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FUNGAL MICROBIOLOGY

An Antarctic Hot Spot for Fungi at Shackleton's Historic Hut on Cape Royds

Robert A. Blanchette · Benjamin W. Held · Brett E. Arenz · Joel A. Jurgens · Nicolas J. Baltes · Shona M. Duncan · Roberta L. Farrell

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Abstract The historic expedition huts located in the Ross Sea Region of the Antarctic and the thousands of artifacts left behind by the early explorers represent important cultural heritage from the "Heroic Era" of Polar exploration. The hut at Cape Royds built by Ernest Shackleton and members of the 1907–1908 British Antarctic Expedition has survived the extreme Antarctic environment for over 100 years, but recent studies have shown many forms of deterioration are causing serious problems, and microbial degradation is evident in the historic wood. Conservation work to reduce moisture at the hut required removal of fodder, wood, and many different types of organic materials from the stables area on the north side of the structure allowing large numbers of samples to be obtained for these investigations. In addition, wood from historic food storage boxes exposed in a ravine adjacent to the hut were also sampled. Fungi were cultured on several different media, and pure cultures were obtained and identified by sequencing of the internal transcribed spacer region of rDNA. From the 69 cultures of filamentous fungi obtained, the most predominant genera were *Cadophora* (44%) followed by *Thielavia* (17%) and *Geomyces* (15%). Other fungi found included *Cladosporium*, *Chaetomium*, and isolates identified as being in *Pezizomycotina*, *Onygenales*, *Nectriaceae*, and others. No filamentous basidiomycetes were found. Phylogenetic analyses of the *Cadophora* species showed great species diversity present revealing *Cadophora malorum*, *Cadophora luteo-olivacea*, *Cadophora fastigiata*, as well as *Cadophora* sp. 4E71-1, a *C. malorum*-like species, and *Cadophora* sp. 7R16-1, a *C. fastigiata*-like species. Scanning electron microscopy showed extensive decay was present in the wood samples with type 1 and type 2 forms of soft rot evident in pine and birch wood, respectively. Fungi causing decay in the historic wooden structures and artifacts are of great concern, and this investigation provides insight into the identity and species diversity of fungi found at the site. The historic woods and other organic materials at this site represent a large input of carbon into the Antarctic environment. This as well as nutrient additions from the nearby Adélie penguin (*Pygoscelis adeliae*) colony and favorable conditions for fungal growth at Cape Royds appear responsible for the significant fungal diversity, and where extensive decay is taking place in wood in contact with the ground.

R. A. Blanchette (✉) · B. W. Held · B. E. Arenz · J. A. Jurgens · N. J. Baltes
Department of Plant Pathology, University of Minnesota,
1991 Upper Buford Circle,
St. Paul, MN 55108-6030, USA
e-mail: robertb@umn.edu

S. M. Duncan · R. L. Farrell
Department of Biological Sciences, University of Waikato,
Hamilton 3216, New Zealand

Present Address:
N. J. Baltes
Department of Microbiology, University of Minnesota,
Minneapolis, MN 55455, USA

Present Address:
S. M. Duncan
Department of Bioproducts and Biosystems Engineering,
University of Minnesota,
Saint Paul, MN 55108, USA

Introduction

Historic wooden structures are important cultural resources, but their preservation is often difficult due to microbial degradation that destroys them [6–8]. Wood can be attacked by a large number of diverse microorganisms and exposure to the environment, especially in historic structures built

long ago, provides conditions that are conducive for decay. Many different types of wood decay have been characterized, and environmental conditions, type of substrate, a source of inoculum, and other biological parameters determine which microbes are able to grow successfully and cause degradation [16, 17, 39]. Wood decay affecting historic structures is best known and most studied in tropical and temperate regions of the world, but it is also a problem in areas with extreme environmental conditions such as in arid and Polar Regions. The microorganisms and decay processes occurring in ecosystems with extreme conditions are not well understood, and information from studies on wooden cultural properties found in these environments is needed for conservation efforts to be successful. Data obtained can also provide a better understanding of the biology and ecology of these little known organisms and lead to greater insight into their role in these unusual ecosystems. There is a global need to increase knowledge on biodiversity and ecosystem functioning in places like Antarctica as well as other cold climates where research studies on microbial ecology have been limited [30, 32, 36, 37].

In February 1908, Ernest Shackleton and members of the British Antarctic Expedition built a hut with an adjacent stable at Cape Royds on Ross Island, Antarctica to serve as a base for their explorations of the South Polar Region and scientific investigations. The hut served this 15-man expedition until March 1909, and stables sheltered the ponies brought with them. The hut was also used in later expeditions by members of Scott's 1910–1913 British Antarctic Expedition and by the Ross Sea Party during Shackleton's 1914–1917 Imperial Trans-Antarctic Expedition [2, 20]. The hut, stables, and thousands of artifacts left at Cape Royds represent an extraordinary legacy from the "Heroic Era" of exploration, and the location is now a protected international heritage site. Although located in Antarctica where extreme cold and dry climatic conditions exist, extensive deterioration has taken place in the hut [4, 10]. Awareness of the decay problems associated with the wooden structure and concern for its preservation has increased in recent years. In 2006, the World Monument Fund listed the hut among the 100 most endangered sites in the world (http://wmf.org/pdf/Watch_List_2006.pdf).

