungi in the respective ecosystems. We employed classical microbiology for isolation of viable organisms, and culture-independent DNA analyses. The research provided baseline data on microbial biodiversity. Principal findings were that there is significant overlap of the yeasts and filamentous fungi isolated from the historic sites, soil, and historic-introduced materials (i.e., wood, foodstuffs) and isolated from environmental samples in pristine locations. Aerial spore monitoring confirmed that winter spore counts were high and, in some cases, similar to those found in summer. Microbial diversity varied between the three Ross Island historic sites, and one historic site showed noticeably higher diversity, which led to the conclusion that this is a variable that should not be generalized. Cultured fungi were cold active, and the broader scientific significance of this finding was that climate change (warming) may not adversely affect these fungal species unless they were out-competed by new arrivals or unfavorable changes in ecosystem domination occur.

Keywords Terrestrial · Climate change · Biodiversity · Adaptation

Introduction

Fungi have been isolated from a wide variety of locations in Antarctica (Vishniac 1996). Onofri et al. (2004) reported that in Antarctica, 0.6% of the known fungal species were water molds (kingdom Chromista) and 99.4% were composed of true fungi including yeasts (unicellular organisms) and filamentous fungi from the phyla Chytidiomycota, Zygomycota, Ascomycota, and Basidiomycota. Paleobiological and paleoecological investigations have demonstrated the presence of Antarctic fossil fungal biota (Taylor and White 1989). Diversity of fungi in the fossil record of the Triassic and Jurassic Periods, as well as the potential relationships that existed among the major groups of fungi, was described by Taylor and White (1989) and included ancestors of endogonaceous mycorrhizal associations, which are widespread today. However, the presence of fungi in extensively degraded organic material (e.g., peat) suggested that they were primarily saprophytic and functioned as major decomposers (Kidston and Lang 1921; Stubblefield and Taylor 1983; White and Taylor 1988). Taylor and White (1989) introduced the hypothesis that the

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fungi in the fossil record were terrestrial saprophytes, degrading organic materials, and evolved on shorelines and in swamps, where organic materials accumulated prior to the evolution of land plants.

Non-Antarctic organic materials were introduced, along with new exotic micro-organisms, so-called aliens that either resided on or had colonized the organic materials, with the establishment of the base sites of the Heroic Era of exploration. The first confirmed and undisputed human landing in Antarctica was 1895 when a Norwegian whaling expedition landed at Cape Adare. One of the Norwegians, Carston Borchgrevink, returned to lead the Southern Cross Expedition (1898–1900), and its remnant prefabricated huts, named after Borchgrevink's mother "Camp Ridley," make Antarctica the only continent with its original dwellings still extant. The British National Antarctic (Discovery) Expedition (1901–1904) led by Robert F. Scott assembled a prefabricated wooden building of timber that originated from Australia, commonly referred to as Discovery Hut, at Hut Point on Ross Island to shelter and store supplies for 48 men for 3 years. Discovery Hut was used extensively by the latter expeditions in the Heroic Era as a key stepping-stone to the southern latitudes and a shelter for those who returned from the South. The British Antarctic (Nimrod) Expedition (1907–1909), led by Ernest Shackleton, followed in 1908 with a prefabricated building from East London, England, the Nimrod Hut, which was assembled at Cape Royds to house a shore party of 15 men. Scott returned in 1911 on the ill-fated British Antarctic (Terra Nova) Expedition (1910–1913). This 25-person expedition erected a large prefabricated building, Terra Nova Hut, which originated from London, England, at Cape Evans to provide accommodation and an additional smaller structure that was framed in wood and lined with asbestos sheeting for taking magnetic observations.

