Diversity and Characterization of Wood Decay Fungi From Historic Wood in Antarctica

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Dedication

This dissertation is dedicated to my lovely wife Chriss. Thank you for your love, patience and support throughout this process.

Abstract

Compared to other biomes, very little is known about fungal diversity in Antarctica and how these important organisms function in this unusual ecosystem. The studies presented in this dissertation are focused on the fungi found in wood on Deception Island, Antarctica, their phylogenetic diversity and role as important decomposers in the polar environment. Chapter 1 describes the fungal diversity associated with historic wood in structures at Deception Island, Antarctica. A diverse fungal assemblage of known wood decay fungi is reported as well as the discovery of several undescribed species. The major group of wood decay fungi identified were species of *Cadophora* which have been previously found in other geographic regions of Antarctica causing a soft-rot type of decay in introduced woods. Unlike other areas of Antarctica that have been studied, several filamentous basidiomycetes (*Hypochniciellum* spp. and *Pholiota* spp.) were also identified that have different modes of degradation including brown and white rot. The conservation of these historic structures poses difficult challenges because of their polar location. Management issues to help preserve the historic woods are considered in Chapter 2. The hostile environment of an active volcano at Deception Island also presents unique issues to consider in protecting the historic whaling station and other structures. Lahars (mudslides from volcanic activity) have partially buried many of the structures. The buried environment and moist, warm soils are conditions conducive for fungal growth that are not found in other regions of Antarctica and facilitate deterioration by the fungi described. In addition, the diverse assemblage of decay fungi and many different

forms of aggressive wood degradation add to the difficulty of conserving these important polar heritage sites.

A number of studies have identified *Cadophora* species, mainly in association with wood, from many areas of Antarctica. A phylogenetic study (Chapter 3) using four gene regions identifies a diverse group of *Cadophora* spp. present at different sites in Antarctica (Ross Sea, Peninsula). *Cadophora malorum*, *C. fastigiata* sensu stricto, and *C. luteo-olivacea* were very similar phylogenetically in these gene regions. Undescribed species were also discovered that were closely related to *C. fastigiata* and these isolates were mainly from Deception Island. Decay studies showed that nearly all the species were able to cause a soft rot type of decay in birch wood wafers and were able to tolerate high levels of salt and copper sulphate. These studies support information that *Cadophora* spp. are well adapted to thrive in extreme conditions and appear to be important decomposers in these biomes.

Table of Contents

List of Tables

List of Figures

Introduction

Compared to other biomes, very little is known about fungal diversity and the biology and ecology of these organisms in Antarctica. Making this even more significant is the fact that microbial diversity is potentially more important in Antarctica because there is a lack of species redundancy compared to species rich systems (Adams et al. 2006). This makes describing fungal diversity and their functional roles a high priority. Human activity has undoubtedly played a factor in the establishment of fungi and their ecology in many parts of Antarctica since fungal introductions likely have taken place for over 100 years. During this time many exotic substrates have been brought to the continent by this activity. One of the major components of exotic materials has been wood, which was primarily used for structures to house early explorers as they made their explorations and travel to the pole (e.g., Shackleton and Scott) and other scientific activities. The wooden structures used in Antarctica served their purpose during the expeditions but were abandoned until organizations like Antarctic Heritage Trust, New Zealand (AHT-NZ) recognized the historical value of the structures on Ross Island and the physical materials that represented the early exploration of one of the most inhospitable places on Earth. We began to work with AHT-NZ on determining the condition of the huts and how best to preserve them for the future. Our research showed that although Antarctica is very cold and dry, there was still abiotic and biotic deterioration taking place (Blanchette 2003; Blanchette et al. 2010; Blanchette et al. 2004; Duncan et al. 2006; Held et al. 2005) and fungi were degrading the wood in specific areas. Management plans were subsequently drafted that detailed what actions should take place in order to preserve the huts for future

generations (A.H.T. 2003, 2004b, a). Research on the fungal species causing decay in the huts and other historic structures on the Antarctic Peninsula as well as in soils has continued over the past decade and has yielded a large culture collection (Arenz and Blanchette 2009, 2011; Arenz et al. 2010; Arenz et al. 2006; Farrell et al. 2011; Held et al. 2006). The past studies have showed that *Cadophora* species are the dominant fungal organism in soil and wood that has been brought to the continent at the sites we investigated. Some *Cadophora* species caused a soft rot in wood that, when the conditions are conducive, can cause extensive degradation of the wood. In most areas where wood was located, *Cadophora* spp. could be recovered, indicating that the genus plays an important role in decomposition. Some preliminary phylogenetic studies of *Cadophora* spp. were previously done when this genus was identified as a significant degrader associated with wood in Antarctica (Harrington and McNew 2003). However, the phylogenetic examination of the many different *Cadophora* spp. isolates from various locations in Antarctica had not been completed and is the objective of one chapter in this dissertation.

Investigations of the fungal diversity at Deception Island have provided the opportunity to identify fungi from this extreme environment that have a detrimental effect on the historic structures. Deception Island, Antarctica, which makes up part of the South Shetland Islands near the Antarctic Peninsula, has also had significant human influences. It began with a whaling station, which was followed by British military activities and later additional structures from the British Antarctic Survey. Many of these wooden buildings still exist today and are listed and protected as International Historic Sites and

Monuments 71 and 76. Although Deception Island is one of the hot spots for biological diversity in Antarctica, little to nothing was known about the fungal community that exists on Deception Island. The studies presented in this dissertation describe fungal assemblages associated with historic wood and other artifacts, provide information necessary for long term management to conserve these sites and present new information on the biology and ecology of these extraordinary organisms living in one of the Earth's most extreme environments.

Chapter 1.

Deception Island, Antarctica harbors a diverse assemblage of wood associated fungi

Introduction

Deception Island, part of the South Shetlands, is a small Antarctic island with unique ecological characteristics, geological features and a rich historical past. The island is an active volcano that has a flooded caldera with narrow entrance to the interior (Fig. 1). This geologic feature is what early sealers and whalers utilized for protection from the open ocean when they visited the island as early as 1820. Historic wooden structures still exist on the island today and are listed as Historic Sites and Monuments. Hektor whaling station (Norwegian) on Whalers Bay was established in 1906 as a land based operation and numerous factory whaling ships used the harbor in subsequent years. Later, in 1944 following the crash of the whale oil market, the British used the site and added a wooden building called Base B. Following that, British Antarctic survey used the site as a base for aerial surveys of the Peninsula, at which time a runway was made and airplane hangar built. Many buildings, structures and remnants from the whaling station and later activities on the island are still present today in varying stages of deterioration.

Pendulum Cove, approximately 4 kilometers north of Whalers Bay is the location of an additional historic site, the destroyed Chilean base, Presidente Pedro Aguirre Cerda Station (Fig. 1). It was built in 1955 and used until 1967 when it was destroyed by volcanic activity. While very little of the station is remaining, the site is also listed as a Historic Site and Monument.

The geological history of the Island includes an eruption of the volcano approximately 10,000 years ago that created Port Foster (the interior bay) (Olsacher 1956). Numerous other eruptions have occurred, including several during the past two

centuries, that have changed the topography of the island significantly. Subsequently, ash has covered glaciers, which occupy 57% of the island. Many areas on the island have obvious geothermal activity that includes fumaroles, heated soils and steaming beaches. These unusual environmental conditions in the polar environment have provided the opportunity for microbial activity to take place and cause extensive decay in the historic wooden structures. It also provides an opportunity for comparative analysis of microbial diversity with other areas of Antarctica where foreign nutrient sources have been deposited, such as the historic expedition huts of the Ross Sea and historic structures on the Antarctic Peninsula. Previous research of fungal diversity and degradation of wooden structures and artifacts in Antarctica has shown that fungi are important decomposers despite the extreme environment (Arenz et al. 2006; Arenz and Blanchette 2009; Arenz et al. 2010; Blanchette et al. 2010; Duncan et al. 2006; Held et al. 2005; Held et al. 2006). The only type of wood decay previously found occurring in Antarctica has been classified as a soft rot caused by ascomycetes and occurs primarily in wood that is in contact with soil on Ross Island (Arenz et al. 2006; Blanchette et al. 2004; Blanchette et al. 2010; Held et al. 2006) and on the Antarctica Peninsula (Arenz and Blanchette 2009). The common types of decay in temperate and tropical areas, brown and white rot caused by basidiomycetes, have not previously been found.

This study was done to identify fungi associated with and causing decay of historic wood on Deception Island and to better understand the microbial diversity and decomposition processes that exist in this unusual polar environment where soil temperatures range from freezing to 90°C. Since this island has had a long history of

having organic materials introduced, these investigations also provide new information on the alien and indigenous organisms established at the site.

Materials and methods

Samples were collected from areas that appeared decayed on wooden structures and artifacts at Whalers Bay and Pendulum Cove, Deception Island (62°57'S, 60°38'W). Small segments (approximately 1 mm x 0.5 mm) of wood were collected and placed in sterile plastic bags and kept cool while transporting them back to the laboratory. Under sterile conditions in the laboratory, the wood samples were cut and placed onto the following types of growth media: malt extract agar (1.5% Difco malt extract), malt extract agar (1.5%) amended with 0.5g of streptomycin sulfate and a semi-selective medium used to culture basidiomycetes (2.0% Difco malt extract, 0.2% yeast extract, 0.006% benlate with 0.2% lactic acid and 0.001% streptomycin sulfate added after autoclaving) (Worrall 1991). Isolations were made from 188 and 30 samples from Whalers bay and the Chilean base, respectively. Isolates were cultured at 8 and 20°C for several weeks after which transfers were made to another plate to obtain pure cultures. DNA was extracted from fungal cultures using an adapted chloroform procedure (Arenz and Blanchette 2011). The internal transcribed spacer (ITS) region of ribosomal DNA was targeted for PCR amplification with the primers ITS1 + ITS4 and LROR + LR5 for large subunit amplification (White et al. 1990). PCR amplifications were done using Amplitaq Gold PCR Master-mix (Applied Biosystems, Foster City, CA) and 1µl of template DNA using the following parameters: 94°C for 5 min, 35 cycles of 94°C for 1

min, 50°C for 1 min, 72°C for 1 min and a final extension step of 5 min at 72°C. PCR amplicons were visualized on a 1% agarose gel using SYBR green 1 (Life Technologies, Grand Island, NY) prestain and a Dark Reader DR45 transilluminator (Clare Chemical Research, Denver, CO). Primers used for PCR were used for sequencing reactions on a ABI Prism 377 automated DNA sequencer using a ABI PRISM Dye Terminator Cycle Sequencing Ready reaction kit (Applied Biosystems). Consensus sequences were assembled using CHROMASPRO software version 1.33 (Technelysium Pty Ltd.) and were subjected to BLAST searches against those in NCBI GenBank to determine the best match. Sequences of ex-type cultures to queried sequences were downloaded for multiple sequence alignments. Multiple sequences were aligned online (http://mafft.cbrc.jp/alignment/software/) using the auto strategy in MAFFT version 7 (Katoh and Standley 2013).

Phylogenetic trees were generated using PAUP version 4.0b10 (Swofford 2002) for neighbor joining and maximum parsimony analysis. Alignment gaps were treated as missing data and all characters were treated as unordered and of equal weight. Maximum parsimony analysis was performed using the heuristic search option and TBR as the branch-swapping algorithm. Support for tree branch nodes was calculated by 1000 bootstrap replicates (Hillis and Bull 1993).

Decay studies using fungal cultures isolated from samples were carried out in microcosms over a 16 week period. Isolates were selected after identification from BLAST searches. Glass filters (55mm) were sterilized by autoclaving and placed in 100 mm petri plates containing media. A malt yeast agar (2% malt extract agar, 0.2% yeast

extract) was used for the basidiomycete cultures and a minimal selective media for soft rot fungi (Worrall et al. 1991) was used for the ascomycete cultures. Wafers measuring 1.5 x 1.5 x 0.3 cm were cut from sound birch and pine wood blocks and dried at 105°C for 24 hours and weighed to determine dry weight. Wafers were then hydrated and sterilized by autoclaving before placing on glass filters in decay microcosms. Plugs (0.4 mm) were transferred from growing fungal cultures and placed on the medium surface adjacent to the filter. After a 16 week incubation period, 10 wafers from each treatment were removed and oven dried to determine biomass loss. Two wafers from each treatment were not oven dried and used for micromorphological study.

The methods for the decay study using ascomycete fungi were the same as the basidiomycete study but a different medium was used. Instead of malt yeast agar, a minimal media consisting of 1.5 g NH_4NO_3, 2.5 g KH_2PO_4, 2 g K_2HPO_4, 1 g $MgSO_4$-$7H_2O$, 2.5 g glucose and 0.1 g thiamine per liter was used (Abrams 1948; Worrall et al. 1991). In addition, instead of using water to hydrate wafers, a solution of same ingredients as the medium exclusive of the agar was used. The incubation period was also 16 weeks.

Wood samples for fungal decay observations were prepared for scanning electron microscopy (SEM) by infiltrating with a 25% TBS™ Tissue Freezing Medium™ (Triangle Biomedical Sciences, Durham, NC, U.S.A.) under vacuum followed by mounting on brass stubs, freezing at -20°C and sectioning in a cryostat freezing microtome. Samples were cut transversely to prepare a clean surface for examination. Cut samples were thawed and rinsed several times in water, air dried before mounting on

aluminum stubs with carbon tape and coated with gold using a sputter coater. Samples were viewed using a Hitachi S3500N scanning electron microscope to determine characteristics of decay and signatures of fungal colonization in the cell wall structure.

Results

Fungal isolation attempts from samples collected at Deception Island yielded 326 isolates from 249 samples. The majority (79%) of the isolates belonged to the Ascomycota and were comprised of 53 taxa, followed by 11 taxa (24%) in the Basidiomycota and 4 Zygomycota taxa (6%). Some of the most relatively abundant genera obtained in the Ascomycota were *Cadophora* (29%), *Phialocephala* (10%), *Lecythophora-coniochaeta* (9%), *Cosmospora* (8%) and *Phoma* (8%). A large number of *Cadophora* species were found including *C. fastigiata* (55%), *C. malorum* (22%), *C.* sp. *NH1-2* (13%), *C. luteo-olivacae* (9%) and *C.* sp. 5R24-1 (2%). Among the most relatively abundant genera in the Basidiomycota were *Hypochniciellum* (55%), *Pholiota* (18%), *Cerinosterus* (11%) and *Tulasnella* (8%). In addition, there were four isolates (including both Ascomycota and Basidiomycota) that matched below 95% sequence identity in GenBank to known taxa: *Mollisia minutella*, *Epichloe typhina*, Uncultured root-associated fungus clone and *Postia* sp. 5AJMH-2010 with a percent sequence identity of 92, 89, 92, and 86 respectively. LSU sequences were used in addition to ITS sequences for *Hypochniciellum* isolates because the nearest percent sequence identity for ITS BLAST searches was a 85% match to *Amyloathelia crassiuscula*. However, BLAST searches using LSU sequence data showed a 99% sequence identity to *Hypochniciellum molle* for which ITS sequence is not

in GenBank. Due to the fact that the LSU region does not resolve species effectively and that sequences of the ITS region for this or other *Hypochniciellum* species were unavailable, the species of this fungus remains uncertain. However, phylogenetic analysis of LSU sequences reveal the isolates most closely resemble *H. molle* in this gene region (Fig. 2). Analysis of the ITS gene region from *Pholiota* spp. sequences form several clades, separate from identified species. One clade is comprised of isolates exclusively from the Chilean base that group with *P. multicingulata*. The second clade has both an isolate from the Chilean base (2Di94-5) in addition to Whalers Bay (isolated from Biscoe house, Di267-2). A second isolate from Biscoe house, Whalers Bay (2Di41-2) does not group with any other isolates from Deception Island or known isolates in GenBank (Fig. 3).

Some of the most common genera among all Ascomycota and Basidiomycota isolated were *Cadophora* (20%), *Hypochniciellum* (13%), *Phialocephala* (7%), *Cosmospora* (5%), *Phoma* (5%), *Pholiota* (4%), *Lecythophora* (3%), *Coniochaete* (3%) and *Cerinosterus* (3%). Most of the taxa isolated from the Chilean base samples (separated by approx. 4 km) were also isolated from Whalers Bay, with the exception of *Arthrobotrys superba*, *Coprinellus micaceu*, *Sistotrema brinkmannii*, *Helotiales* sp. NK251and a fungal endophyte strain (Table 1). However, many that were isolated from Whalers Bay were not isolated from the Chilean base.

Analysis of historic wood at the site indicated that decay fungi are active and extensive decay has taken place. Much of the wood that was near the soil or buried in the soil was severely degraded. However, significant decay was also occurring in many

areas well above the soil (Fig. 4). This occurred extensively on the north side of the Biscoe House and also in the wooden boats on Whalers Bay beach. Microscopic observations of decayed wood samples indicated that three major types of wood decay were found in samples taken at Whalers Bay and Pendulum Cove: white, brown and soft rot. Soft and brown rots were observed at both locations, while white rot decay was identified in wood samples from the Chilean base site and in all cases it was found in wood just beneath the soil surface.

Laboratory decay studies showed substrate weight losses for *Pholiota* sp. 131-2, *Pholiota* sp. 80-3, *Jaapia argillacea*, *Coniophora puteana*, *Hypochniciellum* sp. Di283-3, *Hypochniciellum* sp. Di17-1, *Cerinosterus luteoalbus* and control were 64 ±3.3, 39.9 ±2.0, 52.4 ±2.6, 34.3 ±4.3, 43.3 ±5.4, 36.4 ±2.7%, 0.5 ±0.6 and 0.3 ±0.2%, respectively, on birch and 31.5 ±3.5, 29.9 ±4.8, 30.4 ±2.6, 23.4 ±5.1,13.7 ±3.4, 16.7 ±3.7, 17 ±3.4, 1.4 ±0.2 and 0 ±0.2%, respectively, on pine wafers (Fig. 5). Scanning electron microscopy of decayed samples showed that *Pholiota* spp. and *Coniophora puteana* caused a white rot, degrading all cell wall material (Fig. 6). *Jaapia argillaceae* appears to cause a white rot type of decay but is unusual since it darkens the wood as decay progresses. *Hypochniciellum* spp. and *Coniophora puteana* cause a brown rot type of decay with decay patterns similar to other brown rot fungi found in temperate areas that degrade cellulose and hemicellulose (Fig. 6).