Previous investigations initiated to provide information for long-term conservation plans for several historic structures in the Ross Sea Region identified fungi causing a soft rot type of wood decay in the exterior hut wood and many darkly pigmented filamentous fungi causing stain and disfiguring of wood and artifacts inside the huts [10, 21]. Since the huts were prefabricated with timbers from Europe and rebuilt in Antarctica, brown rot fungi which are the most common destroyers of buildings in temperate areas were expected but not found. The large amount of food stores, fodder, and many other items brought from England and New Zealand by the explorers also would suggest that many opportunities existed for exotic microorganisms that cause degradation of materials to be introduced. However, the most prevalent decay fungi found in recent studies made at the huts were species of *Cadophora*, a member of the *Helotiales* in the *Ascomycota*, and these fungi appear indigenous to Antarctica [4, 10, 21]. Investigations have shown that *Cadophora* species are very prevalent in soils and a common filamentous fungus found at many sites in the Ross Sea Region [4]. Recent investigations have also found *Cadophora* species attacking historic wooden structures in other areas of Victoria Land, the Peninsula Region of Antarctica, and in the Arctic [3, 9, 21, 23].

Although past studies have provided information on a number of fungal taxa from the wood and soils at Cape Royds and other Antarctic historic sites [4, 10], previous sampling was limited. The removal of only minute slivers of wood and sections of other organic materials from a few specific locations was done such that the sampling was always from only inconspicuous places so there would be no adverse impact when sampling from the historic materials. However, recent conservation work at the Cape Royds hut to reduce moisture accumulation from snow and ice melt resulted in excavations around the hut that removed large amounts of materials from the stables and other locations. This included miscellaneous wood from the collapsed stable roof and fragmented storage boxes as well as fodder, rope, canvas, paper, and other organic materials. In addition, unusually low snow levels and greater snowmelt exposed large numbers of fragmented historic wooden boxes in a ravine near the hut. The exposure of these materials provided an extraordinary opportunity to sample widely from many diverse materials at this historic site. This study was done to obtain more accurate information on the filamentous fungi associated with historic woods from Cape Royds by collecting large numbers of samples that were in contact with the ground and to characterize the many different *Cadophora* species obtained from the site. Since previous investigations revealed that filamentous fungi were the important wood decay organisms in this Antarctic terrestrial environment, these investigations focused on the filamentous fungi and not yeasts and bacteria.

Methods

Cape Royds hut on Ross Island, Antarctica is located at geographic location of 77°33′10.7″S, 166°10′6.5″E, and is the site where Ernest Shackleton and his expedition crew from the British Antarctic Expedition built a hut in 1908 (Fig. 1). The historic hut was constructed using pine and

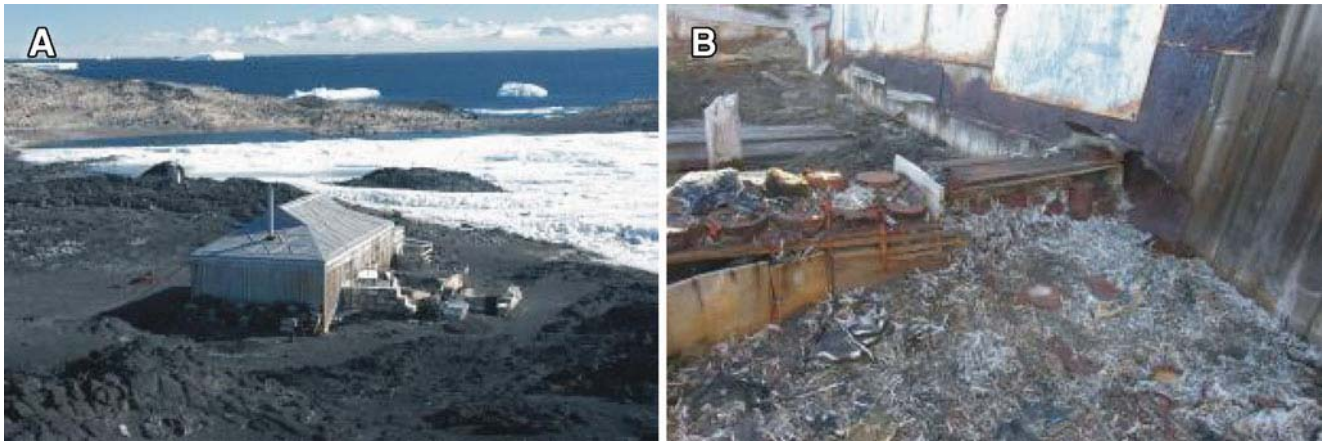

Figure 1 **A** Historic hut at Cape Royds built by Ernest Shackleton and crew of the British Antarctic Expedition in 1908 and surroundings. The stables area that was sampled in this study can be seen along the right (*north*) side of the hut. An Adélie penguin rookery is located between the hut and the Ross Sea. **B** The ground in the stables area showing debris consisting of wood fragments and other materials among layers of old fodder and penguin feathers and guano