The Heroic Era expeditions had the primary goals to discover new land and to be the first to reach the Geographic South Pole, the South Magnetic Pole as well as address scientific objectives. When the expeditions ended and the relief ships arrived, a rapid exodus allowed only essential items to be returned to England. The huts and thousands of items including food stores and fuel depots with unused containers of petroleum products, asbestos materials, and diverse chemicals were left behind (Blanchette et al. 2004a). The three Ross Island historic huts were subsequently occupied by Shackleton's Ross Sea party (under Aeneas Mackintosh) in 1915–1917. The huts were abandoned when the last expeditions left in 1917 and were not revisited until 1947. After establishment of the United States (later named) McMurdo Station in 1955, the "Heroic Era" huts were visited periodically until the early 1960's. Since then, conservators, tourists, base staff, and scientists have visited the huts regularly. Accordingly, decades of human activities have affected these areas. As Discovery Hut is in close proximity (<500 m) of McMurdo Station, possible inputs to this site unique from the other historic sites included windblown debris and foot traffic. Early work on the fungi found in association with the Heroic Era historic huts focused on the long-term survival of organisms in the food supplies and the horse-associated materials (Meyer et al. 1962, 1963; Nedwell et al. 1994).

Long-term survival of any fungi in Antarctica required capability to exist and proliferate in Antarctic conditions, temperature being a major but not the only consideration. Cold tolerant organisms were capable of regeneration at cold temperatures and were defined by Gerday et al. (1997) as psychrophiles or psychrotrophs. A psychrophile was defined as an organism capable of growth at or below 0°C but unable to grow above 20°C, whereas a psychrotroph was capable of growth at around 0°C and also grew well above 20°C (Gerday et al. 1997). Many fungi isolated in Antarctica were cold tolerant strains of mesophiles adapted to grow at temperatures as low as 1°C (Kerry 1990; Azmi and Seppelt 1998). Thomas-Hall et al. (2010) described three novel cold-adapted yeasts found in Antarctica, which were also found in the Italian Alps, and psychrophilic yeasts were isolated from Antarctica as described by di Menna (1960). Recently, Xiao et al. (2010) reported the psychrophilic ascomycete species, *Antarctomyces psychrotrophicus*, isolated from the soils of the maritime and continental areas of Antarctica, suggesting a wide distribution of this fungal species in Antarctica.

This review summarizes our understanding of cold adaptation, diversity, proliferation and impact of Antarctic fungi, from studies of pristine terrestrial locations and at the century-old historic huts of the Heroic Period of exploration in the Ross Dependency. Much of the review is taken from the results of research conducted between 1999 and 2011, which focused on the deterioration and conservation of the historic sites and environs. The research additionally addressed understanding the diversity and mechanisms of cold adaptation and proliferation of Antarctic fungi, using a multidisciplinary approach including microbiology, biochemistry, genetics, molecular biology, geochemistry, geomorphology, and bio-geography, and using molecular techniques to compare the identified fungi of pristine sites and introduced historic huts/artifacts, characterizing their activities and main roles in these environments.

Results and discussion

Study locations and methodology

Samples were taken from the environment (soils, scoria, sandstone, quartz, moss, air, feathers, fecal material, and

melt water) and Heroic Era-introduced non-Antarctic materials (wood, straw, paper, boxes, rope, burlap, textiles, leather, and foodstuffs including flour, peas, beans, milk powder, butter, and biscuits). Heroic Era-introduced materials were sampled at Cape Royds, Cape Evans, Cape Adare, Hut Point, Cape Crozier, and Granite Harbor. Samples were also taken from a wooden crate introduced in November 1959 at New Harbor by scientists who used it as a makeshift workspace and kitchen, which was used intermittently during the past 43 years. This crate remained at the site with various materials left in and around it, and its study expanded the knowledge of microbes colonizing wood brought into the polar environment and provided additional information on deterioration and decomposition processes occurring in Antarctica.