Laboratory decay studies showed weight losses on birch (*Betula* sp.) for *Phialocephala dimorphospora*, *Lecythophora hoffmannii*, *Lecythophora* sp., *Mollisia minutella*, *Mollisia cinera*, *Phoma herbarum*, *Cosmospora vilior* and control were 21.1

±0.69, 20.2 ±0.77, 18.9 ±0.80, 16.6 ±0.97, 10.6 ±0.53, 1.82 ±1.27, 2.1 ±0.46 and 0.28 ±0.18%, respectively. Weight losses of pine were 1.8 ±0.19, 2.5 ±0.31, 2.6 ±0.2, 2.1 ±0.2, 1.7 ±0.28, 2.2 ±0.22, 2.1 ±0.25 and 0.25 ±0.18%, respectively (Fig.7). Scanning electron microscopy analysis revealed unique decay processes in four of the species tested, excluding *C. vilior*, for which no decay was evident. *Lecythophora* sp., *M. minutella* and *P. herbarum* caused a Type 2 soft rot decay, in which the secondary wall layers of the wood cells are eroded, leaving only the middle lamella. *L. hoffmannii* , *P. dimorphospora* and *M. cinera* form cavities in the S_2 region of the cell wall, indicative of a Type 1 soft rot decay. Soft rot cavities are located within the secondary wall and the middle lamella is not affected (Fig. 8).

Discussion

The fungi reported in this study illustrate the diversity of fungi that are associated with wood in the historic structures and artifacts on Deception Island. Other studies on fungal diversity at different locations in Antarctica found similar phyla represented. Fungi isolated from Deception Island sites were, 79% ascomycetes, 15% basidiomycetes and 6% zygomycetes as compared to 82% ascomycetes, 15% basidiomycetes and 3% zygomycetes found on the Antarctic Peninsula (Arenz and Blanchette 2009) and 75% ascomycetes, 21% basidiomycetes and 1% zygomycetes at Ross Island (Arenz et al. 2006). While in a broad sense it seems the phyla represented are generally consistent among these polar locations, a closer look at the genera present shows a drastically different picture. Most significantly, nearly all of the basidiomycetes isolated from the

previous studies were comprised of yeasts whereas the basidiomycetes from Deception Island were filamentous wood decay fungi. Laboratory studies using these basidiomycete isolates showed that they cause a white or brown rot in wood. The occurrence of white and brown rot fungi in Antarctica is quite unique and has not been previously reported on continental or maritime Antarctica. However, other studies have reported decay attributed to saprotrophic basidiomycetes from sub-Antarctic Islands. Deception Island lies in an area that is considered maritime Antarctic, which is characterized as having a cold, moist climate and mean monthly air temperatures of >0°C for 3-4 months of the year (Aleksandrova 1980; Smith 1984). Pegler et al. (1980) reported *Trametes versicolor* (which causes a white rot type of decay) on wood brought to South Georgia that was used in the whaling station there. The wood was introduced, therefore it is likely that the fungus was brought with the wood, and not endemic. However, other decomposer basidiomycetes have been found that presumably exist naturally in bryophyte and grass ecosystems. Smith (1994) reported 37 basidiomycete taxa on South Georgia including species of *Collybia*, *Galerina*, *Omphalina* and *Coprinus* associated with plant litter. *Omphalina antarctica,* found on Deception Island*,* was the first described basidiomycete from maritime Antarctica and was associated with mosses (Singer 1957). While Deception Island is considered maritime Antarctic, it is clear that filamentous basdiomycetous fungi are present and are capable of degrading wood. In temperate and tropical areas filamentous decomposer basidiomycota are the common types of decay fungi found and in those biomes the basidiomycetes out compete and exclude soft rot fungi (Rayner and Boddy 1988). At Deception Island, conditions suitable for growth of

basidiomycetes may be abated enough to allow soft rot fungi to compete for the same resources.

The dominant basidiomycete species and the most common taxon isolated from samples in this study is a species of the brown rot fungus, *Hypochniciellum* sp. Arenz and Blanchette (2009) also isolated a similar fungus (referred to as fungal species BB1) from wood at Wordie House, another historic site on Winter Island on the Antarctic Peninsula. Fruiting bodies of this species are yellowish to pale cream and resupinate in form and were not observed in any areas where samples were taken. Mattsson et al. (2010) showed that *H. molle* was the dominant decay fungus identified in wood from structures on the arctic archipelago Svalbard. It has also been reported as causing deterioration in Norwegian and German buildings (Alfredsen et al. 2005; Schmidt and Huckfeldt 2011), on driftwood in Iceland (Hallgrimsson and Hauerslev 1995) and sporadically distributed in Italy (Bernicchia et al. 2008). It appears that this fungus is adapted to cold environments and may play an important role in nutrient cycling. The adaptive strategy of *H. molle* in cold climates may be the production of thick walled chlamydospores which likely aid in dispersal and survival. Its noted occurrence in Norway suggests a possible source of the fungus isolated in this study since much of the lumber used to build the original Norwegian Hektor whaling station likely originated from Norway. Microcosm studies showed that this fungus is an efficient brown rot causing extensive biomass lost during relatively short laboratory incubation periods. With the abundance of brown rot found at Deception Island and from the frequency with which this fungus was isolated, our study suggests that this is a major cause of the brown rot decay taking place on the

Island.

Fruiting of other basidiomycetes (*Pholiota* spp. and *Omphalina* spp.) have been reported on Deception Island (Singer 1967), which indicates that conditions can be conducive for other basidiomycete species to complete their life cycle. Singer (1967) described a *Pholiota* sp. fruiting body growing on one of the half buried wooden boats at the beach in Whalers Bay. This area is adjacent to thermally heated soil and water, which may have contributed to conditions favorable for fruiting. No fruiting bodies were observed during our field events to Deception Island for these studies. Based on past observations of fruiting bodies, it is interesting that Pholiota spp. was infrequent from samples in Whalers Bay, but was dominant at the Chilean base. The phylogenetic analysis revealing two, possibly three different species likely indicates several different introductions.

Other basidiomyceteous decay fungi were isolated that matched fungi that are commonly found in temperate regions. *Hypochnicium* sp. and *Hyphodontia radula* are known to cause a white rot type of decay (Gilbertson et al. 1975; Larsson et al. 2006), *Coniophora puteana* and a *Postia* sp. that has a close match to *Postia balsamea* are both ubiquitous brown rot fungi that cause significant problems in wooden structures and are important forest fungi in temperate regions. These species were only isolated from one sample each and indicates they are not highly prevalent. Based on high sequence identity matches to known species they were likely introduced. *Tulasnella* is a cosmopolitan genus that has saprotrophic and mycorrhizal characteristics (Preussing et al. 2010).

Cadophora species were the most commonly isolated fungi from wood at

Deception Island. The relative abundance of *Cadophora* spp. obtained in this study was similar to the amount found at other Antarctic Peninsula and Ross Island sites where other investigations studied fungi from other historic sites where wood was present. The distinction about Deception Island, however, is that the diversity of species of *Cadophora* is greater at Deception Island and several possible new species were found. This supports previous research showing the predominance of *Cadophora* spp. functioning as important decomposer fungi in the polar environment (Arenz et al. 2006; Arenz and Blanchette 2009; Arenz et al. 2010; Blanchette et al. 2004; Blanchette et al. 2010; Farrell et al. 2011; Held et al. 2006). While *C. malorum*, *C. fastigiata* and *C. luteo-olivaceae* have been identified in other biomes, their identification in this study points to the proclivity of this group to thrive in polar environments especially, when wood is present as a primary nutrient source. Most of the known *Cadophora* species identified (*C. fastigiata*, *C. luteo-olivaceae* and *C. malorum*) in this study have been shown to cause a Type 1 soft rot decay in wood, in which fungal hyphae create cavities in the S_2 layer of the wood cell wall by enzymatic degradation. Based on preliminary observations, it appears that there are differences in size of cavities produced by the different species and sub groups within the Type 1 soft rot group. More precise measurements are needed to characterize the different forms of soft rot found and whether this may be attributed to aggressiveness of different species. Further research is needed with several strains of each species to confirm this. Some soft rot fungi also cause another form of degradation referred to as Type 2 in which the cell walls are eroded from the inside of the lumen toward the middle lamella.

A large component of fungi isolated from wood at Deception Island was similar to genera that have been classified by other investigators as dark septate endophytes (DSE) associated with plant roots. These include species of *Coniochaete*, *Lecythophora*, *Leptodontium*, *Mollisia*, *Phialocephala*, and *Phoma*. DSE are generally categorized as endophytic fungi having dematiaceous septate hyphae and are restricted to plant roots. They are primarily Ascomycetes, have a wide host range, and have been identified in a many ecosystems ranging from polar to tropical regions (Jumpponen and Trappe 1998; Jumpponen 2001). The broad geographic and host range for these fungi suggests that they have low host specificity (Porras-Alfaro and Bayman 2011). Indeed, a mutualistic relationship has been identified between the much studied DSE fungus *P. fortinii* and species from Pinaceae, Cyperaceae, Ericaceae, Salicaceae, and Rosaceae (Jumpponen 2001) and have been identified in roots of nearly 600 plant species. Unlike true mycorrhizal fungi, DSE frequency in roots does not decrease with an increase in latitude (Upson et al. 2008). Instead, a high incidence of DSE have been found in roots of plants in numerous polar regions that have been studied (Christie and Nicolson 1983; Laursen et al. 1997; Ormsby et al. 2007; Treu et al. 1996; Upson et al. 2008) and appear to be the most widespread root-fungal association at these sites (Newsham et al. 2009). The abundance of DSE in high stress polar environments has led to the hypothesis that they confer tolerance and lead some to hypothesize that they may be more important to healthy ecophysiology functioning in plants than previously realized (Rodriguez et al. 2009). The delineation of DSE species is not well defined, either ecologically or taxonomically, and their function has not been well studied. They are most commonly

isolated from healthy plants but differ from mycorrhizal fungi in that they do not form arbuscules or coils in host roots to obtain nutrients (Newsham et al. 2009).

The study reported here reveals DSE fungi associated with wood, but the more typical niche on Deception Island could be association with local flora (mosses and grasses). Plants found in this area consist of herb-lichen-moss formations with only two vascular plants found on the island: a pearlwort, *Colobanthus quitensis* and a grass *Deshampsia antarctica* (Smith 1984), which DSE fungi have been found associated in different areas. Flora diversity on Deception Island is exceptional with 18 species of bryophytes and lichen that have not been found elsewhere in Antarctica as well as two species which appear to be endemic. In addition, the island has the largest known community of Antarctic pearlwort (Group 2002). Recently, studies have shown that several genera of DSE fungi (*Cadophora, Leptodontium, Lecythophora* and *Phialocephala*) were capable of decomposing bryophyte material (Day and Currah 2011).

Decay studies reported here with DSE species that were isolated from Deception Island show they are efficient decomposers of wood, capable of functioning solely as saprotrophs. Although Menkis et al. (2004) reported species belonging to the DSE genus *Phialocephala* isolated from a wide range of ecological niches, including healthy root tips, decayed coarse roots, live healthy looking stems, decomposing stumps, and fine woody debris, the saprophytic nature of this group of fungi is often overlooked. The capability to live as a saprotroph as well as an endophyte suggests they have highly specialized functions and pronounced ecological plasticity. In Polar environments where spore production, dispersal and colonization of new substrates may be difficult, fungi

with endophytic capabilities would be able to colonize the plant over a long period of time and then capture resources quickly once the plant tissue dies (Porras-Alfaro and Bayman 2011; Jumpponen et al. 1998). As saprotrophs, it appears that this group of fungi employ different decomposition mechanisms as seen in different patterns of decay (Fig. 8). *Mollisia*, *Lecythophora* , *Philocephala* and *Phoma* spp. all cause a soft rot type of decay, but they vary between Type 1 and Type 2 in birch wood. Very little biomass loss occurred on pine, suggesting that these fungi have specific requirements for certain woods (preference for hardwood vs. conifer) for decay to occur.

For nearly two centuries there have been many opportunities for alien fungi to be introduced to Deception Island. The strong anthropogenic effects over the past decades and those continuing today with tourists visiting the sites, has undoubtedly impacted the fungal ecology and diversity on the island. The likely avenue for many of the introductions of wood degrading fungi was the lumber used for buildings and for the wooden boats, barrels and other items. The presence of both brown and white rot at this location and not at other Antarctic locations suggests the environment at Deception Island influences what alien organisms survive.

There are also many reports of live domestic animals that were housed on the island (Smith 1996). In the early 1900's pigs were kept at the whaling station (Hacquebord 1992) as well as an occasional sheep or cow for a food source (Scott Polar Research Institute Archives, unpublished data). The Chilean Base had anywhere from 30-60 sheep brought to the station every year in addition to hens and an occasional pig or cow (Smith 1996). Hay and corn was also brought to feed the animals (Smith 1996). A large penguin

rookery is also located on the south shore of the island approximately four kilometers away from the historic structures in Whalers Bay. These animal populations present a large nutrient source (manure and guano) and points of introduction of microbes that would aid in colonization and persistence of introduced fungi, in an otherwise nutrient lacking volcanic soil.

Also noted by biological reports from the Falkland Island Dependencies Survey (FIDS) was the richness of vegetation around the station, presumably due to the massive nutrient input to the soil from whale carcasses washing ashore and left on the ground (Hacquebord 1992). However, later botanical surveys (1990 and 1991) indicated vegetation in these areas was not visible, likely due to the volcanic activity in 1967 that deposited ash and mud onto the area.

Conclusions

These findings show that all three known types of wood decay (white, brown and soft rot) are active and causing decay in the historic wood at Deception Island, Antarctica. This is in contrast to only soft rot fungi found at other locations in Antarctica. The dynamic nature of the ecosystem of Deception Island with soils that range in temperature from freezing to 90°C and the large amount of wood present at the site providing a carbon source apparently allows many of the introduced fungi to survive. The input of wood also appears to have influenced the indigenous population of fungi such as the DSE types found in native plants to expand their saprophytic existence and colonize wood. Fungal colonizers of the historic wood are causing extensive decay which will gradually result in

degraded and lost structures.

There are fungal isolates from this study which remain unidentified or with poor matches to sequences in GenBank, which suggests that some of these isolates may be indigenous to Antarctica. *Cadophora* spp. were the dominant group isolated from Deception Island as well as other previously studied sites in Antarctica, which further suggests this group of fungi plays an important role in cold ecosystems. Further studies on these fungi are warranted so that their ecology and biology may be better understood.

The wood associated basidiomycete assemblage identified in this study was a key difference to other historic sites previously studied in Antarctica. The frequency at which *Hypochniciellum* spp. were isolated and their presence in the Arctic shows that this genus is not only adapted to, but flourishes in maritime Polar Regions. Studies focused on this genus would perhaps uncover a wider distribution than previously known and aid in understanding its ecology.

Previous research has shown that fungal abundance of Antarctic soils is most positively correlated with the percent of organic carbon compared to other soil characteristics (Arenz and Blanchette 2011). The high degree of fungal diversity associated with historic wood at Deception Island indicates that this large organic carbon input is a driving factor for fungal abundance. In depth studies focusing on background fungal soil communities and plant associated fungi would aid in understanding wood introduced to Deception Island has affected fungal populations.

Table 1. List of taxa isolated in this study including the best BLAST match with percent identity and overall nucleotide overlap of the ITS gene region.