spruce timbers, prefabricated in England and re-erected on site. Adjacent to the north side of the hut, a stables area was built using one wall of the hut and the other walls consisting of a double row of wooden boxes that held stores of food and bales of fodder built to a height of 1.73 m [2]. Many of the wooden boxes were made of birch Venesta board, an early form of plywood. A makeshift roof of wood planks and canvas tarpaulin covered the stables. A photograph taken on February 17, 1911 by a later expedition showed that the roof had already collapsed by this time [2]. Over subsequent years, materials such as leftover fodder, roof planks, fragmented wooden boxes, windblown fragments of materials, and various organic items left by other expeditions in 1910–1913 and 1914–1917 accumulated in this area especially on the side nearest the hut (Fig. 1). During excavation, these materials in layers of penguin feathers, guano, and soil were exposed. Samples were obtained from the wood fragments and other materials above and below the roof planks. In addition, wooden fragments from broken storage boxes that blew away from the hut site and accumulated in a nearby ravine (approximately 200 m west of the hut) were also sampled. Small sections of wood in contact with the ground were collected, placed in sterile bags, and kept cool during transport to the University of Minnesota where they were used for isolations and microscopy. Samples were hand carried from Antarctica and arrived in the laboratory within 2 weeks of collection. Wood segments from the samples were cultured for microorganisms using three types of growth media: 1.5% Difco malt extract agar (MEA), MEA with 2 ml of lactic acid added after autoclaving, and a semi-selective media for Basidiomycetes that included 15 g of malt extract, 15 g of agar, 2 g of yeast, 0.06 g of benlate with 0.01 g of streptomycin sulfate, and 2 ml of lactic acid added after autoclaving. All ingredients for each media type were added to 1 l of deionized water. Incubation was at 20°C to 22°C since previous studies have shown filamentous fungi from Polar Regions are primarily psychrotrophs or mesotrophs and can grow above 20°C [3, 4, 14, 21, 31]. Cultures of fungi were transferred to individual plates, and pure cultures were obtained.

DNA was extracted from pure cultures using a Qiagen DNeasy Plant Mini-kit following manufacturer's instructions (Qiagen Sciences Inc., Germantown, MA, USA). After DNA extraction, the internal transcribed spacer (ITS) region of ribosomal DNA was targeted for polymerase chain reaction (PCR) amplification with the primers ITS1 and ITS4 [19]. A 25-µl total volume reaction was prepared including 12.5 µl Amplitaq PCR Gold Mastermix, 9.5 µl sterile distilled H20, 1 µl 10 µm ITS1, 1 µl 10 µm ITS4, and 1 µl DNA template. Details on the thermocycler and PCR profile are described by Arenz and Blanchette [3]. Following PCR amplification, amplicon size was verified (500–600 b) on a Dark Reader DR45 with a SYBR green 1 prestain. Sequence was obtained using an ABI PRISM Dye Terminator Cycle Sequencing Ready reaction kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 377 automated DNA sequencer. Following sequencing of both forward and reverse strands and alignment using ChromasPro software, fungi were identified by comparison to the GenBank database via BLASTn [1] searching.

Sequences were aligned using DS Gene v1.5 (Accelrys Inc., San Diego, CA, USA), and phylogenetic analysis of the DNA alignments was performed with PAUP 4.04b [34]. The alignment was subjected to parsimony analysis, and bootstrap values were determined using 1,000 replications, only retaining groups with greater than 50% support. These values were inserted into trees generated by neighbor

joining to obtain branch lengths. *Tapesia cinerella* and *Dactylella lobata* were used as outgroup taxa. Sequences of fungi obtained in these investigations were deposited in GenBank. Segments of wood samples with visible evidence of decay were prepared for scanning electron microscopy by hydrating the samples in TBS™ Tissue Freezing medium™ (Triangle Biomedical Sciences, Durham, NC, USA) under vacuum, mounting on brass stubs at −20°C in an OM 2488 Minotome® microtome-crystat (International Equipment Company, Needham Heights, MA, USA) and cutting the section transversely to create a clean face for viewing. After thawing and air-drying for 48 h, the specimens were coated with gold in an EMS 76M Ernest Fullum sputter coater (Ernest F. Fullam, Inc., Schenectady, NY, USA). Samples were examined using a Hitachi S3500 N (Hitachi, Tokyo, Japan) scanning electron microscope.