Soil samples were obtained from the historic sites of Ross Island, from the ice-free mountainous regions at Mt. Fleming, which contain significant amounts of fossilized wood, Allan Hills, McKelvey Valley in the Upper Dry Valleys as well as the Lake Fryxell Basin in the McMurdo Dry Valleys (the latter collected by Professor Diana Wall, Colorado State University). Historic environs sampling details were given in Blanchette et al. (2004b), Held et al. (2006), Duncan et al. (2006, and 2008); soil sampling locations were given in Arenz et al. (2006), Pointing et al. (2009) and Arenz and Blanchette (2011); air sampling details were given in Duncan et al. (2010). Details of biodiversity plots were described in Arenz et al. (2011). Approximately, 100–200 g of soil were taken with a sterile scoop at each sampling site. Splinters (<2 cm × 0.5 cm × 0.5 cm) of structural wood and other wooden artifacts were taken aseptically from inconspicuous locations and placed into sterile containers, and sterile swabs were wiped over any object where fungal growth was conspicuous (Fig. 1a, b), and also objects with no conspicuous fungal growth. All samples were placed in sterile bags or tubes and stored at below 0°C until processed in the laboratory.

Classical mycological isolation and taxonomic keys were used to identify fungi from the aforementioned samples, and molecular tools including the extraction and sequencing of deoxyribonucleic acid (DNA) were used. Also conducted for making species diversity assessments and identification of cryptic organisms were denaturing gradient gel electrophoreses (DGGE) using the internal transcribed spacer (ITS) regions of ribosomal DNA, as described by Arenz et al. (2006).

Environmental conditions in historic huts

Pasanen et al. (1992) showed evidence that the environmental conditions conducive to fungal growth were temperatures above 0°C and relative humidity above 80%. Held et al. (2005) demonstrated that the temperature and relative humidity (RH) of each of the interiors of the three Ross Island historic huts were quite different; Discovery and Terra Nova Huts demonstrated 0 and 569 h, respectively, for these conducive conditions for fungal growth during the 2-year period from 2000 to 2002. Terra Nova Hut had significant snow accumulation around the exterior of the hut leading to frost formation on the interior wall in several areas. As the temperatures increased to above freezing in the summer months, free water forms on wood surfaces and the moisture accumulation coupled with temperatures above freezing enabled fungal proliferation in those areas (Held et al. 2005). Held et al. (2005) demonstrated that each of the historic huts interiors can not be considered as a single environment, as micro-environments exist, evidenced from the considerably different results recorded by data loggers in different locations within a single hut. For instance, in the Terra Nova Hut, the location near the entrance was determined to be warmer and more

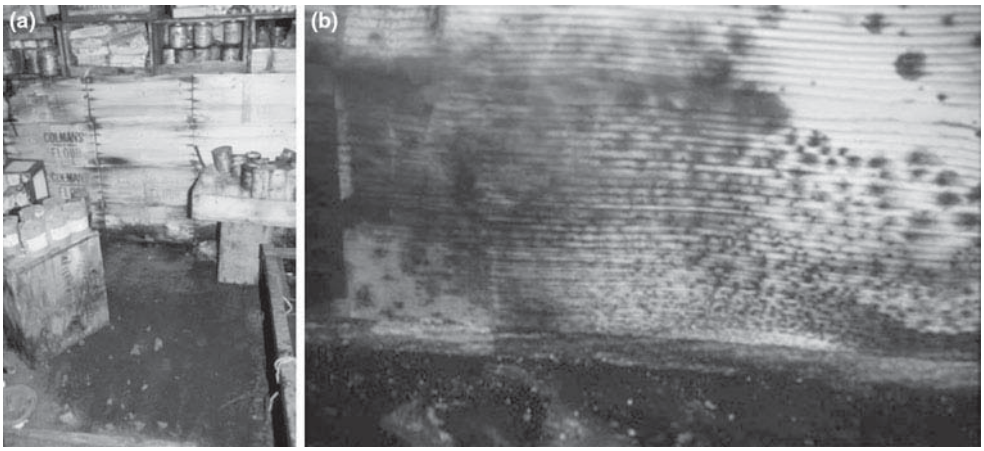

Fig. 1 **a** Crates in kitchen area of Terra Nova Hut **b** Close-up of crate showing significant black fungal mold

humid than other locations, and this was speculated to be due to close proximity to the door where it was likely influenced by more humid air in the annex. The data logger under a bunk recorded higher relative humidity, which may be due to decreased airflow in that area. Therefore, within the historic huts were created micro-environments, which must be considered as isolated ecosystems when considering biological communities and diversity. Whether or not human impact was significantly affecting the environments of the huts creating conditions more favorable for microbial colonization was studied; temperature and RH collected on the days before, during and after large numbers of visitors entered the huts suggested that the current visitation did not cause a rise in RH during the visit or after several days (Held et al. 2005). The amount of moisture in the air inside the huts appeared to be more greatly influenced by frost build up, snow melt water, lack of air movement within some parts of the huts, and exterior environmental influences.