Best BLASTn Match	Percent Identity	Overlap	#	# of Samples		GenBank Accession #
				Whalers Bay	Chilean Station	
Ascomycetes						
Acremonium atrogriseum (AB540569.1)	99	515/518	6	6	-	KC514842
Antarctomyces psychrotrophicus (AJ133431)	99	465/469	2	2	-	KC514843
Arthrobotrys superba (EF445988)	98	539/547	1	-	1	KC514844
Arthrinium sacchari (EF076712.1)	95	466/491	1	1	-	KC514845
Ascocoryne solitaria (HM152545)	100	520/520	1	1	-	KC514846
Ascomycete sp. HK-S209 (AM084476)	99	459/461	1	1	-	KC514847
Ascomycota sp. PV Wi 0b (EU740392)	99	447/451	5	5	-	KC514848
Axenic grass root isolate (AJ430225.1)	99	482/483	1	1	-	KC514849
Cadophora fastigiata (AY781232)	100	495/496	21	18	3	KC514850
Cadophora luteo-olivacea (GU212374)	100	522/522	6	6	-	KC514851
Cadophora malorum (GU212434)	100	512/512	14	12	2	KC514852
Cadophora melinii	99	461/465	14	14	-	KC589024
Cadophora sp. NH1-2 (AY371513)	100	468/468	8	6	2	KC514853
Cadophora sp. 5R24-1 (DQ317330)	100	468/468	1	1	-	KC514854
Cladophialophora minutissima (EF016384.1)	99	488/490	1	1	-	KC514855
Coniochaeta ligniaria (AY198390)	99	532/535	9	2	7	KC514856
Cosmospora vilior (GU726751.1)	97	506/524	17	15	2	KC514857
Epichloe typhina (L78298.1)	89	415/464	2	2	-	KC514858
Exophiala sp. BC36 (DQ317336)	100	516/516	1	1	-	KC514859
Fungal endophyte sp. ECD-2008 (EU686037)	99	476/480	1	2	-	KC514860
Fungal endophyte voucher Hur ANT-MOSS (HQ335296.1)	100	467/467		-	2	KC514861
Geomyces pannorum (DQ189229.1)	98	528/536	3	3	-	KC514862

Best BLASTn Match	Percent Identity	Overlap	#	Whalers Bay	Chile Station	Genbank Accession #
Geomyces sp. BEA-2010 isolate A2 (HM589249.1)	100	424/424	3	2	1	KC514863
Geomyces sp. JZ-174 (HQ637306.1)	99	520/523	1	1	-	KC514864
Helotiales sp. NK251 (FR846472.1)	96	413/432	3	-	3	KC514865
Helotiales sp. PIMO_102 (HQ845745)	99	466/469	1	1	-	KC514866
Holwaya mucida (DQ257357.1)	95	496/517	2	2	-	KC514867
Hormonema dematioides (AY253451)	100	581/581	1	1	-	KC514868
Hyaloscypha aureliella (EU940229.1)	99	352/358	1	1	-	KC514869
Iceman fungal clone T2709 (X88771.1)	98	448/458	1	1	-	KC514870
Lecythophora hoffmannii (AY805566.1)	100	456/456	11	9	2	KC514871
Leptodontidium elatius (AY805569.1)	99	470/472	3	3	-	KC514872
Mollisia sp. aurim650 (DQ069036.1)	98	482/491	5	4	2	KC514873
Mollisia minutella (EU314678.1)	92	420/453	2	2	-	KC514874
Oidiodendron truncatum (AF062809)	99	513/522	2	2	-	KC514875
Phaeosphaeria sp. (HQ324780.1)	97	492/510	2	2	-	KC514876
Phaeosphaeria pontiformis (AF439499.1)	97	505/521	1	1	-	KC514877
Phialocephala dimorphospora (AF486121.1)	99	449/455	23	22	1	KC514878
Phialophora lagerbergii (AF083197.1)	99	474/478	2	2	-	KC514879
Phoma herbarum (AY293800.1)	100	501/501	8	8	-	KC514880
Phoma sp. 2 (AF218789.1)	99	565/566	9	9	-	KC514881
Pseudeurotium sp. olrim976 (AY787729.2)	100	432/432	2	2	-	KC514882
Sclerostagonospora sp. (DQ286767.1)	96	516/543	3	3	-	KC514883
Stogonospora foliicola (AF439510.1)	96	501/525	1	1	-	KC514884
Thelebolus microsporus (DQ028268)	99	451/452	2	2	-	KC514885
Ulocladium atrum (AF229486)	100	480/480	1	1	-	KC514886
Uncultured *Clathrosphaerina* clone (HQ212333.1)	97	506/519	2	1	1	KC514887
Uncultured fungus clone IH (EU292240)	98	517/527	3	1	2	KC514888
Uncultured *Lachnum* clone 6_h03 (HQ211775.1)	99	512/519	2	2	-	KC514889

Best BLASTn Match	Percent Identity	Overlap	#	Whalers Bay	Chile Station	Genbank Accession #
Uncultured Sordariomycetes clone (FJ475724.1)	98	499/512	1	1	-	KC514890
Uncultured root-associated fungus clone (FJ362275)	92	426/564	7	7	-	KC514891
Uncultured Thelebolales clone (GU910625.1)	99	521/526	1	1	-	KC514892
Xenopolyscytalum pinea (HQ599581.1)	99	470/474	1	1	-	KC514893
Basidiomycetes						
Hypochniciellum molle (GU187667.1)*	99	866/867	44	40	4	KC514894
Cerinosterus luteoalbus (AY618667.1)	100	446/446	9	7	2	KC514894
Coniophora puteana (AM946631.1)	100	1295/1295	1	1	-	KC514900
Coprinellus micaceus (HM240519.1)	96	619/647	1	-	1	KC514901
Hyphodontia radula (GQ411525.1)	99	561/564	1	1	-	KC514902
Hypochnicium bombycinum (FN552537.1)	92	483/525	1	1	-	KC514903
Jaapia argillacea (GU187524.1)	98	560/566	1	1	-	KC514904
Pholiota multicingulata (HQ533029.1)	95	628/660	13	4	9	KC514905
Pholiota multicingulata(HQ533029.1)	97	860/863	1		1	KC514906
Postia pelliculosa (JX090101)	99	558/560	1	1	-	KC514907
Sistotrema brinkmannii (DQ899094.1)	99	568/569	1	-	1	KC514908
Tulasnella violea (DQ457643.1)	99	485/487	6	6	-	KC514909
Zygomycetes						
Mortierella alpina (FJ161918.1)	100	595/595	17	10	7	KC514910
Mortierella parvispora (EU484279.1)	100	567/567	1	1	-	KC514911
Mortierella amoeboidea (GU559984.1)	99	531/532	3	2	3	KC514912
Mortierella sarnyensis (FJ161927.1)	97	460/477	1	1	-	KC514913

*Large subunit sequences were used for these isolates because ITS sequences were not in GenBank for this species.

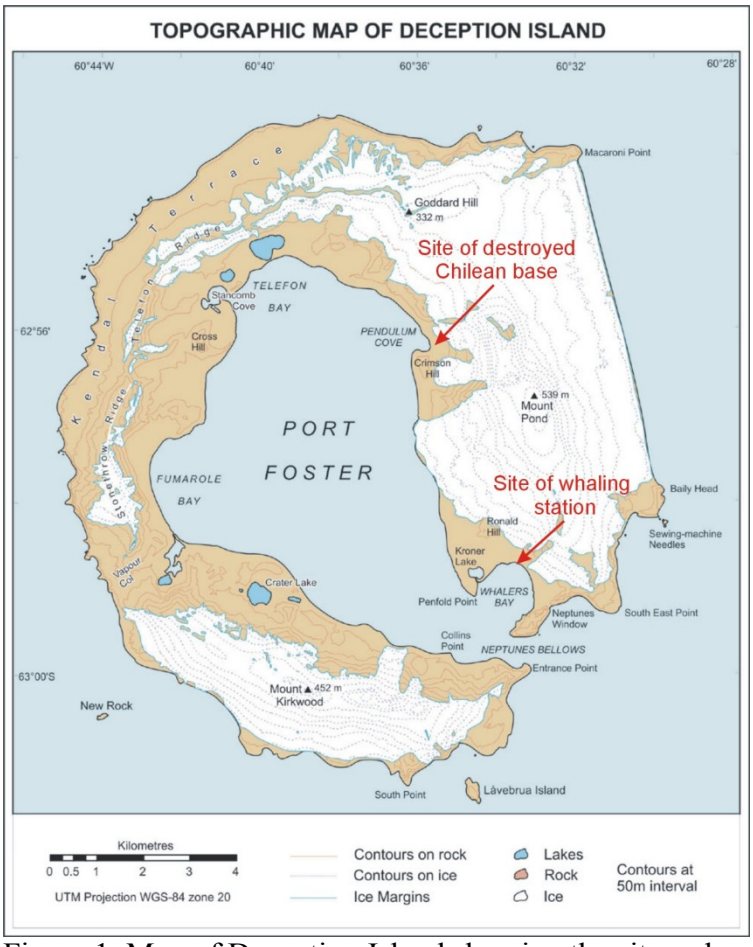

Figure 1. Map of Deception Island showing the sites where samples were collected.

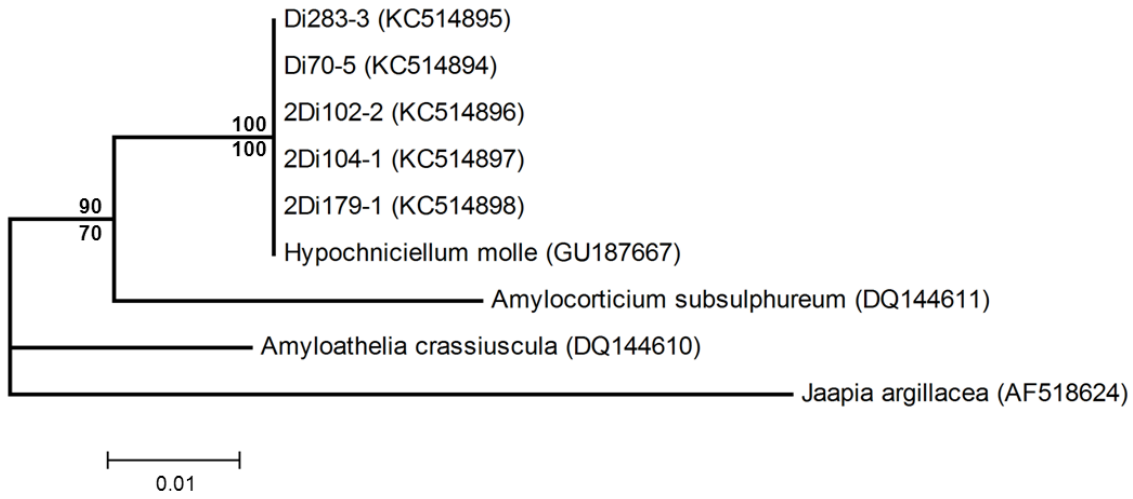

Figure 2. Neighbor joining tree constructed using LSU basdiomycete sequences showing *Hypochniciellum* spp. and related genera. GenBank accession numbers follow known isolates. Bootstrap values for neighbor joining and maximum parsimony (NJ/MP) above 50% are shown. *Jaapia argillacea* was used as the outgroup. Scale bar = substitutions per site.

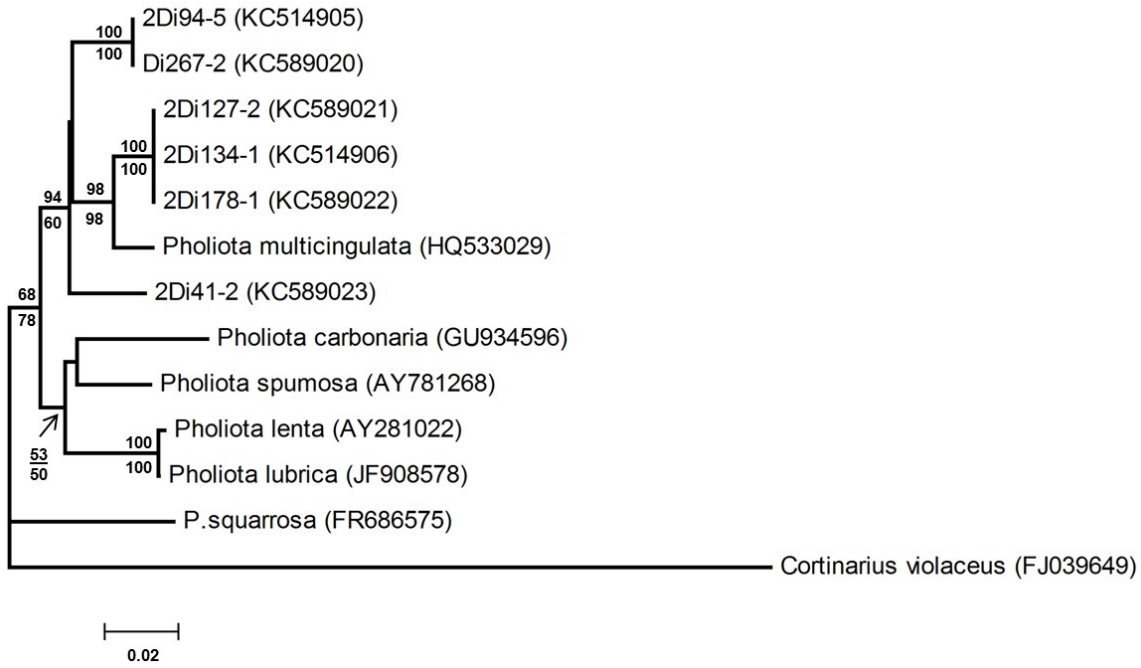

Figure 3. Neighbor joining tree constructed using ITS sequences of *Pholiota* spp. GenBank accession numbers are in parentheses. Bootstrap values from neighbor joining (above) and maximum parsimony (below) analysis above 50% are shown at nodes. *Cortinarius violaceus* was used as the outgroup. Scale bar = substitutions per site.

Figure 4. The side of the Biscoe House, a building at Whalers Bay on Deception Island, showing extensive decay of wall boards and other timbers.

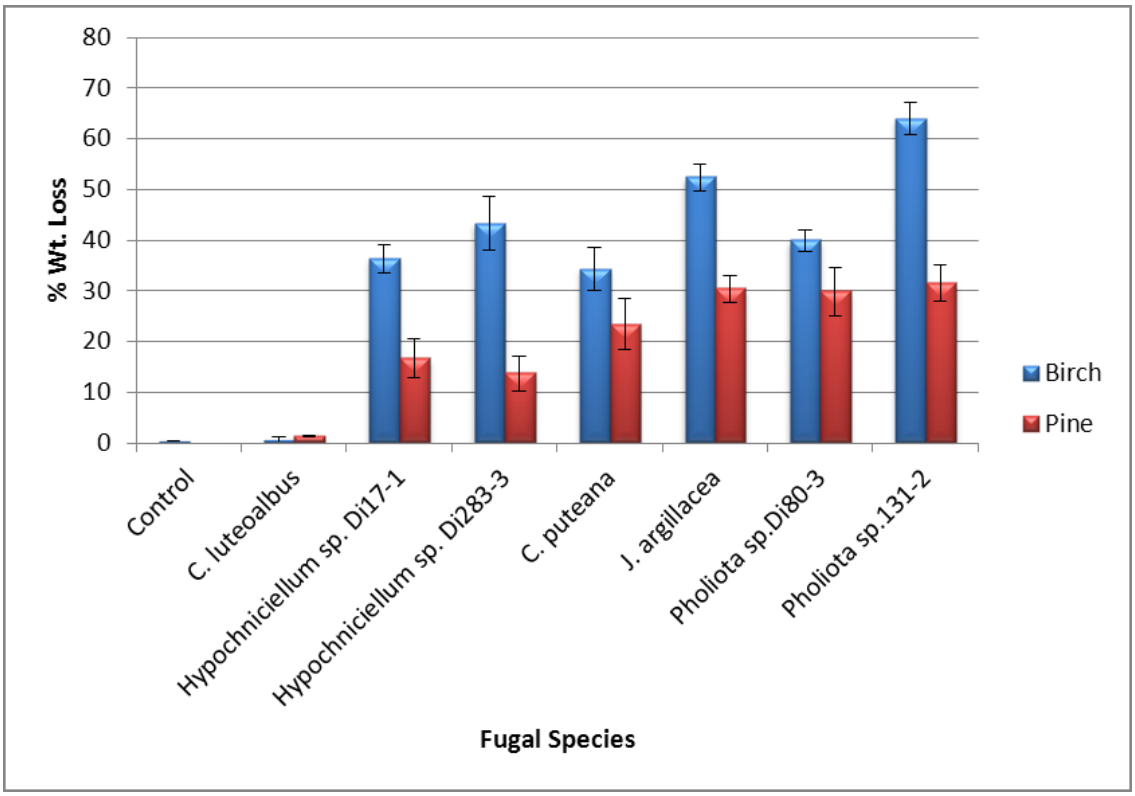

Figure 5. Weight losses of birch and pine wood wafers after decay in the laboratory by basidiomycete species isolated from Deception Island, Antarctica.

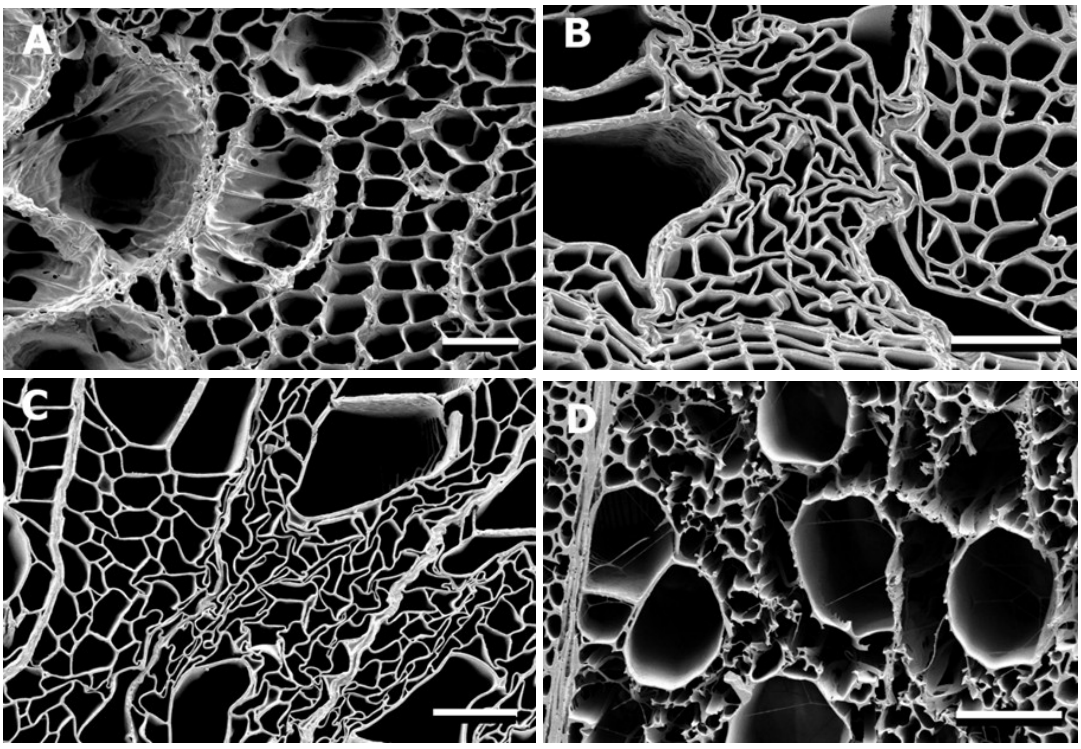

Figure 6. Scanning electron microscope images of transverse sections of samples from the basidiomycete decay study showing decay patterns of tested fungal strains. (A) Pine wood decayed by *Jaapia argillacea* showing complete disintegration of the cell walls characteristic of a white rot (bar=125µm). (B) Birch wood decayed by *Jaapia argillacea* showing alteration of the cell wall and loss of cell structure (bar=50 µm). (C) *Hypochniciellum* sp. causing a brown rot on birch wafers showing fiber cell walls that have been weakened and collapsed from the loss of cellulose (bar=100 µm). (D) Typical white rot decay occurring on birch caused by *Pholiota* sp. showing all cell wall layers attacked (bar=50 µm).