Results

This historic site at Cape Royds contained an unusually large amount of introduced wood, fodder, and other organic materials that had been at the site for nine to ten decades. These materials were mixed with windblown soil, penguin feathers, and other debris, and the north side of the hut provided a somewhat protected area where moisture accumulated from snowmelt in the austral summer (Fig. 1). The ravine sampling site was also protected from the wind and had increased moisture due to snowmelt in comparison to other areas at the site. Most of the wood samples collected were soft with extensive decay. Microscopic observations revealed various stages of soft rot were present in all of the woods examined. Many of the wood samples contained advanced decay, and wood cell walls were severely degraded. In samples of pine wood, type 1 soft rot was evident with cavities formed within the secondary cell walls (Fig. 2a). Individual cavities in the secondary wall were evident as well as cavities that coalesced forming large voids in the wood cells. In samples of hardwood, such as in the birch venesta boards, a type 2 soft rot was evident consisting of fiber cells with eroded secondary cell wall layers. Wood with advanced stages of decay had secondary walls that were completely degraded, and only middle lamella between cells remained (Fig. 2b). Wood cell integrity was greatly compromised, and wood from the samples collected was sponge-like and could easily be crushed. Fiber cells in these samples were often collapsed and altered due to the severely reduced strength properties of the wood. Some woods had dark discoloration present, but no other forms of decay, such as white or brown rot, were observed.

Isolations in media from different wood samples yielded 69 cultures of filamentous fungi (Table 1). The most predominant organisms were *Cadophora* (44% of the cultures) followed by *Thielavia* (17%) and *Geomyces* (15%). Many other fungi were also found in smaller numbers such as *Cladosporium* and *Chaetomium*, while others had BLAST matches that were for isolates previously described in broad taxonomic groups of Ascomycete sp., *Pezizomycotina* sp., *Onygenales* sp., and *Nectriaceae* sp. Some BLAST matches had low similarity to other sequences in GenBank such as the culture matching *Solenopezia* that had only an 87% similarity of base pairs. The *Cadophora* species were mainly isolated from wood, but a few were also obtained from soil, paper, rope, and straw. Phylogenetic analyses confirmed the identity of the

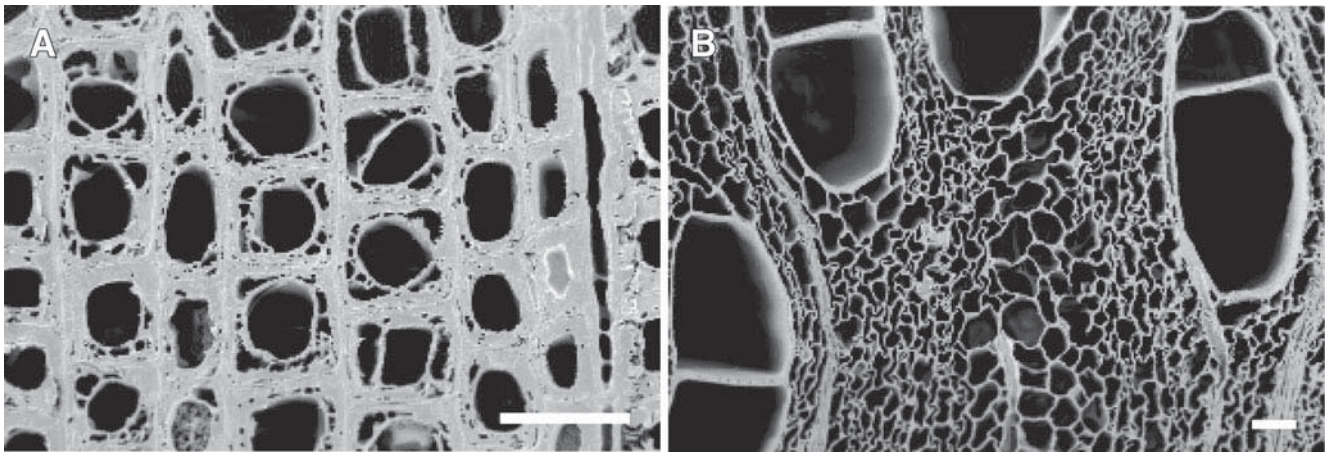

Figure 2 Scanning electron micrographs from transverse sections of pine (**A**) and birch wood (**B**) showing the two forms of soft rot that were found in the historic woods sampled. Type 1 soft rot produced cavities within the secondary cell walls layers (**A**). These cavities merged together in advanced stages of decay resulting in large voids in the cell walls. Type 2 soft rot caused a general erosion of the fiber secondary cell wall layers but did not degrade the middle lamella region (**B**). Advanced decay had cells where the entire secondary wall was degraded leaving only a weak lattice of middle lamella that collapsed and appeared distorted. Bar=25 μm

Table 1 List of taxa isolated from wood samples from Cape Royds, Antarctica including best blast match with percent identity and overall nucleotide overlap of the internal transcribed spacer (ITS) region