Fungi isolated at historic sites

Despite the dry and cold climate of Antarctica, deterioration from both abiotic and biotic causes occurred at the historic sites, which led to concerns for the long-term preservation of the historic structures (Blanchette et al. 2002). Fungal degradation of wood and other organic matter was demonstrated to be common in the historic huts and surrounding introduced organic materials such as remnant foodstuffs and boxes (Held et al. 2003; Farrell et al. 2004, 2008).

Micromorphological examinations indicated just one type of wood decay, a soft-rot, present in deteriorated woods, as shown in Fig. 2. Soft-rot fungal decay was discovered in some areas of the Terra Nova and Nimrod Huts

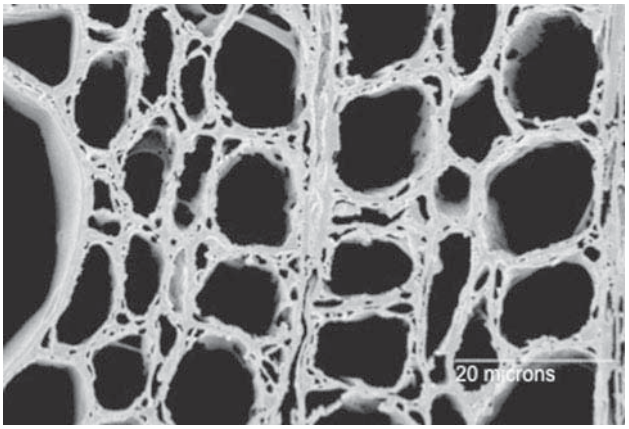

Fig. 2 Scanning electron micrograph of transverse sections of Terra Nova Hut exterior wood that was in ground contact, decayed by soft rot fungi

where the wood was in ground contact (Blanchette et al. 2004b). Pure cultures obtained from these woods were identified by morphological characteristics and phylogenetic analysis to be species of *Cadophora* including *C. malorum, C. luteo-olivacea,* and *C. fastigiata* as well as several previously undescribed *Cadophora* species designated *C. species H and C. species E* (Blanchette et al. 2004b). Prior to taxonomic revision by Harrington and McNew (2003), the *Cadophora* genus of the phylum Ascomycota was described as *Phialophora-like*. Though no soft rot decay was found in Discovery Hut, isolates of *Cadophora* sp. were cultured from samples of exterior wood of the hut that was in ground contact (Blanchette et al. 2004b; Arenz et al. 2006). Arenz et al. (2006) also isolated *Cadophora* species from five of eight petroleum contaminated soil samples around a historic fuel depot at the Terra Nova Hut (Blanchette et al. 2004a), consistent with the *Phialophora (reclassified as Cadophora)* which Aislabie et al. (2001) reported as the dominant species in oil contaminated sites in the McMurdo Sound region. Arenz et al. (2006) reported the most frequently isolated fungal genera from historic woods, and artifacts were *Cadophora* (21%), *Cladosporium* (18%), *Geomyces* (17%), *Cyptococcus* (8%), *Hormonema* (6%), *Rhoturula* (3%), and *Fusarium* (3%); the only taxon isolated with significant frequency (nine times) from wood or other artifact samples but not found in soil samples was *H. dematioides*.

In addition to *Cadophora* species, fungi reported from sampling artifacts and structural wood inside Terra Nova Hut included *Cladosporium cladosporioides, Hormonema dematioides, Penicillium echinulatum, P. expansum, Geomyces* sp. (Held et al. 2005), *Penicillium roquefortii*, and other *Penicillium* sp. (Duncan et al. 2006). In a detailed study of 30 samples taken at Discovery Hut, 156 filamentous fungi were isolated and seven were studied in detail, belonging to three genera, *Cladosporium, Geomyces,* and *Gliocardium* (Duncan et al. 2008). *Geomyces* spp. were isolated from a range of diverse samples including wood, straw, fur, biscuits, flour, and paper (Arenz et al. 2006).