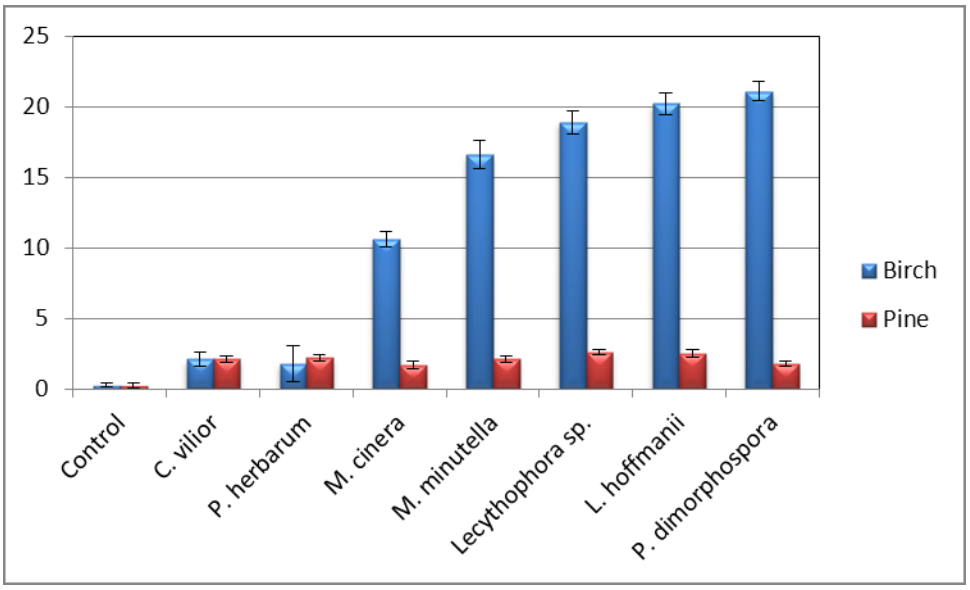

Figure 7. Weight losses in birch and pine wood wafers following a 16 weeks of decay in laboratory using various DSE fungi isolated from Deception Island, Antarctica.

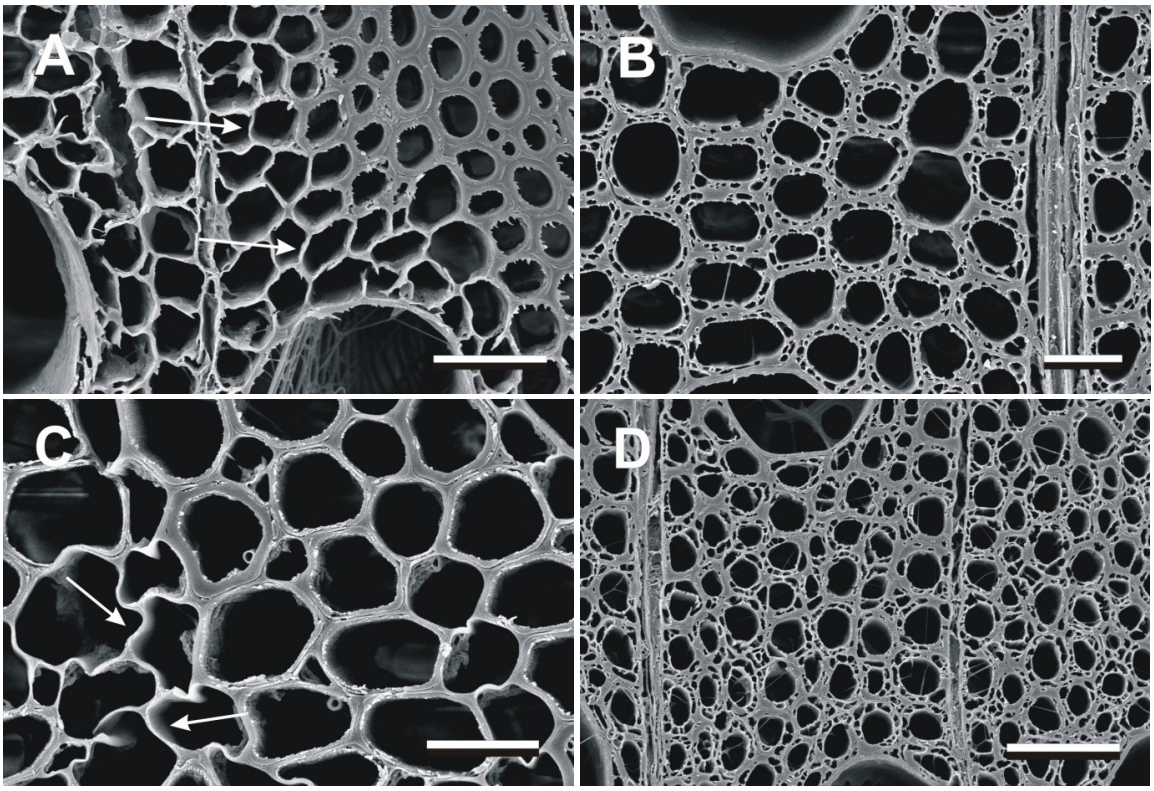

Figure 8. Scanning electron microscope images of transverse section from historic wood showing decay patters of various DSE related fungi after growing on wood wafers (birch) in a microcosm decay experiment. (A) An example of a Type 2 soft rot decay where cell walls are eroded leaving the middle lamella intact (arrows) caused by *Lecythophora* sp. (bar=25 µm). (B) Type 1 soft rot cavities formed in the S2 layer of the secondary wall caused by *Lecythophora hoffmanii* (bar=25 µm). (C) Type 2 soft rot caused by *Phoma herbarum* (bar=25 µm). Arrows indicate areas where the secondary cell has been eroded down to the middle lamella. (D) *Phialocephala dimorphospora* causes a Type 1 soft rot, showing numerous cavities in cell walls (bar=50 µm).

Chapter 2

Factors influencing the deterioration of historic structures at Deception Island, Antarctica

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Introduction

Deception Island, part of the South Shetland Islands in Antarctica, has a long history of human activity. It is an active volcano and is one of the rare locations where ships may enter a sunken caldera flooded with seawater (Figure 1). This unique geologic feature has provided protection to mariners, which lead to the first human visitation to the island by sealers in the early 1800's. The island later became the site of a whaling station in 1906 (Figure 2). In 1931, whaling operations ceased and in 1944 the British Navy established a wartime base called "Base B" at the site of the whaling station. After WWII, the Falkland Island Dependency Survey (FIDS) operated from the site for many years followed by occupation by the British Antarctic Survey (BAS). A volcanic eruption in 1969 forced the site to be abandoned. Many structures and remnants from the whaling station and various bases are still present today and are deteriorating.

Three structures the Whalers barracks, dispensary/store and the magistrate's residence, were built in Whalers Bay by the Norwegian whaling company operating there for use as part of the land-based whaling station. These were prefabricated wooden structures some of which were also used by the British Antarctic Survey in later years. Pine (*Pinus* spp.) and spruce (*Picea* spp.) were the main types of wood used to construct the buildings. Many other artifacts, such as wooden boats, oak barrel remnants and processing equipment remain at the site from the whaling period. The British later added a generator house to the Whalers barracks and renamed it "Biscoe House", built another structure called the "Hunting Lodge" (1955) and also a hangar for the Havilland DHC-3 Otter that was used for conducting survey work. The historical significance of the site

was recognized by the international community and is now designated under the Antarctic treaty system as Historic Site and Monument No. 71.

The Chilean base, Pedro Aguirre Cerda Station, at Pendulum Cove was established in 1955 and served as a site where meteorological and volcanological studies were carried out. It consisted of several structures that were used until volcanic eruptions produced lahars (mudflows of pyroclastic material and water) in 1967 and 1969 which demolished the base and forced abandonment. The remains of the station at the site consist primarily of foundation material made of wood and metal. It is now listed as Historic Site and Monument No.76.

Previous research on wooden structures and artifacts in Antarctica has shown that significant deterioration takes place over time and resilient microbes function in this extreme environment, albeit at a slow rate (Arenz and Blanchette 2009; Held et al. 2006; Blanchette et al. 2004). Wood decay is occurring at the historic huts located on Ross Island where wood is in contact with the ground. Extensive fungal growth has also occurred inside the historic structures where deterioration of wood and other cultural properties has taken place (Held et al. 2005). The wooden materials at the Deception Island site are situated in a unique and hostile environment and are also being significantly affected by a diverse and aggressive assemblage of wood decay fungi. This, coupled with the volcanic activity on the island, has had a profound influence on the deterioration of the structures and artifacts. Comparisons made with results from investigations of decay occurring at other Polar locations indicate that the historical structures and artifacts at Deception Island are being affected by a larger number of more

diverse microorganisms causing different forms of degradation and a more rapid rate of wood decay is taking place. This paper provides new information about the factors influencing deterioration of this important historic polar heritage site.

Condition of historic woods at Deception Island

The wooden buildings and artifacts at Whalers Bay and wood remnants at Pendulum Cove have deteriorated extensively. In most areas where wood is in contact with the ground, significant decay has resulted. The decay commonly extends up from the soil to about a meter in the standing structures (Figure 3). Sufficient moisture and the presence of aggressive decay fungi have resulted in extensive damage. One structure exhibiting significant decay is the north side of the Whalers barracks (Biscoe House). This location has advanced stages of decay that has resulted in large holes formed in several areas of the wall (Figure 3). Many other parts of this structure are also affected and the damage is so severe the structure is likely to collapse in the near future. A significant amount of mud has also entered the north side of the structure and filled many areas. Much of the wood in contact with the soil is being affected by decay fungi.

The dispensary/store was partially destroyed and buried by a lahar resulting from the volcanic eruption in 1969. Sections of the structure have collapsed and others are also precariously close to collapse. Mud has also filled in and around the structure which has led to significant decay of wood in contact with the soil as well as in the wood well above ground level. Support posts in this structure are also severely decayed.

The Magistrate's residence and Hunting Lodge, a prefabricated hut built by the British in 1955, are structures at the site that are in comparatively better condition than the others. This is in part because the buildings are on foundations that are above the soil (Figure 4). Without contact of the wood to the soil, decay is limited by reduced moisture and less contact with nutrients and soil microbes. Both of these structures did not suffer from major damage from the lahar, however the ground on the northwest corner of the hunting lodge has been cut away by soil erosion and that part of the building is in danger of collapse. The roof of the Magistrate's residence has blown off and is lying nearby. The inside of this building is now exposed to the elements.

In a Southeasterly direction from the buildings along the beach, a number of different wooden artifacts can be found (Figure 5). Many of them are half buried in the soil. Most notable are a number of small boats (used by whalers for transporting freshwater to whale processing ships) and wooden barrel depots. The boats are located in a wide area along the beach and are half buried in soil. The boats are extensively decayed not only in areas that are below ground but also in areas well above the soil. There are also several large areas with remnants of oak barrels and this wood is also decaying. The Chilean station at Pendulum Cove was completely destroyed by lahars in 1967 and 1969 and only a foundation of metal and wood structural elements remain. The wooden remains at this site have decay that is similar to the wood degradation taking place at Whalers Bay with significant decay in wood at ground contact and wood that has been buried.

Decay and fungi present

Scanning electron microscopy was used to examine samples obtained from the site and ascertain what forms of degradation were present. The results show that several types of decay are occurring in affected woods, with many samples displaying an extensive soft rot type of attack and others a brown rot form of wood degradation (Figure 6). Soft rot fungi are known to function in very harsh environments which exclude other types of decay fungi (Blanchette 2003). Brown rot fungi degrade wood differently from soft-rotters and are well known to cause severe strength loss early in the decay process. Brown rot fungi cause a rapid depolymerization of wood constituents primarily targeting cellulose which causes the rapid loss of strength (Eriksson et al. 1990; Zabel and Morrell 1992). Brown rot decay fungi commonly affect wooden structures in temperate climates and are very important in forest ecosystems as decomposers of organic material. However, while not common in Polar environments, at Deception Island many of the woods inspected had advanced stages of brown rot. A third type of wood decay found was a white rot affecting buried wood from the destroyed Chilean station at Pendulum Cove. Extensive mycelial cords formed white rhizomorphs on the wood. White rot fungi can degrade all cell wall components and some cause a preferential attack on lignin. The white rot found at Deception Island appears to cause a selective attack on the wood resulting in a bleached appearance and a white-pocket rot form of decay. Generally, strength loss is not significant until advanced stages of decay are reached. No previous evidence of brown or white rot fungi has been reported from other historic wooden

structures in Antarctica and both forms of decay by these basidiomycetes on Deception Island are an unusual occurrence in a Polar environment.

Determination of the species of fungi attacking the wood was made by isolating fungal cultures from samples and sequencing rDNA from pure cultures. Small wood sections were aseptically placed on various culturing growth media. Fungi that grew out from these sections were transferred and pure cultures obtained. Identification of the cultures was done by extracting DNA and amplifying and sequencing the ITS (internal transcribed spacer) region of rDNA using previously described techniques (Arenz and Blanchette 2009; Gardes and Bruns 1993). Fungi that were isolated that are known to cause a soft rot in wood include species of *Cadophora, Lecythophora, Phialocephala* and *Phialophora*. *Cadophora* spp. are common fungi isolated and identified from wood in many Polar environments (Arenz and Blanchette 2009; Arenz et al. 2006; Blanchette et al. 2010; Held et al. 2006). Phylogenetic analysis of *Cadophora* isolates cultured from wood at Deception Island revealed a very diverse species population (data not shown) was present. The most prevalent brown rot fungus obtained does not match other sequences in public DNA databases (GenBank) and cultural characteristics suggest this may be a previously unreported species. The white rot fungus is related to other *Pholiota* species and also appears to represent an undescribed species.

Factors influencing decay

As previously mentioned, the extent and severity of wood decay identified in the wooden structures and artifacts at Deception Island are much more extensive than has been found

in historic structures located in other Antarctic locations (Arenz and Blanchette 2009; Blanchette et al. 2010; Blanchette et al. 2004; Held et al. 2006). There are several primary factors that are contributing to the rapid and extensive decay taking place at Deception Island including:

1. Diverse population of decay fungi
2. Environmental conditions conducive for decay
3. Volcanic activity that has covered historic woods with soil
4. Large amounts of exotic wood from many different areas brought to the island during the last century

Diverse fungi in Deception Island's unique ecosystem

A unique ecosystem exists on Deception Island. Active volcanoes are rare in Antarctica and only two, Mt. Erebus and Deception Island, have had activity with recent eruptions. Deception island is home to exceptionally rare flora including eighteen species of plants not found elsewhere in the Antarctic (Group 2002). Our investigations show that fungi associated with historic wood is also remarkably diverse and unusual. The research we carried out has identified approximately 71 taxa of fungi including what appear to be many previously undescribed species. In addition, wood decay fungi have been found that cause three major types of wood degradation: white, brown and soft rots. While the damage caused by the white rot fungi do not appear to be widespread among the wooden materials, the fungi causing brown and soft rots are active and very aggressive, causing extensive damage to many woods. Past research suggests that soft rot fungi should be

the dominate decay organisms at this Antarctic location, but because of the diversity of decay fungal species, coupled with moderate temperatures and moisture at the site, it has led to significant decomposition in the historic woods (Blanchette et al. 2010; Held et al. 2006). The origin of the many different decay fungi at Deception Island is not clear. A strong case could be made that fungi were introduced on the timbers or were carried to the island on other materials brought by the many inhabitants over the past decades. They could have also been brought in on birds or as wind disseminated spores. However, with many isolates found being genetically different from known species, there is a strong possibility that some of these fungi are endemic to Antarctica. Whatever the mode of introduction to the island, one underlying factor fueling the decay is the abundant carbon source (coming from all the introduced woods) and conducive temperature and moisture conditions available for colonization and continued survival of the fungi and decomposition of the substrates.

Arenz and Blanchette (2009) described fungal diversity and deterioration at nine different historic sites on the Antarctic Peninsula with wood structures. While these sites have environmental conditions similar to Deception Island, the diversity profile of fungi (including decay fungi) isolated from wood is quite different. In addition, less decay was observed in wood at these other Peninsula sites compared to Deception Island.

Environmental Conditions

Deception Island has a polar maritime climate with a mean annual temperature of -3°C. Temperature ranges from -28°C to +11°C and an average high temperature during the

months from October to April is 1°C. The mean annual precipitation is 560 mm. Comparing Deception Island to that of Ross Island where the historic huts of Scott and Shackleton are located shows considerable differences in climactic conditions (Table 1). At Ross Island, the average yearly temperature is -19°C, average high temperature from October to April is -8°C and there is an average yearly precipitation of 190 mm. The maritime conditions of Deception Island favor warmer temperatures and increased precipitation. Since adequate moisture and temperatures above freezing are necessary for fungi to function, the conditions at Deception Island are allowing microbes to be active many more days per year than at Ross Island. In addition, the precipitation that occurs will likely be a heavier wet snow or rain/snow mix at Deception Island. Observations at the site during our assessment showed that a heavy wet snow accumulated on the siding of the structures during storms and subsequent melting left considerable moisture on the wall boards and other above ground woods (Figure 7). It was apparent that this moisture absorbing into the wood was providing sufficient moisture for fungal growth and is the reason why decay extends up from the soil into the above ground wood structures. In areas where the bases of structures are buried in mud, decay begins and advances from the ground upward into the structure. Once decay occurs, the porosity of wood changes and the wood becomes more absorbent, leading to even more water retention. This exacerbates the problem and allows for increased decay.

Volcanic Activity

Lahars, specific to areas of volcanic activity, can cause dramatic destruction. Lahars occurred with recent eruptions in 1967 and 1969 and damaged structures at Whalers Bay and destroyed the Chilean base buildings at Pendulum Cove. There are clear implications that this phenomenon has contributed to the severe degradation and condition of the structures. There is a two-fold effect from lahars. First is the destructive force behind the flowing mud and water that damages or obliterates objects in its path. Second, there is a change of environment that affects degradation processes in wood. The process of partially burying structures and artifacts brings soil, moisture and microbes that inhabit the soil into contact with the wood substrate and creates an environment conducive for decay by microorganisms (Figure 8). This has led to advanced decay in buried wood and also leads to decay occurring in wood above ground where there is sufficient moisture. These damaging effects are also evident on other artifacts like the barrel remnants and wooden boats on the shore. Several boats are nearly buried and are breaking apart due to the loss of structural integrity caused by decay fungi. In contrast, the Magistrate's residence and Hunting Lodge, largely unaffected by the lahars, have far less decay. Generally, the wood of these structures is above the soil line and little to no decay is taking place. However, the Magistrate's residence has soil in contact with wood on the north side of the building that has allowed decay to occur in that area.