Best BLAST match	Percent identity	Overlap	Location	Sample	Culture No.	GenBank accession No.
Ascomycete sp. BC20	99	515/516	Stables	Wood	7R10-4	GU212367
Ascomycete sp. BC20	99	526/531	Stables	Wood	7R13-2	GU212368
Cadophora fastigiata	100	515/515	Stables	Straw	7R28-1	GU212369
Cadophora fastigiata	99	522/523	Stables	Wood	7R52	GU212370
Cadophora fastigiata	100	544/544	Stables under roof boards	Wood	7R121-1	GU212371
Cadophora fastigiata	99	490/491	Stables	Wood	7R122-9	GU212372
Cadophora fastigiata	99	505/506	Stables	Wood	7R124-1	GU212373
Cadophora luteo-olivacea strain 18	99	623/624	Near hut wall, stables	Fodder/soil	7R38-4	GU212374
Cadophora malorum	100	568/568	Stables	Wood	7R10-1	GU212375
Cadophora malorum	100	547/547	Near hut wall , stables	Fodder/soil	7R38-3	GU212376
Cadophora malorum	100	532/532	Ravine	Wood	7R74-3	GU212377
Cadophora malorum	100	565/565	Ravine	Wood	7R77-1-8	GU212378
Cadophora malorum isolate PhiK3II	100	400/400	Hut wall, stables	Wood	7R25	GU212379
Cadophora malorum isolate PhiK3II	100	509/509	Ravine	Wood	7R77-2	GU212380
Cadophora malorum isolate PhiK3II	100	530/530	Stables under roof boards	Wood	7R120-1	GU212381
Cadophora malorum strain CCF3784	100	509/509	Stables	Paper	7R46-3	GU212382
Cadophora malorum strain CCF3784	100	561/561	Stables	Rope	7R56	GU212383
Cadophora malorum strain CCF3784	99	542/546	Ravine	Wood	7R70-2	GU212384
Cadophora malorum strain CCF3784	100	532/532	Ravine	Wood	7R76	GU212385
Cadophora malorum strain CCF3784	100	591/591	Stables under roof boards	Wood	7R120-4	GU212386
Cadophora malorum strain CCF3784	100	589/589	Ravine	Wood	7R134	GU212387
Cadophora sp. 6e51-2	100	614/614	Near hut wall, stables	Wood	7R3-1-8	GU212388
Cadophora sp. 6e51-2	100	607/607	Stables	Box packing material	7R29-1	GU212389
Cadophora sp. 6e51-2	98	597/604	Near hut wall, stables	Wood	7R119-4	GU212390
Chaetomium globosum isolate 18a-1	100	372/372	Near hut wall, stables	Corn	7R26-1	GU212391
Cladosporium cladosporioides	100	532/532	Stables	Corn	7R44	GU212392
Cladosporium cladosporioides strain MUCC551	100	501/501	Near hut wall, stables	Corn	7R26-2-8	GU212393
Cladosporium cladosporioides strain STE-U 3683	100	447/447	Ravine	Wood	7R77-1-8(2)	GU212394
Dothideomycete sp. 7666	100	527/527	Near hut wall, stables	Dried food Stuffs	7R65	GU212395
Geomyces pannorum strain VKM FW-2260	99	546/547	Stables	Wood	7R41-8	GU212396
Geomyces pannorum strain VKM FW-2260	99	555/556	Stables	Wood	7R6-2	GU212397
Geomyces pannorum var. *pannorum* strain VKM FW-2264	99	553/554	Near hut wall, stables	Fodder/soil	7R38-2	GU212398
Geomyces pannorum VKM FW-2260	99	553/554	Stables	Fodder/soil	7R11-3	GU212399
Geomyces sp. BC7	100	491/491	Near hut wall, stables	Paper	7R46-2	GU212400
Geomyces sp. BC7	99	544/549	Stables	Canvas	7R125-1	GU212401
Geomyces sp. BC7	100	547/547	Stables	Wood	7R128-3	GU212402
Geomyces sp. T489/9b	99	555/556	Near hut wall, stables	Fodder/soil	7R38-1	GU212403
Geomyces sp. T489/9b	99	554/555	Near hut wall, stables	Fodder/soil	7R38-6	GU212404
Onygenales sp. BC8	99	557/562	Stables	Wood	7R17-3	GU212405
Onygenales sp. BC8	98	560/566	Stables	Wood	7R18-1	GU212406
Onygenales sp. BC8	99	513/517	Stables	Fodder/soil	7R7-3	GU212407
Onygenales sp. BC8	98	567/573	Stables	Fodder/soil	7R11-2	GU212408
Pezizomycotina sp. BC11	99	484/486	Ravine	Cork	7R133-1	GU212409
Solenopezia solenia	87	454/518	Roof board of stables	Wood	7R39-4	GU212410

Table 1 (continued)