Conservation work at Nimrod Hut, led by Antarctic Heritage Trust in 2007 and 2008, allowed for extensive sampling of historic artifacts and the hut in 2009; this sampling was done in sites with large amounts of organic matter and increased water due to snowmelt (Blanchette et al. 2010). From the 69 cultures of filamentous fungi found, the dominant genera were *Cadophora* (44%) followed by *Thielavia* (17%) and *Geomyces* (15%). *Thelavia* is known to cause soft rot, and this was its first report in the Ross Sea Region (Blanchette et al. 2010) though it had been isolated from lichen on King George Island (Stchigel et al. 2001). Additional fungi found included *Cladosporium, Chaetomium, Pezizomycotina, Onygenales, Nectriaceae,* and others. No filamentous basidiomycetes were

found from these isolations, but Blanchette et al. (2010) concluded the diversity of fungi and species richness found at the Cape Royds historic site was remarkably large and apparently influenced not only by the huge input of carbon from the introduced materials brought to the area by the early explorers but also the site conditions, as the hut was built in a protected location where moisture from snowmelt accumulated. Blanchette et al. (2010) completed a phylogenetic analysis of the *Cadophora* species isolated from the Nimrod Hut and the surrounding environment. Significant species diversity was demonstrated including *Cadophora malorum, C. luteo-olivacea, C. fastigiata,* as well as *C.* sp. 4E71-1, a *C. malorum*-like species, and *C.* sp. 7R16-1, and a *C. fastigiata*-like species. It was speculated that the nearby Adélie penguin colony was responsible for the *Cadophora* robustness since soft rot fungi thrive with increased nitrogen. Interestingly, *Cadophora* spp. also composed 35% of all isolates made from samples collected at Port Lockroy, built in 1944 directly on an active Gentoo penguin colony (Arenz and Blanchette 2009).

Fungi isolated at locations other than historic sites

Soft rot decay was found in the wooden crate that has resided at New Harbor since 1959, and *Cadophora* sp., *Cladosporium cladosporioides, Hormonema dematoides, Penicillium mali,* and *Geomyces* sp. were isolated from wooden splinters taken from the crate (Held et al. 2005).

Some species that caused degradation in the historic huts, such as *C. malorum, C. luteo-olivacea, C. cladosporioides*, and *Geomyces* sp. were also found in pristine (very remote) locations where soils were sampled (Lake Fryxell Basin, Mt.Fleming, and Allan Hills sites). *Geomyces* spp. were previously isolated from pristine areas (Kerry 1990) and areas with both little biotic influence and seal-influenced soil samples such as Peterson Island, off the Windmill Islands (Azmi and Seppelt 1998).

Arenz et al. (2006) compared the soils from around the historic huts in the Ross Sea area to those at the Dry Valley and mountain sites and showed that *Cadophora* and *Geomyces* were the two most commonly isolated genera in the Ross Island and New Harbor soils, whereas in the Dry Valley area and mountain soil samples, the most common fungi were the yeast *Cryptococcus* and *Epicoccum*, a cosmopolitan saprophyte of worldwide distribution. Nonetheless, some *Cadophora* species were identified from all of the pristine sites sampled except the Allan Hills.

In the Dry Valley samples, the only fungi detected by culturing methods were yeasts such as *Cryptococcus antarcticus, C. friedmannii, C. vishniacii*, and *Candida parapsilosis*, though the use of DGGE detected six additional yeast species and 8 additional filamentous fungi (Arenz et al. 2006). Identifications from samples taken from the Lake Fryxell Basin, Allan Hills, and Mt. Fleming sites had equal numbers of filamentous fungi (50%) and yeasts (50%). These equal proportions were not exactly the same as reported by previous investigators who found the fungal diversity of Dry Valley soils to have a higher abundance of yeasts (Vishniac 1996). Samples taken from the Ross Island and New Harbor locations produced a higher proportion of filamentous micro-fungi (76%) than yeasts (24%). Overall, from 164 samples taken from the three Ross Island historic huts, New Harbor, Allan Hills, Mt Fleming, and Lake Fryxell Basin, the major groups identified included filamentous ascomycetes (74%), basidiomycetous yeasts (21%), ascomycete yeasts (1%), and zygomycetes (1%) (Arenz et al. 2006).