There are several areas on the island that have heated geothermal soils with abundant and rare bryophyte communities (Lewis Smith 2005). It is possible that these areas are harboring some of the decay fungi that are normally found functioning in more temperate

ecosystems (e.g., white and brown rot fungi). Since there is abundant plant material and a range of soil temperatures, these fungi (exotic or indigenous) could be thriving in this area and have spread from these sites to the locations with historic wood. In addition, a report of a mushroom fruiting in one of the large bryophyte stands indicates there is a resident population of basidiomycetes that exist among the live and dead bryophytes (Lewis Smith 2005).

Wood is an introduced substrate to Deception Island and Antarctica and the amount of wood present at Whalers Bay is substantial. This abundance of wood from many different places around the world brought in over more than a century has allowed populations of wood decay fungi to become established and proliferate. Fungal populations have most likely changed dramatically since the introduction of wood material and human activity on the island. The addition of wood substrate in the volcanic ecosystem of Deception Island adds a tremendous amount of nutrients to an otherwise very nutrient poor environment. Although it is not yet known what fungi are indigenous to Antarctica and which were brought in on the wood or on other materials, these sites constitute some of the most significantly diverse areas in Antarctica for fungi. It is clear that the decay fungi found are well adapted to the site, capable of degrading wood and extremely efficient decomposers.

Management Considerations

Many of the wooden resources at Deception Island are so deteriorated that very little can be done to preserve them. Among them, Biscoe House and the dispensary are

precipitously close to collapse. Removing soil away from the base of the structures and artifacts where feasible is recommended to help arrest decay by limiting contact with soil moisture. Perhaps the most appropriate procedures are those that focus resources where conservation work could be feasibly carried out. For instance, repairing the roof of the magistrate's residence would help protect one of the original structures at the site that is still in relatively good condition. Replacing the roof would protect the interior from further damage and the structure could possibly be used to house interpretive material. Also repairing the foundation of the Hunting Lodge that has washed away would greatly improve the structural stability of this building (Figure 9).

The use of preservatives on the wood to stop fungal attack is not an option for several reasons. Most of the effective preservatives contain heavy metals or other compounds that can be toxic in the environment and should not be used in Antarctica. Also, some of the fungi causing decay, such as the *Cadophora* species, are tolerant of preservatives (Daniel and Nilsson 1988) and they would not be effectively controlled by them. Conservation standards would also not allow treatments that are not reversible to be used and the long term effect of material treated with preservatives is unclear. Finally, it would be difficult to successfully treat and infiltrate wood at Deception Island under the extreme Antarctic conditions that can occur there. At the present time, the only effective method of control is to reduce moisture. Therefore, wherever possible conservation measures should work to reduce moisture in wood to limit the rate and extent of decay.

The wooden structures and artifacts at Deception Island provide a legacy of rich polar history that warrants preservation efforts by the international community. The factors influencing decay described in this paper indicate why rapid deterioration has taken place over the last few decades. Understanding decay mechanisms threatening the wood resources is the first step to making informed conservation decisions to protect these and other important polar historic sites. The information provided here can be used to provide more precise management plans to limit decay and preserve the historic woods long into the future.

Table 1. A comparison of climatic data between Deception Island and Ross Island, Antarctica. Deception Island has much higher precipitation and higher temperatures compared to Ross Island, enabling decay fungi to function for a longer period of time.

Location	Climatic Data			
	Ave. Yearly Temp.	Ave. High Oct-April (°C)	Months Ave. High Temp >=0°C	Annual Precipitation (mm)
Deception Island	-3	1	5	560
Ross Island	-17	-8	0	190

Figure 1. Whalers Bay inside the caldera of Deception Island where structures of the historic whaling station are located along the shore.

Figure 2. Structures at the whaling station at Whalers Bay, Deception Island are deteriorating. In addition to historic structures, there are a number of other wooden artifacts and equipment from the whaling period as well as activities by the British Antarctic Survey located on the island that are also decaying rapidly.

Figure 3. Left: The north side of the Biscoe House showing advanced decay of the wall boards. Right: Decay is occurring in many areas well above the soil line, as shown in these wall boards of the dispensary structure.

Figure 4. The Magistrate's residence has a solid foundation that has aided in avoiding deterioration by keeping the structure well above the soil. However, the roof has blown off exposing the interior to moisture.

Figure 5. Left: One of several large areas where remnants of barrels are located along the shore at Whalers Bay. Along with other wooden structures and artifacts, these areas serve as an enormous nutrient source for decay fungi. Right: One of several water boats that remain on the beach area that is partially buried and showing advanced deterioration.

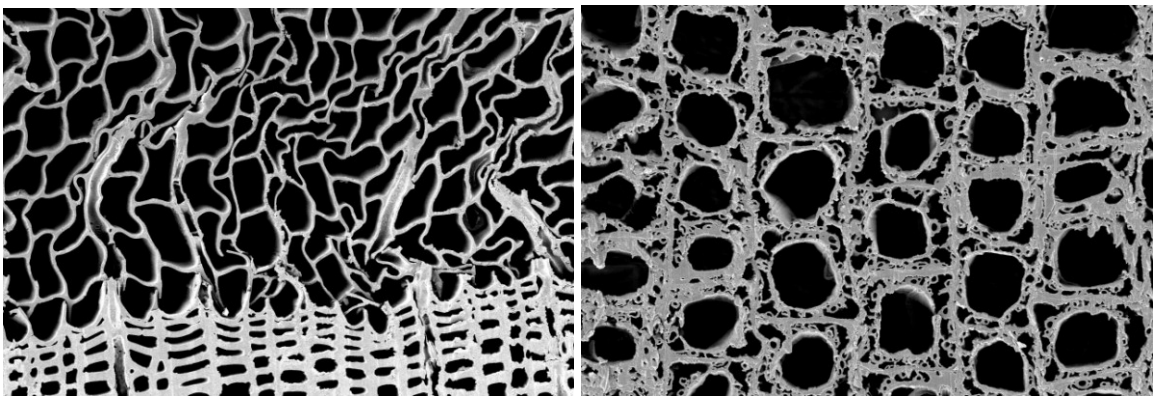

Figure 6. Scanning electron micrographs of transverse sections of wood from the whalers barracks at Whalers Bay affected by fungal decay. Left: Brown rot in spruce wood showing the severe degradation and attack on the cell walls resulting in significant reduction in wood strength and loss of structural integrity of the timbers. Right: Soft rot decay showing cavities in the wood cells caused by the fungus. Many cavities occur in the cell walls and as they enlarge the cavities coalesce causing large holes and significant degradation of the wood substrate.

Figure 7. The north side of the dispensary at the end of a precipitation event showing accumulation of snow sticking to the wall boards. When this snow melts it is sufficient moisture to support fungal growth and decay.

Figure 8. The dispensary/store has been inundated with mud from a lahar. The mud holds moisture and microbes which creates a conducive environment for decay to take place.

Figure 9. The Hunting Lodge has significantly less deterioration than other structures at the site partly due to a high foundation keeping the wood well above the soil. However the left corner shows erosion of the foundation that is in danger of collapse.

Chapter 3

Diversity of *Cadophora* species from historic sites in Antarctica

Introduction

Species of *Cadophora* Lagerberg & Melin are dematiaceous hyphomyces that have been associated with decomposition, plant disease, and endophytic relationships in plants. They can also be found in extreme environments such as Antarctica and the Arctic (Arenz and Blanchette 2009; Blanchette et al. 2010; Blanchette et al. 2004; Held et al. 2006; Jurgens et al. 2009). The type species, *C. fastigiata*, is described as having solitary phialides and hyaline collarettes. Eight *Cadophora* species became synonymous with *Phialophora* in 1937 (Conent 1937) but Gams (2000) returned *Cadophora* as a separate genus and established affiliations of teleomorphs in the Helotiales rather than Chaetothyriales. Harrington and McNew (2003) also supported this revision with morphological and rDNA phylogenies and proposed other revisions for this group.

Cadophora species have been identified in many ecological niches. Several *Cadophora* such as *C. luteo-olivacea*, *C. malorum* and an unidentified species have been implicated with die-back, cankers and fruit rot of kiwi (Di Marco and Osti 2008; Prodi et al. 2008; Riccioni et al. 2007) and declining and diseased grapevines in different regions of the world (Gramaje et al. 2011; Halleen et al. 2007; Overton et al. 2005; Rooney-Latham 2005; Abreo et al. 2008; Manning and Munday 2009). *Cadophora malorum* has been shown to cause side rot of pear (Sugar and Spotts 1993) and infection of apples while on trees, leading to losses during storage (McColloch 1944). It has also been used as a biocontrol against water hyacinth (one of the most problematic aquatic weeds worldwide) on which it causes a leaf blight (Dagno et al. 2011). *Cadophora finlandia* has been described as a dark septate endophyte (DSE) because of its mycorrhizal

relationships in roots (Vralstad et al. 2002) and *C. malorum* was recently identified as an endophyte in orchids (Chen et al. 2010). Several *Cadophora* species have been isolated frequently from soils contaminated with heavy metals and hydrocarbons, indicating a tolerance to compounds that usually limit the growth of other organisms (Gorfer et al. 2009; Kerry 1990; Omar et al. 2009; Utmazian et al. 2007; Aislabie et al. 2001).

Cadophora species may be best known as saprophytes and for their association with wood and decomposition. Many species are known to cause a soft rot type of decay (Nilsson 1973; Morrell and Zabel 1985; Worrall et al. 1997; Blanchette et al. 2004; Held et al. 2006; Blanchette et al. 2010). Soft rot fungi are known to cause decay in wet, dry or extreme environments that usually exclude other decay fungi. One such environment is Polar Regions where *Cadophora* species are commonly isolated from wood, soil and other organic materials. At sites where wood was brought by early explorers to the Ross Sea Region of Antarctica and to the Antarctic Peninsula, they are commonly isolated (Arenz and Blanchette 2009; Arenz et al. 2006; Arenz et al. 2010; Blanchette et al. 2010; Held et al. 2006; Blanchette et al. 2004; Kerry 1990; Möller and Dreyfuss 1996). In most of these environments *Cadophora* species are capable of decaying wood during repeated freeze thaw cycles and when temperatures are above freezing for only a few weeks per year. Although decay may be slow in the Polar environment, *Cadophora* species have the capacity to be efficient lignocellulosic decomposers (Worrall et al. 1991; Worrall et al. 1997) and dominant recycling organisms when associated with cellulosic substrates (Held et al. 2006; Blanchette et al. 2010).

Many *Cadophora* species have been found in Antarctica but the taxonomy of this group is unclear and phylogenetic analysis to better understand the diversity of this fungus has not been undertaken. *Cadophora* isolates used in these studies have been isolated from many areas in the Ross Sea Region as well as the Peninsula of Antarctica. The phylogenetic structure of this genus in Antarctica is important as we begin to better understand the role of this decomposer in this unusual biome. Mechanisms for adaptive strategies were also tested in these investigations with studies on hyper-salinity and heavy metal tolerances. In addition, the type of wood decay was characterized and amount of decay (mass loss) were tested for known species as well as for new, undescribed species identified in this study.

Materials and Methods

Samples from wooden structures and artifacts from many historic sites located in Antarctica were collected. Small segments of wood were obtained and placed in sterile plastic bags and kept cool while transporting them back to the laboratory. Once in the laboratory and under sterile conditions, sections of the wood samples were cut and small segments placed onto malt extract agar (1.5% Difco malt extract) or malt extract agar (1.5%) amended with 0.5 g/L of streptomycin. Isolates were cultured at 20°C for several weeks from which transfers were made to another plate to obtain pure cultures. DNA was extracted from fungal cultures using an adapted chloroform procedure (Arenz and Blanchette 2011). Several genes were amplified, sequenced and used for phylogenetic analysis. The complete internal transcribed spacer (ITS) region of ribosomal DNA as

well as the 5.8S gene was targeted with primers ITS1 and ITS4, as well as a partial sequence of the large subunit rDNA with primers LROR and LR5 (White et al. 1990) using Amplitaq Gold PCR Master-mix (Applied Biosystems, Foster City, CA) and 1µl of template DNA using the following reaction conditions: 94°C for 5 min, 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min and a final extension step of 5 min at 72°C. The translation *elongation factor 1 alpha* locus (*EF-1α*) was amplified using primers EF1-728F and EF1-986R (Carbone and Kohn 1999) with the following conditions: 94°C for 5 min, 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min and a final extension step of 5 min at 72°C. The actin (ACT) locus was amplified using primers ACT-512F and ACT-783R (Carbone and Kohn 1999) with the following conditions: 94°C for 5 min, 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min and a final extension step of 5 min at 72°C. PCR amplicons were visualized on a 1% agarose gel using SYBR green 1 (Molecular Probes, Eugene, OR) prestain and a Dark Reader DR45 transilluminator (Clare Chemical Research, Denver, CO). Primers for PCR were used for sequencing reactions on an ABI Prism 377 automated DNA sequencer using a ABI PRISM Dye Terminator Cycle Sequencing Ready reaction kit (Applied Biosystems). Consensus sequences were assembled using CHROMASPRO software version 1.33 (Technelysium Pty Ltd.) and were subjected to BLAST searches against those in NCBI GenBank to determine the best match. Sequences of ex-type cultures with close matches to queried sequences as well as other representative species were downloaded and used for multiple sequence alignments. Multiple sequences were aligned online using the E-INS-i strategy in MAFFT version 7 (Katoh and Standley 2013). The

best fit substitutional model for neighbor joining (NJ) analysis was determined using Modeltest v.3.7 (Posada and Crandall 1998) from the Akaike information criterion (AIC).

Phylogenetic trees estimated using NJ and maximum parsimony (MP) analysis were constructed using PAUP 4.0b10 (Swofford 2002), gaps were treated as missing data and all characters were treated unordered and of equal weight. Maximum parsimony analysis was performed using the heuristic search option and TBR as the branch-swapping algorithm. Support for tree branch nodes was calculated by 1000 bootstrap replicates (Hillis and Bull 1993).

Decay studies using fungal cultures isolated from samples were carried out in microcosms over a 16 week period. Glass filters 55 mm in size were sterilized by autoclaving and placed in sterile 100 mm Petri plates containing a medium consisting of 1.5 g NH_4NO_3, 2.5 g KH_2PO_4, 2 g K_2HPO_4, 1 g $MgSO_4$-$7H_2O$, 2.5 g glucose and 0.1 g thiamine per liter (Worrall et al. 1991; Abrams 1948). Wafers measuring 1.5 x 1.5 x 0.3 cm of birch and pine were cut from sound wood blocks, dried at 105°C for 24 hours and weighed to determine dry weight. A solution made with the same ingredients as the medium, excluding agar, was used to hydrate the wafers. The wood wafers were then sterilized before placing on filters in decay microcosms. Three small plugs were transferred from a growing fungal culture and placed on the medium surface adjacent to the filter. After a 16 week incubation period, 10 wafers from each treatment were removed and oven dried to determine biomass loss. Two additional wafers from each treatment were not oven dried and were used for micromorphological study.

Wood samples for fungal decay observations were prepared for scanning electron microscopy (SEM) by infiltrating small segments of the wood with a 25% TBS™ Tissue Freezing Medium™ (Triangle Biomedical Sciences, Durham, NC, U.S.A.) under vacuum followed by mounting on brass stubs, freezing at -20°C and sectioning in a cryostat freezing microtome. Samples were cut transversely to prepare a clean surface for examination. Cut samples were thawed and rinsed several times in water and air dried then mounted on aluminum stubs with carbon tape and coating with gold using a sputter coater. Samples were viewed using a Hitachi S3500N scanning electron microscope to determine characteristics of decay mechanisms.

A carboxymethylcellulose (CMC) Congo red assay was used to screen *Cadophora* species for cellulolytic activity. Small plugs from growing plates of representative species of *Cadophora* were transferred to the center of plates containing *Trichoderma viride* medium A (14 ml of 10% $(NH_4)\ 2SO_4$, 15 ml of 1 M KH_2PO_4, 6 ml of 35% urea, 3 ml of 10% $CaCl_2$, 3 ml of 10% $MgSO_4 \cdot 7H2O$, 1 ml of Trace elements solution (10 ml of concentrated HCl, 0.51% $FeSO_4$, 0.186% $MnSO_4 \cdot 4H2O$, 0.166% $ZnCl_2$, 0.2% $CoCl_2$, 2 ml of Tween 80, 0.2% (w/v) carboxymethylcellulose, 1.5% (w/v) agar) (Mandels et al. 1962). Three plates of each isolate were made for each incubation temperature, 8 and 22°C. After 14 days at 22°C and 37 days at 8°C, plates were flooded with approximately 15 ml of 0.1% Congo red and incubated for 30 minutes, followed by a 60 minute destaining period with a 1 M NaCl solution (Teather and Wood 1982). An index of relative enzyme activity was determined by making two perpendicular

measurements of the cleared zone (area where staining did not occur) as well as in the area of fungal growth and dividing the two numbers, respectively.

The same set of isolates screened for cellulolytic activity was also screened for lignin peroxidase and Mn-dependent peroxidase activity by using an Azure B media assay (Archibald 1992). The assay was carried out by transferring a small plug from an actively growing culture to a plate with a medium consisting of 0.01% w/v Azure B and 16 g of agar in 1 L of water (Pointing 1999). The assay was carried out at two temperatures, 8 and 22°C, with three plates of each isolate at each temperature. The plates were monitored for any decolorization of the medium (which indicates the production of lignin peroxidase or Mn-dependent peroxidase) for 37 days.

Tolerance of high concentrations of salt and heavy metal in *Cadophora* isolates was tested by culturing representative species on media having varying concentrations of NaCl and $CuSO_4$ and measuring growth at 6, 8, 12, 17 and 20 days. The concentrations of NaCl consisted of 2% (0.34 M), 5% (0.86 M), 8% (1.37 M) and 12% (2.05 M) with 15 g agar in 1L of de-ionized water. Copper sulphate concentrations were 0.05% (3 mM), 0.25% (20 mM) and .50% (30 mM) with 15g agar in 1 L of de-ionized water. The pH was adjusted to approximately 5.0 using NaOH. Control plates were made using 1.5% malt extract with 15 g agar in 1 L of de-ionized water. Other fungi tested for comparison to *Cadophora* included *Geomyces pannorum*, *Lecythophora* sp., *Phialocephala dimorphospora* and *Phoma herbarum* from Antarctica.