Best BLAST match	Percent identity	Overlap	Location	Sample	Culture No.	GenBank accession No.
Thielavia hyalocarpa	99	478/482	Stables	Wood	7R123-1	GU212411
Thielavia sp. B27	99	547/552	Stables	Wood	7R4-1	GU212412
Thielavia sp. B27	99	546/551	Stables	Fodder/soil	7R11-4	GU212413
Thielavia sp. B27	99	546/551	Stables	Wood	7R5-1	GU212414
Thielavia sp. B27	98	546/552	Near hut wall, stables	Wood	7R8-1	GU212415
Thielavia sp. B27	98	519/526	Stables	Wood	7R10-6	GU212416
Thielavia sp. B27	99	546/551	Stables	Wood	7R12-1	GU212417
Thielavia sp. B27	99	511/516	Stables	Wood	7R17-1	GU212418
Thielavia sp. B27	99	523/528	Stables	Wood	7R20-1	GU212419
Thielavia sp. B27	99	546/551	Stables	Wood	7R22-2	GU212420
Thielavia sp. B27	99	536/540	Stables	Cloth	7R126-2	GU212421
Thielavia sp. B27	99	538/542	Stables	Straw	7R127-1	GU212422
Fungal sp. AB25	100	549/549	Stables	Wood	7R19-1	GU212423
Fungal sp. AB34	100	494/494	Roof board of stables	Wood	7R39-2	GU212424
Fungal sp. AB34	100	470/470	Stables	Wood	7R122-13	GU212425
Fungal sp. AB47	98	550/559	Stables	Leather	7R16-2	GU212426
Fungal sp. AB47	98	518/528	Stables	Leather	7R16-1	GU212427
Fungal sp. AB52	100	531/531	Stables	Wood	7R4-2	GU212428
Fungal sp. AB52	100	531/531	Near hut wall, stables	Fodder/soil	7R38-5	GU212429
Fungal sp. AB9	100	591/591	Stables	Box packing material	7R21-1	GU212430
Fungal sp. AB9	100	591/591	Roof board of stables	Wood	7R39-1	GU212431
Fungal sp. AB9	100	588/588	Near hut wall, stables	Dry beans	7R42	GU212432
Fungal sp. AB9	100	558/558	Ravine	Wood	7R70	GU212433
Fungal sp. AB9	100	588/588	Ravine	Wood	7R73	GU212434

Cadophora species with sequences from known isolates (Fig. 3). The greatest numbers of *Cadophora* species obtained were identified as *Cadophora malorum* followed by a group of species identified as *Cadophora fastigiata*. Fungi with the best BLAST match of Fungal sp. AB9 from GenBank were identified as *C. malorum*, while Fungal sp. AB47 isolated from samples 7R16-1 and 7R16-2 were found to be most closely related to *C. fastigiata* (Fig. 3). Other *Cadophora* species were confirmed to be *Cadophora luteo-olivacea* and others a *C. malorum*-like species that formed a separate phylogenetic group labeled *Cadophora* 4E71-1, a fungus that had been previously identified from Scott's Cape Evans historic hut in Antarctica.

Discussion

The removal of debris, soil, penguin guano, and other materials from the Cape Royds hut stables by conservators to drain standing water that accumulated during the austral summers and remove the large amount of organic matter in contact with the exterior hut wall provided many miscellaneous fragments of wood and other materials for isolation of fungi. The large number of *Cadophora* species obtained from different samples indicates that this fungus is widely distributed throughout the area and is the dominant fungus colonizing wood and the other organic substrates. Wood collected from the ravine was also colonized primarily by *Cadophora* species. This fungus had been previously found in wood at this site and also at the Hut Point and Cape Evans historic huts [4, 10]. A significant finding from these investigations is the large number of different *Cadophora* species found at the sampling sites at Cape Royds. Phylogenetic analyses of the ITS region of rDNA revealed many isolates of *C. malorum* as well as isolates of *C. luteo-olivaea* and *C. fastigiata*. A group of isolates related to *C. malorum* but showing distinct sequence differences matched an isolate previously only found on wood at Cape Evans and has been labeled *Cadophora* sp. 4E71-1. This group of *Cadophora* isolates may represent a new species; however, additional taxonomic investigation is needed to determine this. Isolates of *C. fastigiata* also are variable in their ITS sequence, and one 7R16-1 is separated from the other sequences of the *C. fastigiata* group. Two isolates of this fungus were found from two different pieces of leather, and isolate 7R16-1, used in the phylogenetic analyses, matched

Fungus sp. AB47 as a best BLAST match of sequences in GenBank (Fig. 3 and Table 1). Fungus AB47 is an isolate found on wood and other organic material at an historic site on Horseshoe Island and from wooden buildings at Duse Bay in the Peninsula region of Antarctica [3]. The diversity of *Cadophora* species found within this relatively small area at Cape Royds is great and represents most of the species previously found from many sampling sites throughout Antarctica.