Molecular analysis of soil, sandstone, and quartz at McKelvey Valley revealed previously unreported fungi. Ascomycota (Dothideomycetes and Sordariomycetes) and Basidiomycota (Cystobasidiomycetes) occurred only in endolithic and chasmolithic communities (Pointing et al. 2009). Connell et al. (2008) found a large proportion of yeasts isolated from soil in Taylor Valley, Mt Discovery, Wright Valley, and two mountain peaks in South Victoria Land and of these basidiomycetous yeasts comprised 89%. Connell et al. 2010 additionally found 13 basidiomycetous yeast strains in Taylor Valley, characterized as "obligate psychrophiles" since they failed to grow above 20°C. The finding of basidiomycetes in the Dry Valleys demonstrated significantly different fungal biodiversity than that found on Ross Island and the role of the basidiomycetes in the Dry Valley environments has yet to be explained.

Growth characteristics and extracellular enzyme activity of selected fungi

The roles of the fungi found in the historic huts was examined by considering their biochemistry, most specifically growth characteristics as a function of temperature and ability to produce the extracellular enzymes for secondary metabolism and degradation of wood. Duncan et al. (2006) demonstrated that filamentous fungi, identified to four genera and seven taxa, isolated from Terra Nova Hut, were cold active and all the fungi tested grew at 4°C and at 25°C. *Cadophora sp.*, *Geomyces sp.*, and *Cladosporium sp.* produced more biomass (based on weight of biomass per volume culture) when cultured at 4°C rather than 10, 15, 20, or 25°C and could sustain growth at 4°C after 3 repeated inoculations.

Endo-1, 4-β-glucanase (EC 3.2.1.4), a cellulase catalyzing the hydrolysis of cellulose, is a requisite enzyme for catalysis of wood degradation. Duncan et al. (2006) used a cellulose plate screening technique on a selection of filamentous fungi isolated from historic hut samples, and 26% of the fungal isolates were positive for cellulase activity.

Duncan et al. (2006) further demonstrated extracellular production of endo-1,4-β-glucanases at 4 and 15°C by *Cadophora*, *Geomyces*, *Penicillium,* and *Cladosporium* sp., thereby demonstrating that the enzyme responsible for wood degradation was made by fungal isolates when cultured at the cold temperatures typically experienced in the huts during the Austral summer. Enzyme activity was measured in units defined as micromoles glucose released per minute per mg of protein in the extracellular supernatant of the culture. *Cadophora malorum* produced 120 units when cultured at 4°C, which was the highest amount of activity recorded of any species at any temperature. *Penicillium roquefortii* produced the next highest amount of 105 units when cultured at 15°C. Of the 18 fungi tested for endo-1,4-β-glucanases activity production, eight produced more when cultured at 4°C than at 15°C, one produced at 4°C and not at 15°C, and one did not produce endo-1,4-β-glucanase activity when cultured at 4°C; these results indicated that the fungi were cold-adapted to produce endo-1,4-β-glucanase activity.

Statistical analysis showed that more biomass was required in a 4°C culture to produce the same units of endo-1, 4-β-glucanase activity as in a 15°C culture; there is yet no explanation for this, but it may be a result of cold adaptation, including a reduced efficiency of growth at 4°C, or perhaps as a result of stress, or different extracellular enzymes produced between the two temperatures. The extracellular endo-1, 4-β-glucanase complex was determined by SDS Page electrophoresis and hydroxyethyl cellulose zymogram activity gels, the latter showing two independent fungal isolates of *Penicillium roquefortii* produced different migrating cellulose-degrading bands when the fungi were grown at 4°C versus 15°C, indicating either differential gene expression or post translational modification as a function of temperature (Duncan 2007).