Results

Phylogenetic analysis

Cultures from a number of Ross Sea Island and Antarctic Peninsular sites that were used in the phylogenetic analysis are listed in Table 1 designated GenBank accession numbers for gene sequences. Aligned ITS sequences had 513 total characters of which 155 were variable parsimony uninformative and 138 were parsimony informative. The substitution model that best fit the data was (GTR+G) (proportion of invariable sites (I) = 0; gamma distribution shape parameter (G) = 0.4588; base frequencies: $\pi_A = 0.2395$, $\pi_C = 0.2540$, $\pi_G = 0.2170$, $\pi_T = 0.2896$) for ITS analysis. A distance phylogram is shown (Figure 1) with both MP and NJ bootstrap values from 1000 replicates. The most parsimonious tree total length (TL), consistency index (CI), retention index (RI) and homoplasy index (HI) were 495, 0.790, 0.945, 0.210, respectively.

The LSU alignment resulted in 803 characters having 110 parsimony-uninformative characters and 350 parsimony-informative characters. The substitution model that best fit the data was TrN+I+G (proportion of invariable sites (I) = 0.5241; gamma distribution shape parameter (G) = 0.5692; base frequencies: $\pi A = 0.2541$, $\pi C = 0.1986$, $\pi G = 0.3062$, $\pi T = 0.2411$). The heuristic search analysis resulted in four most parsimonious trees (TL= 710, CI=0.800, RI=0.880, HI=0.200). The NJ phylogram generated from LSU data is shown in Figure 2, with bootstrap from NJ and MP analysis run with 1000 replicates shown at nodes.

The *EF-1α* sequence alignment comprised 756 characters, of which 475 were parsimony uninformative and 243 were parsimony informative. The substitution model

that best bit the data was TrN+G (proportion of invariable sites (I) = 0; gamma distribution shape parameter (G) = 1.3950; base frequencies: πA = 0.2660, πC = 0.2755, πG = 0.2082, πT = 0.2503). The heuristic search analysis resulted in four most parsimonious trees (TL= 1181, CI=0.846, RI=0.920, HI=0.153). The NJ phylogram generated from *EF-1α* data is shown in Figure 3, with bootstrap from NJ and MP analysis run with 1000 replicates shown at nodes.

The actin alignment was 315 characters, of which 27 were parsimony uninformative and 243 were parsimony informative. The substitution model that best fit the data was HKY+I+G (proportion of invariable sites (I) = 0.2212; gamma distribution shape parameter (G) = 1.8666; base frequencies: πA = 0.2302, πC = 0. 3109, πG = 0.2036, πT = 0.2553). The heuristic search analysis resulted in one most parsimonious tree (TL= 1181, CI=0.605, RI=0.739, HI=0.395). The NJ phylogram generated from actin gene data is shown in Figure 4, with bootstrap from NJ and MP analysis run with 1000 replicates shown at nodes.

The sequence analysis revealed very little genetic variation among isolates of *C. luteo-olivacea* or *C. malorum*. Isolates matching the GenBank accessions from ex-type *C. fastigiata* and *C. melinii* also showed no variation. However, from ITS sequence data there are six distinct clusters of isolates that are near *C. fastigiata* ex-type. Four of these clusters contain only Antarctic isolates, while the other two share similarity with isolates retrieved from GenBank. However, the sister clade to Di253, Di2-3 and Di255-2, contains accessions that were isolated from maple sap in Canada, grapevines in Spain and silver birch in Sweden, which indicate close associations with woody plants. Most

distantly related species from *C. luteo-olivacea*, *C. malorum* and *C. fastigiata* are *C.* sp. 5R24, *C.* sp. H37 and one other isolate (2Di-181-2) that grouped together, Di62-7 that grouped closest with *Xenopolyscytalum pinea* and *C.* sp NH1-2, which was closer to *Collophora* spp. rather than *Cadophora* sp. ex-types. LSU phylogeny agrees with ITS tree topology with respect to *C. luteo-olivacea*, *C. malorum*, *C. fastigiata*, *C.* sp. NH1-2 and those matching *C.* sp. 5R24-1. Also, BLAST searches with LSU sequences showed *Mycochaetophora gentianae* having high sequence similarity to *C. malorum* and *C. luteo-olivacea* and because of this the sequence from GenBank was included in the analysis. It groups with *C. malorum, C. luteo-olivacea* and *C. fastigiata*, but is nearest *C. malorum* and *C. luteo-olivacea*. Unfortunately the ITS sequence for *M. gentianae* was not available in GenBank and could not be included in the analysis.

The phylogenetic tree of *EF-1α* data showed similar topology as that for ITS. As with the ITS region, showed no variation among the *C. malorum* isolates. The *C. luteo-olivacea* clade was split into two weakly supported groups and *C. fastigiata* was also similar to isolates that matched the ex-type strains. The *EF-1α* tree topology was also similar to the ITS region tree topology for the clades related to *C. fastigiata*. Di243, which grouped with one of the new clades of *C. fastigiata* in the ITS analysis, but grouped with 2Di181-2, which is the same as *C.* sp. 5R24-1 and *C* sp. H37, in the *EF-1α* analysis. Isolates of *C.* sp. NH1-2 were also the most distal to all other species.

The actin sequence analysis did not coincide with the sequence analysis of the other loci sequenced (ITS, LSU and EF). Isolates that were placed with strong support in clades defined using ITS sequences were not grouped according to defined species

parameters using actin gene sequence data. For example, a *C. fastigiata*-like isolate (EB19-1) grouped with *C*. sp. NH1-2, but based on data from other loci, were phylogenetically distant from another. Isolates of *C. luteo-olivacea* were in various different clades with *C. fastigiata* and also in a sister clade to *C*. sp. NH1-2. The largest clade consisted of nine isolates of three species (*C. fastigiata*, *C. luteo-olivacea* and *C. malorum*) from wide geographical regions.

Wood Decay Studies

The wood decay microcosm studies showed a range of weight loss among the *Cadophora* species tested (Figure 5). Using birch wafers, *C. luteo-olivacea* caused the most weight loss (27.7 ±1.2%), followed by *C. malorum* with 20.3 ±1.3%, *C. fastigiata* with 18 ±1.4% and others in the *C. fastigiata* complex between 9.4 and 12.3%. *Cadophora* sp. H and *Cadophora* sp. NH1-2 had negligible weight loss (below 5%). Mass loss using pine wafers for all species was between 1.8 and 3.2%. SEM examination of wafers showed that all the species that caused weight loss above 5% caused a soft rot type of decay (Figure 6). Wafers decayed by *C. luteo-olivacea,* had the most mass lost, had more of the secondary cell wall removed than those decayed by *C*. sp. 106-1, which had the least mass loss of the isolates causing decay.

Cellulase Assay

All the isolates screened in the cellulase assay showed cellulase activity at 8 and 22°C with the exception of *C*. sp NH1-2 at 22°C (Fig. 7). *Cadophora* sp. 4E71-1 had the

highest relative activity of all tested isolates at 8°C, while C. sp. 4E71-1, *C. luteo-olivacea* and *C*. sp 5R24-1 all had similar activity at 22°C. *Cadophora luteo-olivacea* had the most cellulase activity among the isolates that showed soft rot abilities in the wood decay study. Comparisons were not made among isolates at different temperatures since the assays at each temperature were not conducted at the same time because of the slower growth at 8°.

Lignin-peroxidase study

None of the isolates tested in the lignin-peroxidase medium showed a positive reaction (clearing).

NaCl and $CuSO_4$ Media Tolerance

All *Cadophora* species tested grew on the medium containing 5% NaCl (Fig. 8). In medium having 8 and 12% NaCl, no fungal growth occurred for *C*. sp 5R24 and was negligible for *C. fastigiata*, and *C*. sp. NH1-2, *C. luteo-olivacae*, *C*. sp. E, and *C. malorum* had 0.17 cm, 0.48 cm, 0.63 cm and 1.35 cm of growth, respectively. The only growth that occurred on the 12% NaCl medium was that of *C. malorum* (0.05 cm) and *C*. sp. E (0.10 cm). All other isolates tested had no growth at this concentration. Other commonly isolated fungal species were tested to serve as a comparison to *Cadophora* spp. *Lecythophora* sp. only grew at 2% NaCl and *G. pannorum*, *P. dimorphospora* and *P. herbarum* all had similar tolerance and grew on medium with up to 8% NaCl, but not 12%.

The *Cadophora* species that showed tolerance to $CuSO_4$ were *C. fastigiata*, *C. malorum* and *C. luteo-olivacea*, which had 1.05 cm, 0.88 cm and 0.64 cm of growth over a 20 day period on 0.05% $CuSO_4$ amended media (Fig. 9). Other than *Cadophora* spp., the only species tested that showed any $CuSO_4$ tolerance was *Phialocephala dimorphospora,* which had 0.40cm of growth over the same period. No growth was seen for any isolates tested at the higher concentrations of 0.25% and 0.50% $CuSO_4$.

Discussion

The phylogenetic analysis used in this study shows that a complex of diverse *Cadophora* species exist in Antarctica. The phylogenetic analysis also separated isolates related to *C. fastigiata* that may represent new species. New clades were discovered in isolates closely related to *C. fastigiata* that are diverse and mainly (with one exception) from Deception Island, near the Antarctic Peninsula. This is supported by ITS, LSU and *EF-1α* loci analyses. The isolates in the undescribed clades are closely related to GenBank accessions associated with other woody hosts, maple, birch and grapevines, from a wide geographical range (Canada, Spain and Sweden). The decay and enzyme studies also support similar decomposition function, and their ability to function as a primary decomposer of lignocellulosic material (soft rot). Morphological descriptions of the isolates in the *C. fastigiata* complex are needed to determine if they can be distinguished from *C. fastigiata* and if morphological data are in agreement with molecular data.

Cadophora malorum, C. luteo-olivacea and *C. fastigiata* sensu stricto showed only slight phylogenetic variation in the ITS and *EF-1α* gene regions, indicating no

phylo-geographical patterns in the isolates tested in these gene regions. However, using actin sequence data, several isolates from the same location shared a well supported clade. Sequencing other gene regions or using other genetic markers may help to further distinguish the phylogenetic structure more precisely. The placement of *Mycochaetophora gentianae* (Figure 2, LSU data) near *C. malorum* and *C. luteo-olivacea* has not been reported previously. Nekoduka et al. (2010) describe *M. gentianae* as a pathogen that causes a brown leaf spot on gentian (*Gentiana* spp.). However, better phylogenetic resolution with additional loci is needed to determine more precise placement.

Analyses of ITS and LSU sequence data from *C*. sp. NH1-2, *C*. sp. H37 and *C*. sp 5R24-1 suggest these isolates are not in the genus *Cadophora*. *C*. sp. NH1-2 groups closest to *Collophora* species with high bootstrap support in MP and NJ analyses for both loci. *Collophora* is in the Leotiomycetes and was originally reported and described from cultures isolated from necrotic tissue of *Prunus* trees in South Africa (Damm et al. 2010). *Collophora hispanica* was more recently described from diseased almond trees in Spain (Gramaje et al. 2012). *C.* sp. H37 and *C*. sp. 5R24-1 are the same species based on these analyses. However, they group near *Xenopolyscytalum pinea* Crous. sp. nov. with high bootstrap support, indicating their taxonomic placement should be reconsidered. Little is known about this species with regard to its ecology other than it was isolated from pine needles in the Netherlands (Crous et al. 2010). Nonetheless, this species also shares an affinity to a woody host, as do *Cadophora* species.

The phylogenetic analyses revealed incongruence between the actin gene phylogeny and the ITS, LSU and EF phylogenies. The actin tree could be considered an anomalous gene tree in which genealogical histories lead to differences in topology compared to species trees (Degnan and Rosenberg 2006). From these studies, it is not clear what factors account for this incongruence. In fungi, actin plays a critical role in exocytosis, endocytosis, organelle movement and cytokinesis (Berepiki et al. 2011). Due to the important role of these functions, especially considering how they may relate to adaptation to differing environmental conditions and substrates, it would seem plausible that this gene region may have diverged from other loci analyzed. It could be informative to sequence other gene regions that may uncover cryptic species or relationships not uncovered by the current loci.

Cadophora species have been isolated in a wide range of environments, many considered to be extreme. One such environment is that of Polar Regions with high salinity (i.e., low water availability). Antarctica has many high saline environments on rock and soil surfaces and ponds (Des Marais 1995; Nishiyama 1977) and some isolates used in this study were isolated from Ross Island, where this is especially true. Thick crusts of salts can form on the soil surface and high concentrations can be found in snow (Blanchette et al. 2004). The tolerance to high salt conditions of several *Cadophora* species shown here supports their occurrence in saline environments (Goncalves et al. 2012; Burgaud et al. 2009). Freezing dehydrates cells by way of reduced water absorption and conduction, and high salinity has the same effect through osmotic imbalances. It has been inferred that organisms that are tolerant of drought or freezing are

well adapted to environs of high salinity (Gunde-Cimerman et al. 2003). Based on the findings here and their occurrences in environments with these conditions, it appears *Cadophora* species also have these characteristics. Tolerances to extreme conditions, in part, have enabled *Cadophora* species to thrive where other, more sensitive species cannot. This tolerance also appears to be somewhat shared with other fungi referred to as dark septate endophytes (DSE), such as *Phialocephala* and *Phoma* species.

Previous work has shown *Cadophora* (syn. *Phialophora*) species have been isolated frequently from treated wood (CCA) and can tolerate toxicity of heavy metals (Karunasekera and Daniel 2013; Daniel and Nilsson 1988). Further, *C. finlandica,* shown to form mycorrhyizal associations with ercoid hosts, are commonly found in habitats that contain heavy metals (Vralstad et al. 2002). This work provides further evidence of metal tolerance (copper) for other *Cadophora* species and shows the upper limits of that tolerance. However, several species were shown to be intolerant to copper. It is interesting that *C*. sp. E., which is closely related phylogenetically to *C. malorum* and *C. luteo-olivacae,* would not grow on copper sulphate amended media. It is not surprising that *C*. sp. H and *C*. sp. NH1-2 did not exhibit copper tolerance because, as stated earlier, these strains do not appear to be in the genus *Cadophora*. However, *Lecythophora dimorphospora,* considered to be a DSE, did show tolerance and grew at the lowest $CuSO_4$ concentration (0.05%). The high salt and copper medium may be suitable as selective medium for screening materials for the presence of *Cadophora* species from other environments where many other saprophytic fungi that can outcompete *Cadophora* are found.

The results presented here, with those from previous studies (Arenz and Blanchette 2009; Arenz et al. 2006; Arenz et al. 2010; Blanchette et al. 2010; Held et al. 2006; Blanchette et al. 2004), provide strong evidence that supports the hypothesis that *Cadophora* species play a significant role in decomposition of lignocellulosic material in the Antarctic environment. The laboratory decay studies showed the ability of many species of *Cadophora,* including the undescribed *C. fastigiata*-like isolates reported here, to cause a Type 1 soft rot decay in wood. In this type of decay, hyphae form cavities in the secondary wood cell wall in which it degrades mainly cellulose and hemicellulose via secretory enzymes. The cavities can coalesce and become large and cause significant strength loss. However, decay is usually limited to the outer surfaces of affected wood, which may indicate the need for higher O_2 conditions (Duncan 1960). While it is clear that they can be efficient decomposers of the historic wood that was brought to Antarctica, it is not well understood what role these species play in the ecology of nutrient cycling in areas without introduced wood in Antarctica. Vegetation in Antarctica is limited to only two vascular plants (one grass species and one pearlwort species) and many species of bryophytes. Day and Currah (2011) showed that *C. luteo-olivacea* was able to degrade bryophyte material from Canada, which indicates the possibility of a wider ecological role in the decomposition of bryophyte plant material.

Three of the *Cadophora* species (*C*. sp. NH1-2, *C*. sp. H37 and *C*. sp. 4E71-1) that did not show significant decay in the laboratory decay tests, had relative enzyme activity equal to or greater than that of the species that did cause decay. A complex of enzymes is needed to degrade lignocellulose materials (Hatakka and Hammel 2010).

Carboxylmethylcellulose, a derivative of cellulose in wood, used in this study is more easily degraded than native cellulose that is bound in the wood cell walls in a matrix with lignin and hemicellulose. Therefore, these species are apparently efficient at producing cellulases in the presence of this type of cellulose but lack the mechanisms to degrade the cell wall in which cellulose is bound with lignin and hemicellulose. The chemistry of soft rot degradation has not been studied to the same extent as white and brown rots. It has been shown that some soft rot fungi (mainly Type 1) are able to degrade and or modify lignin, but the mechanism has remained elusive (Eslyn et al. 1975; Kirk and Farrell 1987; Nilsson and Daniel 1989). One study on the degradation of cellulose and lignin of beech wood by the soft rot fungus *Chaetomium globosum*, placed it as an intermediate between brown and white rot fungi (Seifert 1966). One would expect some lignin degradation/modification in some of the samples with substantial mass loss, but it is not clear from the negative results of the lignin-peroxidase screening study whether lignin is not being substantially degraded or if it is being degraded via another enzyme pathway (i.e., manganese peroxidase, laccase or others). Further studies aimed at detailing the secretome of soft rot fungi and comparing it to that of brown and white rots are warranted to elucidate their mode of degradation.