Environmental conditions dictate which microorganisms can successfully colonize and degrade a substrate, and in Antarctica, the extreme weather conditions, UV radiation, high salt concentrations, and large amounts of penguin guano in soils at Cape Royds undoubtedly have an influence on which fungi can grow. The widespread occurrence of *Cadophora* species found indicates that they are well suited for not only survival in these conditions but they appear to flourish in the Antarctic environment. Investigations of soft rot fungi have also demonstrated that external sources of nitrogen are often needed for significant amounts of decay to occur [18, 38]. An Adélie penguin colony at Cape Royds is adjacent to the hut and likely is responsible for contributing

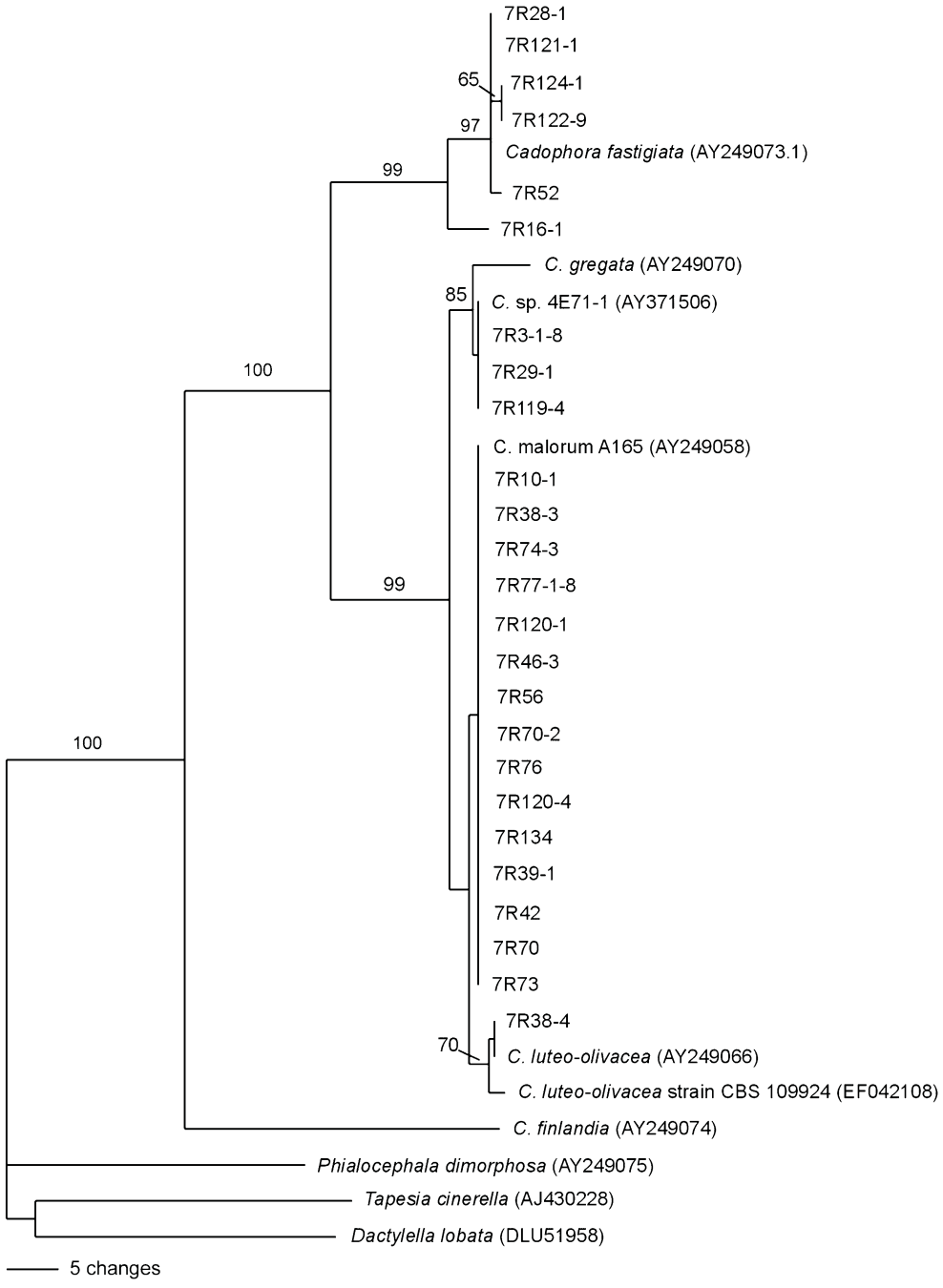

Figure 3 Phylogenetic tree based on parsimony analysis of the ITS1, 5.8S, and ITS2 regions of the rDNA of *Cadophora* isolates obtained in this study. Bootstrap values greater than 50% are shown

nutrients that affects fungal growth resulting in advanced decay occurring at this site. In contrast, Discovery hut located at Hut Point on Ross Island is older (built in 1901), but there is no significant penguin activity near it, and this hut has fewer decay problems, and only a small percentage of isolations made at this site have yielded isolates of *Cadophora* [4, 10]. A similar finding was made at historic sites on the Antarctic Peninsula; of nine sites visited, the highest percent occurrence of *Cadophora* spp. (35% of total isolates) was found to occur at Port Lockroy, a British base built in 1944 which is surrounded by a Gentoo penguin (*Pygoscelis papua*) colony [3]. Although these observations only provide anecdotal evidence, they do suggest that the relatively high nutrient contents of these ornithogenic soils may be promoting the growth and activity of *Cadophora* species.