Survival and air sampling for spore detection

Robinson (2001) reported that Antarctic fungi used a variety of physiological mechanisms to survive including cold tolerance, accumulation of stress protectants such as trehalose and cryoprotectant sugars, production of polyol, change of cell membrane composition, secretion of antifreeze proteins, secretion of exopolysaccharides, and biochemical adaptation. Additional physiological adaptations were suggested including morphological mechanisms to ensure survival such as cold avoidance rather than tolerance with reestablishment in spring/summer from spores produced before winter (Marshall 1997), re-colonization from fungal material from outside Antarctica (Marshall 1998), acclimation due to slow cooling of the environment, abbreviated life cycles, dominance of sterile fungi in the cold environments, and dominance of dark hyphae due to melanin production (Onofri et al. 2004). The one major conclusion from studies of cold fungal communities is that there is not one specific adaptation that confirms survival in adverse conditions.

Airborne microorganisms were studied in Antarctica as an indication of robustness of the organisms, their mechanisms of spreading, and association with human impact in this environment (Marshall 1997). Duncan et al. (2010) air sampled during late Austral summers (January 2006, 2007, 2008, 2009) and Austral winter (2007) and demonstrated that the numbers of spores present after the winter in some locations were not significantly different than during late summer. *Cladosporium cladosporioides, Pseudeurotium desertorum, Antarctomyces psychrotrophicus, Geomyces, and Thelebolus* sp. were present in the air environment within the historic huts. A significant source of aerial spores was the hay fodder across from the main entrance of Discovery Hut; 26, 280 and 8,800 colony forming units per cubic meter of air were found in front of the fodder in summer and in winter, respectively, and the colonized yeasts in the hay fodder were detected by scanning electron microscopy. Duncan et al. (2010) concluded that demonstration of fungal aerial spores present in both summer and winter was consistent with the hypothesis that spores are both a method to survive unfavorable conditions and for dispersal of fungal material to new nutrient sources. The amount of fungal material collected by air sampling was expected to be greatest at the end of summer (January), since at that time, the fungi would have experienced the longest period of conducive temperatures and relative humidity in which to grow and produce reproductive structures (Held et al. 2005). This would also be consistent with increased human impact as the frequency and numbers of visitors increase during October–January with visitors peaking in January (Harrowfield 1989). Both summer and winter viability and quantity of fungal material were significant and the impact of human visitation appeared to be a negligible factor considering the amount of aerial fungal spores present in the huts (Duncan et al. 2010).

An unique class of hypolithic systems, composed largely of fungal filaments, in the Antarctic Upper Dry Valleys, was discovered (Pointing et al. 2009); these systems appeared to exist all year round as filamentous structures and could be an example of a key survival mechanism due to incorporation in the lithic niche. Pointing et al. (2009) proposed that endoliths, which supported the greatest diversity and number of phylotypes and which were very long-lived, acted as a reservoir for terrestrial microbiota.

Key drivers of fungal presence

The key drivers for fungal presence were studied by Arenz and Blanchette (2011) in a survey of sites in the Antarctic

Peninsula where they found fungal abundance was most consistently correlated with the percent carbon and nitrogen composition of the soil. Soil moisture, salinity, and pH were also analyzed and found to have less consistent correlations with fungal abundance and varied by location. *Geomyces* and *Cadophora* genera were among the most frequently isolated fungal genera in this study.