Cadophora species appear well adapted to the extreme nature of the environment in Antarctica (high salts, strong UV radiation, extreme temperatures and frequent freeze thaw cycles) and are the major decomposers, in contrast to the brown and white rot fungi, that are found in temperate and tropical environments. It is likely, based on the worldwide occurrence and role in many different ecological capacities of *C. luteo-*

olivacea, *C. malorum* and *C. fastigiata*, that these species were introduced along with wood and/or other materials by anthropogenic activities or proliferated due to the large nutrient input from these activities. Their current distribution, despite their origin, shows they are now endemic and have tolerated selection pressures that have limited other species. As research of microbial life in Polar regions continues, focus is needed on the role of fungi in these environments. Only a small fraction of Polar ecosystems have been studied in this regard, compared to other biomes worldwide, leaving a tremendous opportunity to study a model system in which microbes dominate. All indications are that Antarctica may be one of the unusual ecosystems in the world where Ascomycetes are the major group of fungi decomposing lignocellulosic material. This raises interesting questions about microbial succession in degradation of lignocellulose and nutrient cycling, due to the unique nature of the plant and microbe communities in a harsh environment.

Conclusions

Cadophora species are adapted to a number of environments, some of them extreme, around the globe. Research has shown that *Cadophora* species have a three-fold association with plants and plant material: as pathogens (*C. fastigiata*, *C. luteo-olivacea*) on a variety of plant species, in forming mycorrhizal associations (*C. finlandia*, *C. malorum* and *C. luteo-olivacea*) and as a saprotroph, in which case *Cadophora* species are found decaying wood or other cellulosic materials, even in harsh environments. The ecological plasticity of *Cadophora* spp. suggests the genus has important functions,

which are not well understood. Studies aimed at following the possible successive role of *Cadophora* species as plant endophyte to saprophyte could begin to detail these functions. In addition, discovery of more specific adaptive strategies enabling function in extreme environments, which would give insight how these adaptations, are taking place.

In Antarctica, a complex of *Cadophora* species is found in association with wood that has been introduced to the continent. Some of these species may have been brought to Antarctica by human activity but others may be endemic and have been successful at long term survival in this extreme environment. Their impact on and role at the landscape scale has yet to be discovered. Soft rot fungi, among which *Cadophora* species may be classified in their saprotrophic lifestyle, are not well understood in ecological terms. Antarctica provides a good model to further study decomposition by soft rot fungi due to lower nutrient inputs and reduced microbial complexity compared to temperate systems. This would provide insight into more complex biomes in which fungal species and lignocellose abound.

Table 1. List of *Cadophora* isolates and associated accession numbers for loci sequenced from this study. ITS=internal transcribed spacer region of ribosomal DNA, EF= translation *elongation factor 1 alpha* locus (*EF-1α*), LSU=large subunit of ribosomal DNA and ACT=actin

Species	**Isolate Number**	**Region**	**Accession Numbers**			
			ITS	**EF**	**LSU**	**ACT**
Cadophora malorum	ArgH1-1	Peninsula	KF053538	--	--	--
	BE04-1	Pen.	KF053539	--	--	--
	DP11-1	Pen.	KF053540	--	--	--
	DP6-3	Pen.	KF053541	KF053608	--	KF053644
	DB1N-4	Pen.	KF053542	KF053609	--	KF053645
	DE22-1	Pen.	KF053543	---	--	--
	Di3-4	Pen.	KF053544	KF053610	--	--
	2Di43-1	Pen.	KC514852	KF053611	--	KF053647
	Dnco8-2	Pen.	KF053545	KF053612	--	KF053648
	Pg1-1	Pen.	KF053546	KF053613	KF053582	
	EB97-3	Pen.	KF053547	KF053614	--	KF053649
	HI2-1	Pen.	KF053548	--	--	--
	HI4-1	Pen.	KF053549	--	--	--
	HDD3A-1	Pen.	KF053550	--	--	KF053650
	VP14-2	Pen.	KF053551	--	--	--
	WH19-1	Pen.	KF053552	--	--	--
	6E69-1	Ross Island	KF053553	KF053615	--	KF053651
	7E68-1	R.I.	KF053554	--	--	--
	7R78-2	R.I.	KF053555	KF053616		KF053652
	A149	R.I.	--	KF053606	KF053583	KF053642
	A163	R.I.	--	KF053607	KF053584	KF053643
	Di270-1	Pen.	--	--	--	KF053646
C. luteo-olivacea	PL12-3	Pen.	KF053556	--	--	--
	EB46-4	Pen.	KF053557	--	--	--
	EB54	Pen.	KF053558	KF053618	KF053586	--

Species	Isolate Number	Origin	Accession Numbers			
			ITS	EF	LSU	ACT
	EB09-2	Pen.	KF053559	KF053619	KF053587	
	DE24-1	Pen.	KF053560	--	--	--
	DE26-3	Pen.	KF053561	KF053620	--	--
	Di13-1	Pen.	KF053562	KF053621	KF053588	--
	Di90-5	Pen.	KC514851	KF053622	KF053589	KF053655
	Di279	Pen.	--	KF053623	--	KF053656
	EB60-2	Pen.	--	--	--	KF053653
	DBIN-3	Pen.	--	--	--	KF053654
C. fastigiata	Di76-3	R.I.	KF053563	--	KF053590	--
	Di252-1	Pen.	KC514850	KF053624	--	KF053657
	EB40-1	Pen.	KF053564	KF053626	KF053591	KF053658
	EB92-1	Pen.	KF053565	--	--	--
	EB95-1	Pen.	KF053566	--	--	--
	7R121-1	R.I.	KF053567	KF053627	--	KF053659
	7R122	R.I.	KF053568	KF053628	--	--
	7R124-1	R.I.	--	KF053629	KF053593	--
	DB19-1	Pen.	--	KF053625	--	KF053660
	7R52	R.I.	--	--	KF053592	--
Cadophora species	Di2-3	Pen.	KF053578	KF053636	KF053600	KF053663
	Di3-8	Pen.	KF053576	--	KF053598	--
	Di102-1	Pen.	KF053572	--	--	--
	Di106-1	Pen.	KF053571	KF053631	KF053594	--
	Di254-1	Pen.	KF053569	--	--	--
	Di273	Pen.	KF053573	KF053633	KF053596	KF053661
	2Di100-3	Pen.	KF053574	KF053634	KF053597	KF053662
	Di274	Pen.	KF053577	KF053635	KF053599	--
	EB19-1	Pen.	KF053570	--	--	--
	HB16-2	Pen.	KF053575	--	--	--

Species	Isolate Number	Origin	Accession Numbers			
			ITS	EF	LSU	ACT
	Di255-2	Pen.	KF053579			
	Di94-1	Pen.	--	KF053630	--	--
	Di103-4	Pen.	--	KF053632	KF053595	--
	Di253	Pen.	--	--	--	--
	Di253-1	Pen.	--	KF053637	KF053601	--
	Di255-1	Pen.	--	--	KF053602	--
C. sp. NH1-2	Di90-1	Pen.	KF053580	KF053638	KF053603	KF053664
	2Di94-7	Pen.	KF053581	KF053639	KF053604	KF053665
	2Di102-3	Pen.	KC514853	KF053640	--	KF053666
	Di62-7	Pen.	KC514893	--	--	--
C. sp. H	2Di181-2	Pen.	KC514854	KF053641	KF053605	--
C. sp. 4E71-1		R.I.	AY371506	KF053617	KF053585	--

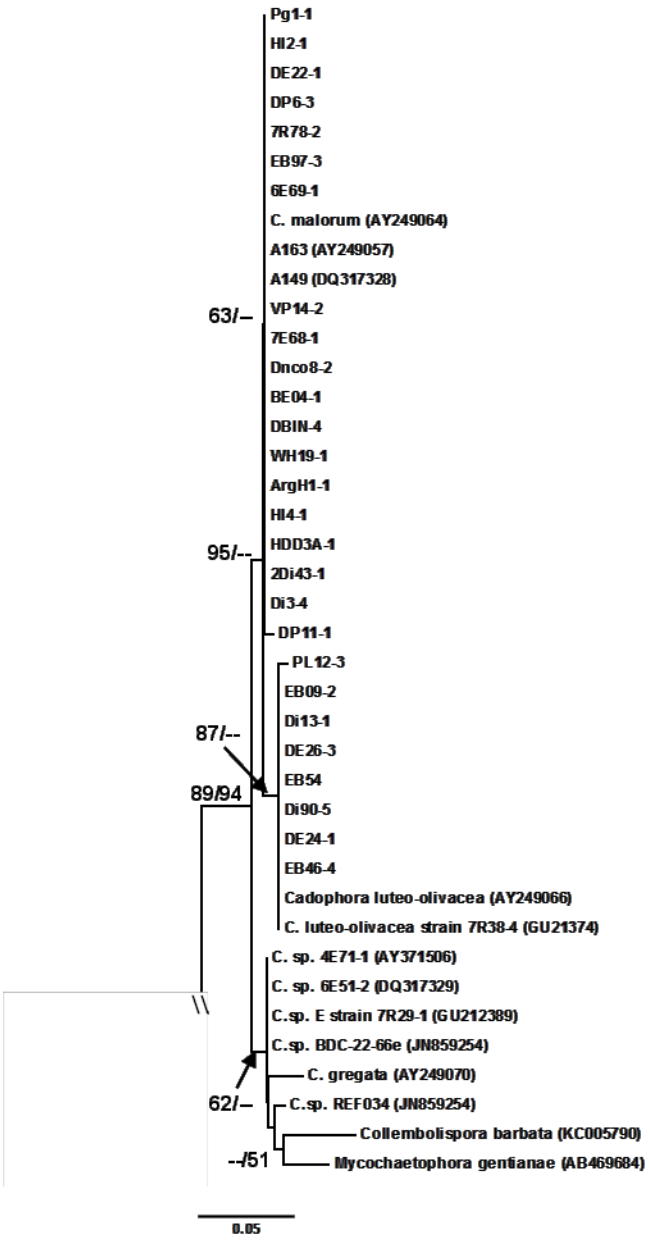

Figure 1. Distance tree generated by NJ analysis based on the ITS region of rDNA. Data from species from previous studies and sequences downloaded from GenBank are followed by accession numbers. Bootstrap values of 1000 replicates of NJ and MP are shown, respectively. *Peziza phyllogena* was used as the outgroup. Scale bar = substitutions per site.

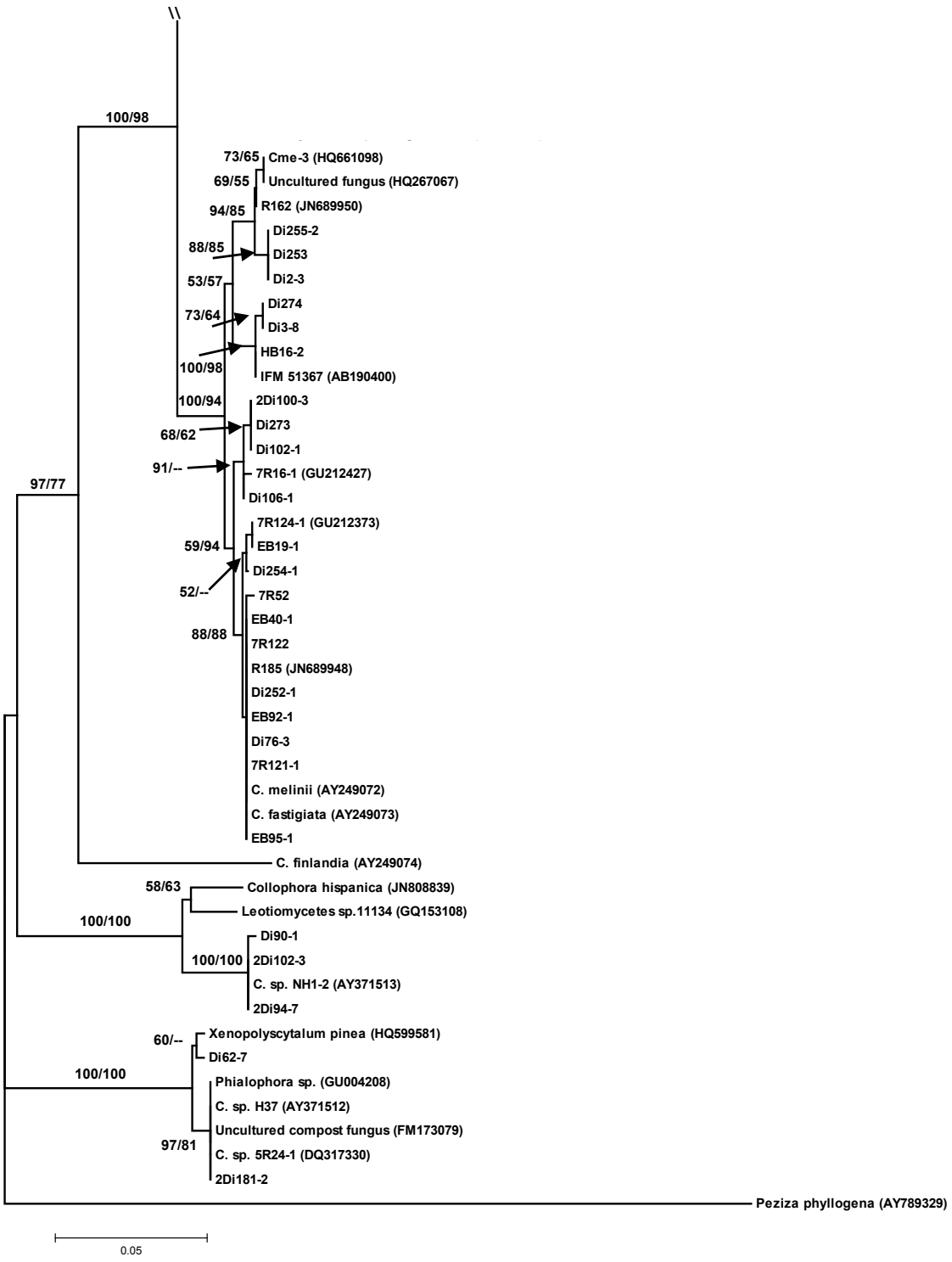

Figure 1, continued.

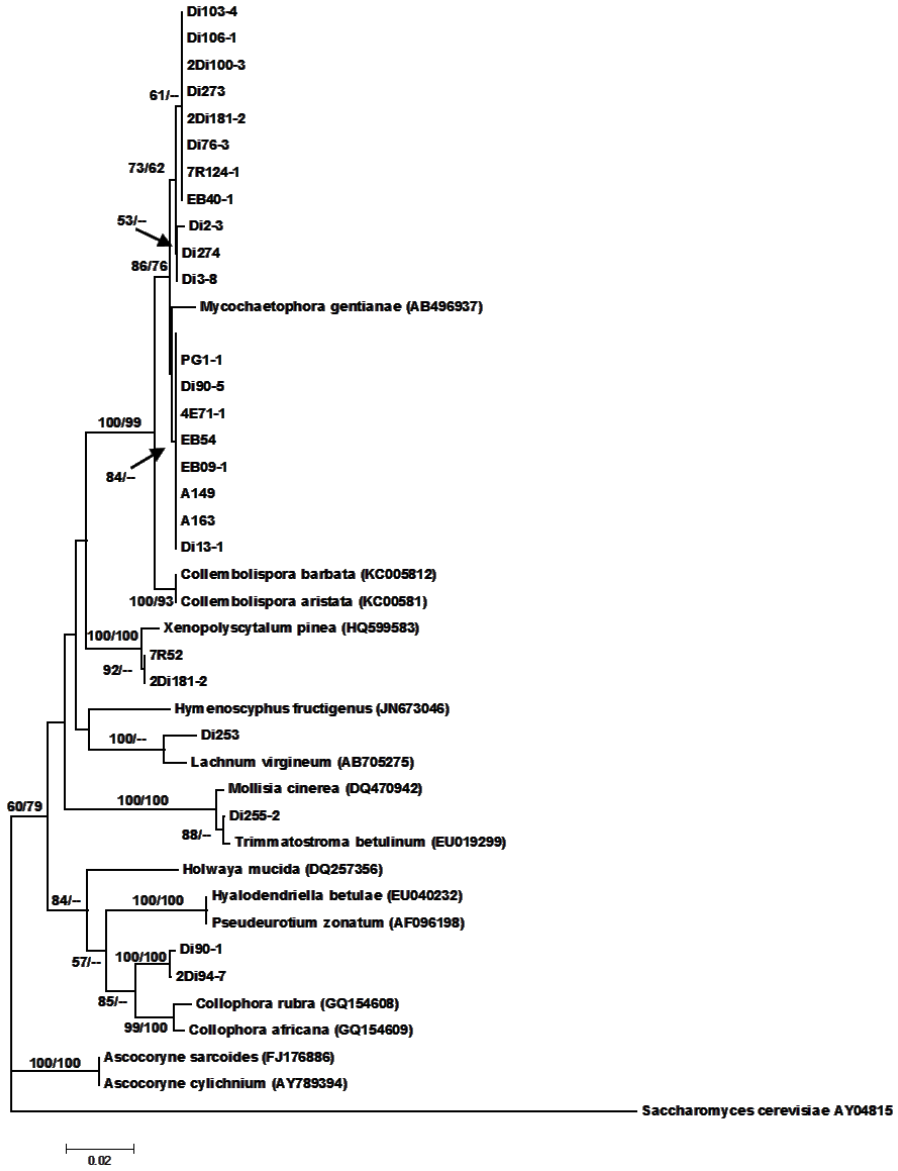

Figure 2. Distance tree generated by NJ analysis based on partial large subunit of rDNA sequence data. Species from previous studies and sequences downloaded from GenBank are followed by accession numbers. Bootstrap values of 1000 replicates of NJ and MP are shown, respectively. *Saccharomyces cerevisiae* was used as the outgroup. Scale bar = substitutions per site.

Figure 3. Distance tree generated by NJ analysis based on partial EF-1α gene sequence data. Species from previous studies and sequences downloaded from GenBank are followed by accession numbers. Bootstrap values of 1000 replicates of NJ and MP are shown, respectively. Peziza vesiculosa was used as the outgroup. Scale bar = substitutions per site.

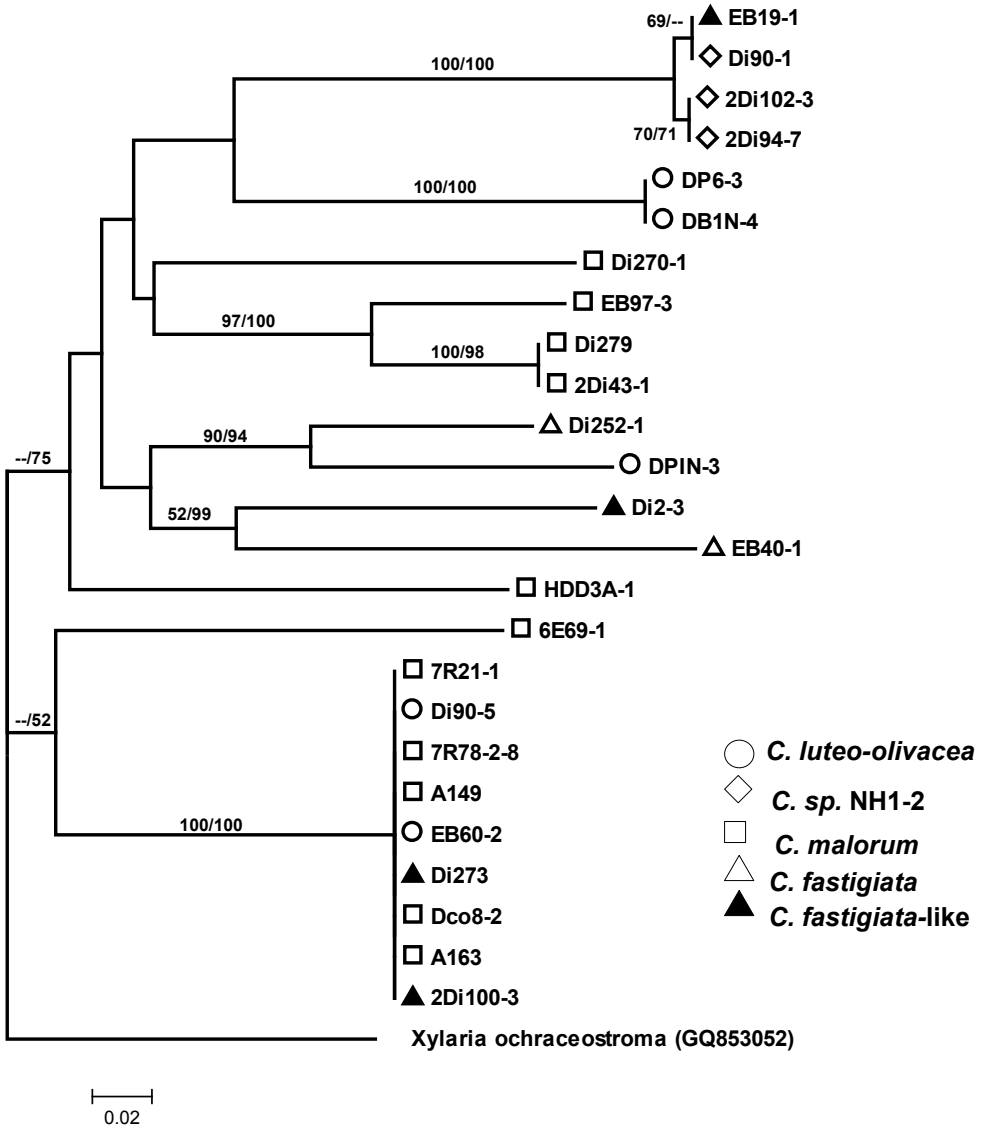

Figure 4. Distance tree generated by NJ analysis based on partial actin gene sequence data. Species from previous studies and sequences downloaded from GenBank are followed by accession numbers. Bootstrap values of 1000 replicates of NJ and MP are shown, respectively. *Xylaria ochraceostroma* was used as the outgroup. Scale bar = substitutions per site.

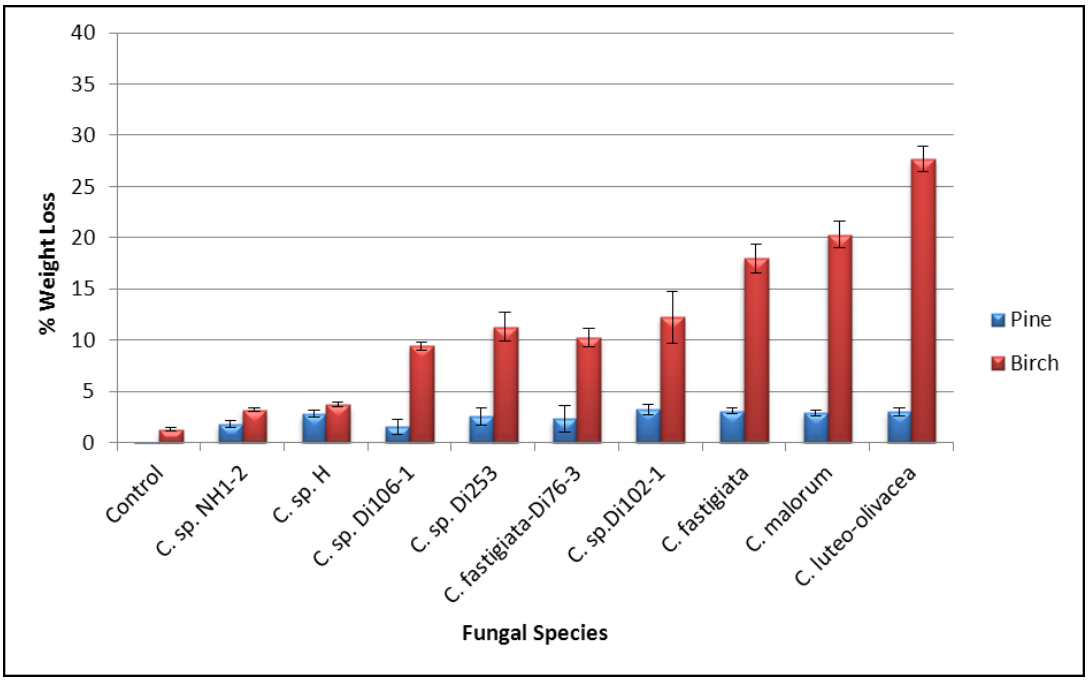

Figure 5. Graph showing mass loss of birch and pine wafers after a 16 week microcosm decay study.

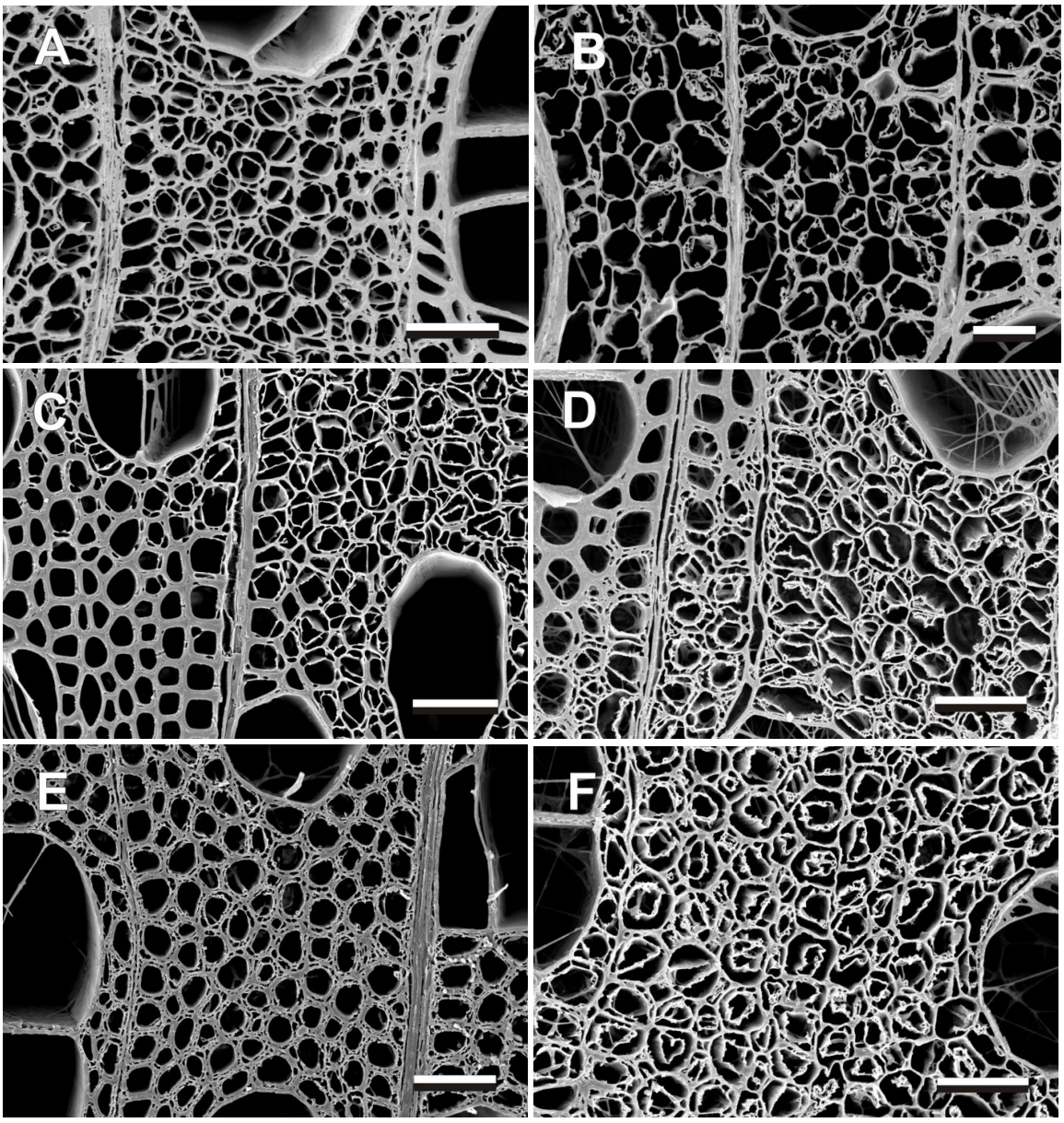

Figure 6. Scanning electron micrographs of wood wafers (birch) from the decay and mass loss study of different *Cadophora* species showing soft rot type of decay affecting cell wall structure. A: *Cadophora malorum* B: *C. luteo-olivacea* C: *C. fastigiata* D: *C.* sp. Di253 (*C. fastigiata* like) E: *C.* sp. Di106-1 (*C. fastigiata*-like) F: *C.* sp. Di102-1 (*C. fastigiata*-like). The decay across all species is very similar and is preferentially degrading the cellulose rich secondary cell wall leaving the middle lamella and primary

cell walls. *C*. *luteo-olivacea* (B) shows the most advanced degradation while *C*. sp. 106-1 (E) shows smaller cavities than others tested in the secondary wall. Bars=50 μm

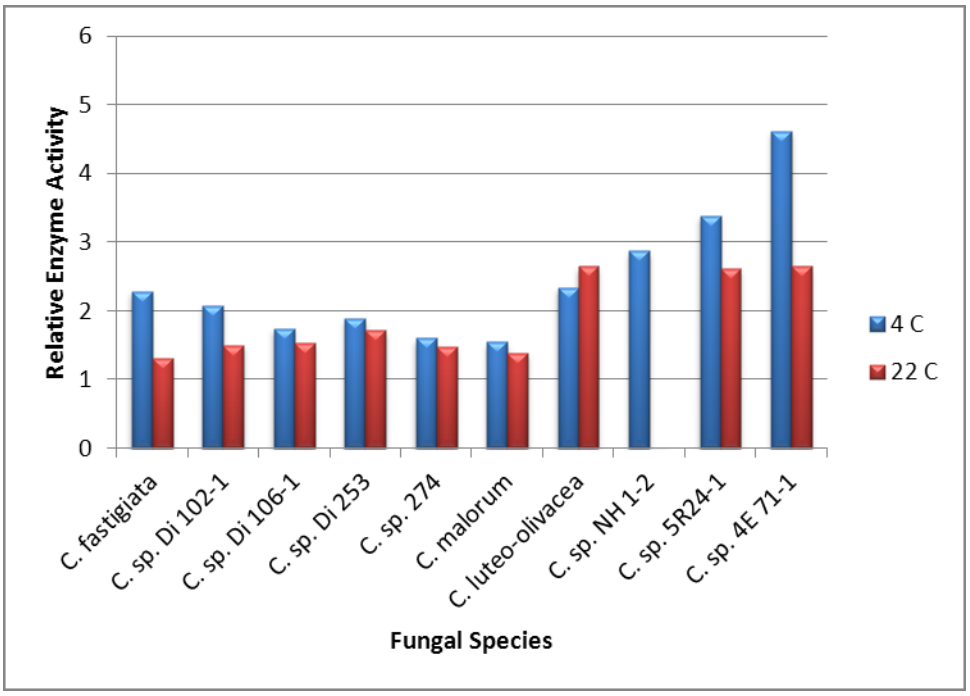

Figure 7. Graph showing the relative cellulase enzyme activity of *Cadophora* species.

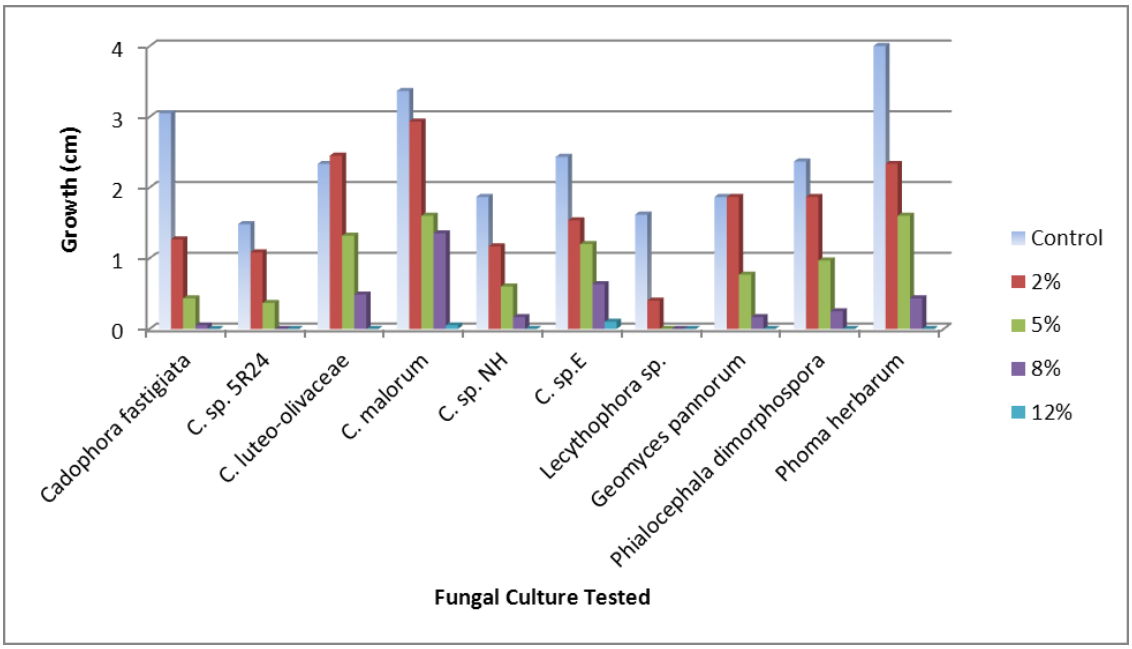

Figure 8. Growth (cm) of different *Cadophora* species and other commonly isolated fungi from Antarctica after 20 days on media amended with 2, 5, 8, and 12 % w/v (0.34, 0.86, 1.37 and 2.05 M respectively) NaCl.

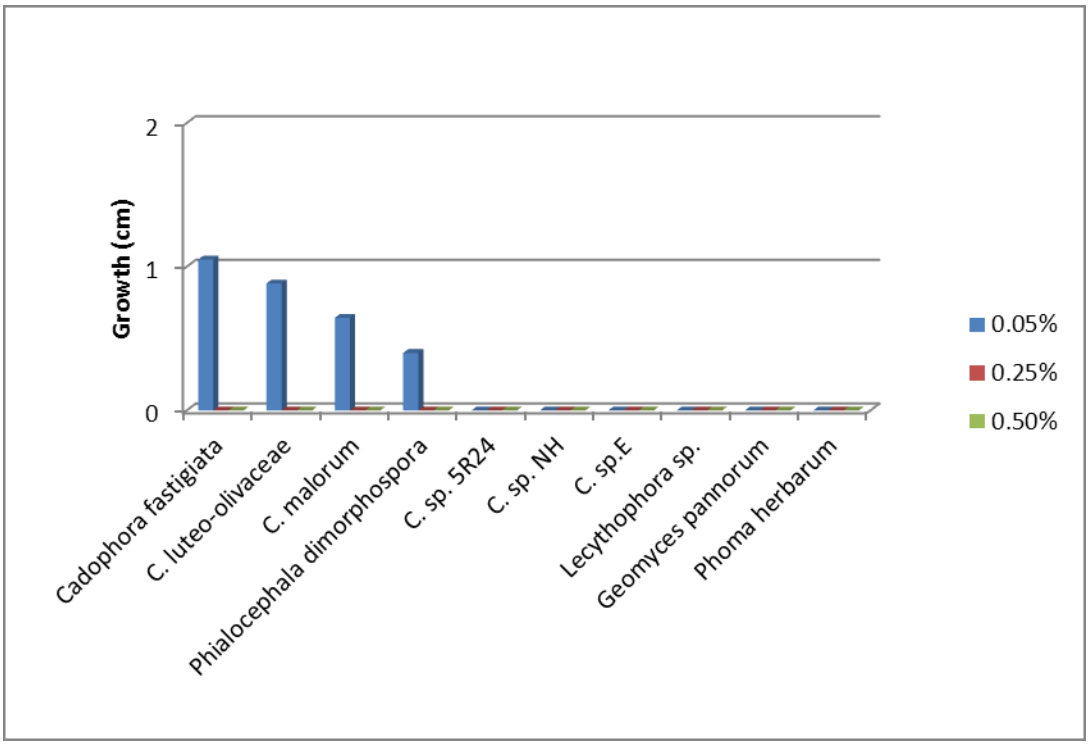

Figure 9. Growth (cm) of *Cadophora* and other fungal species commonly isolated from Antarctica after 20 days on media amended with 0.05, 0.25 and 0.50 % w/v (3, 20, and 30 mM, respectively) of $CuSO_4$.

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