A concurrent multi-year biodiversity study based on a fungal baiting experiments using buried sterile wood and cotton as potential nutrient substrates was conducted at both Ross Island and Antarctic Peninsula locations (Arenz et al. 2011). The results of this study indicated that soil in direct contact with these substrates, 4 years after the baits had been buried, had 3–4 orders of magnitude higher fungal abundance than surrounding soils at non-baited study locations. These results indicated that lack of organic matter in most Antarctic soils may be one of the primary limiting factors affecting indigenous fungal populations. Though Vishniac 1996 reported that Antarctic fungi were either endemic or introduced, the term indigenous is suggested to be used since many isolated fungi were not technically endemic as Antarctica was not the only place they were found (Blanchette et al. 2008, Arenz and Blanchette 2009). Addition of nutrient rich substrates to these soils such as wood and cotton appeared to allow fungal population numbers to approach levels usually associated with temperate soils. It was also interesting that the two fungal genera with the highest abundance represented by substrate isolations in the baiting experiments were *Geomyces* and *Cadophora*. The finding that introduction of sterile, exotic nutrient sources to these soils had a larger effect on fungal genera that were widely believed to be indigenous than it did on possible exotic species supported the hypothesis that exotic fungal species were largely restricted by polar environmental conditions not directly related to nutrient availability. The fact that these two genera were most associated with growing in and around the historic huts on Ross Island provided further support for this. *Geomyces pannorum* is a species widely reported from many locations throughout Antarctica, previously suggested to be an indigenous (Vishniac 1996) and as it is keratinophilic, its abundance may be explained by the presence of feathers, rich in keratin, used as nutrient source (Marshall 1998). Arenz et al. (2006) concluded that the widespread occurrence of *Geomyces* strongly suggested that it has a role in decomposition and nutrient cycling in Antarctica. *Cadophora* spp were also reported on environmental samples, e.g., Antarctic mosses (Tosi et al. 2002), a mummified seal carcass (Greenfield 1981), skua feathers, and soil (Del Frate and Caretta 1990) from the Ross Sea area. Investigations that found *Cadophora* species attacking historic wooden structures in Victoria Land, the Peninsula Region of Antarctica, and in the Arctic demonstrated their wide distribution on the Antarctic continent (Blanchette et al. 2008; Arenz and Blanchette 2009; Jurgens et al. 2009).

Conclusions

Very few extremophile fungi have been isolated, and little is known about how they adapt and survive in unusual environments. Baseline data of fungi from multiple locations living in extreme conditions ranging from areas of relatively intense human activity occurring over the past 30 years as well as at pristine sites that have had limited human interactions has now been accumulated.

Our research has led to an understanding of the fungal diversity in both the historic hut environs and in pristine environments of the Ross Dependency with considerable overlap of the most frequently isolated species. For some of these fungal species, spore production was an over-winter survival mechanism. We addressed the question "How did endemic and indigenous Antarctic microorganisms respond to introduced organic materials, i.e., wood, seeds, leather, paper?". We found that endemic and indigenous Antarctic microorganisms responded to at least one introduced organic material, wood, by extensive colonization and use of wood as a source of carbon and nitrogen. Exotic fungal species were certainly brought to Antarctica with the exotic substrates (i.e., wood and other organic artifacts). The experimental evidence indicated that it was the indigenous soil fungi, *Cadophora* and *Geomyces* that dominated and were the major organisms that exploited these substrates as nutrients. Will climate change alter the diversity and hierarchy of dominant organisms? Further research will be required. However, the fact that many of the genera studied were cold tolerant and also capable of growth at higher temperatures suggests that climate change (warming) may not adversely affect them. New molecular methodology including next generation sequencing and functional gene microarrays will advance our understanding of the role these microbes have in the Antarctic ecosystem, where water content, temperatures and areas of increased carbon and nutrient inputs provide a unique field laboratory to study how global change affects polar organisms, their interactions and ecological processing. It is clear that any increase in degree-days as a consequence of any environmental changes will be utilized by the microorganisms to continue their degradative processes.

Since *Cadophora* sp. were found in soils in areas away from high human impact and because of the high ITS region genetic diversity of Antarctic specimens (Blanchette et al. 2002; Arenz et al. 2006), it is possible that these isolates may be native Antarctic saprophytes. Key research in the future will be to trace the emergence of the

Cadophora species in Antarctica on a geological time-scale—this would both provide for a better understanding of the past and a projection of the survival and proliferation of this species in Antarctica in the future. Most importantly, it may demonstrate a link between the saprophytic *Cadophora* species currently isolated in Antarctic soils and organic substrates and the terrestrial saprophytic fossil fungi proposed by Taylor and White (1989).

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