Other studies have reported fungi from Cape Royds historic woods [4, 10, 21], but the study presented here lists nine taxa not previously found at the site and demonstrates the benefits from sampling greater numbers of samples so that a better determination of microbial diversity can be realized. Since sampling historic wood and artifacts must be done with extreme care and only minute samples taken to avoid excessive destructive sampling, the large numbers of miscellaneous samples available from this present study allowed for a more complete survey of the fungi at the site. In addition to *Cadophora*, several other fungi in the Ascomycota have been found such as *Cladosporium*, *Chaetomium*, *Geomyces*, and *Thielavia*. *Cladosproium* and *Chaetomium* are genera of fungi known to cause soft rot in wood [29], and the *Cladosporium* may also be contributing to the decay taking place in the historic woods. The *Chaetomium* isolate, however, was only found in a sample of corn from the stables and not from wood. *Cladosporium* has been previous reported as a serious problem inside the Ross Sea expedition huts where it causes deterioration of materials due to the dark and disfiguring discoloration caused on wood, paper, textiles, and other materials as it grows [21]. *Geomyces* has also been commonly reported in microbial surveys of Antarctica and has been shown to utilize keratin-based substances [11, 26, 27]. Transport of this fungus by birds and penguins in Antarctica has been suggested by Marshall [26], and soil isolations made along transits by Connell et al. [12] found this fungus widely distributed in the coastal and central zones of the Taylor Valley in Antarctica. Large amounts of feathers were found among the sampled materials originating from the penguin colony near the historic hut (Fig. 1a, b) that likely contributed to the large number of *Geomyces* isolates obtained. *Thielavia* has also been isolated from lichen on King George Island, Antarctica [33] but was not found in previous studies completed in the Ross Sea Region [4]. The production of cellulases and other degradative enzymes by species of *Thielavia* found in other places [15] and its taxonomic placement in the *Chaetomiaceae* suggests that this cold tolerant *Thielavia* deserves more research investigation into its decomposition and recycling ability in Polar environments.

Although Antarctica is often considered to have a "simple environment with the lowest species diversity on earth" [37], the diversity of fungi and species richness found at the Cape Royds historic site was remarkably large and apparently influenced by the huge input of carbon from the introduced wood and other materials brought to the area by the early explorers. Other important factors were the site conditions at the Cape Royds hut. The hut was built in a protected location, and the north-facing side of the hut where the stables are located was an area were moisture from snowmelt accumulated. Snow accumulation in the stables area and the layers of fodder as well as other organic matter may have influenced soil temperatures at the site. The pooling of water from snowmelt in the stables as well as in the ravine sampling location likely provided more favorable conditions for fungal growth. Many of the fungi found may likely be indigenous to Antarctica, and selection pressure of the environment, such as extreme cold, rapid and multiple freeze thaw cycles, high ultra violet radiation, high salt concentrations, as well as many other limiting factors, greatly influenced the microorganisms that dominate the site. Studies carried out in the McMurdo Dry Valleys of Antarctica have shown that temperature, moisture, and substrate availability influence soil respiration, and laboratory simulations of soil carbon inputs of external organic matter can produce high rates of microbial activity [5]. Cape Royds represents an extraordinary experimental field site and provides an opportunity for further studies of microbial dynamics and cycling of carbon and other compounds at a location where large inputs of organic matter were added into the Antarctic environment 100 years ago.

Several *Cadophora* species have been shown to cause a soft rot form of wood decay in laboratory studies [10, 22], and previous microscopic studies of wood from the huts have shown that a type 1 soft rot attack was evident in various woods. The type 1 form of soft rot is characterized by the formation of cavities within the cell walls (Fig. 2a) [16, 17]. As decay progresses, the cavities increase in number and merge together causing large holes to form in the wood cells. Observations made from the wood samples collected in this study show that in addition to type 1 soft rot attack, there was also a type 2 form of soft rot found (Fig. 2b). Decayed cells had secondary cell walls that were eroded, and in advanced stages of degradation, the entire secondary wall was removed. The middle lamella, however, was not degraded, and remaining cells consisted of only a fragile network of middle lamella regions found between cells. Some soft rot fungi produce both type 1 and 2 within cells of woody substrates [13, 29], but in the samples

observed for this study, type 1 was most commonly found in coniferous wood and type 2 in various hardwoods.

No filamentous basidiomycetes were found at this site despite their abundance and widespread distribution as decay organisms in temperate regions of the world. Previous investigations have shown that most basidiomycota reported from continental Antarctica are yeasts, and very few studies report the presence of filamentous basidiomycetes [11, 12, 24, 25, 35]. Instead, ascomycetes such as *Cadophora* that have been previously found in Antarctica in mosses and lichens and in soils from remote areas with little to no human influences [28, 33] are the major decay fungi. It is intriguing to speculate on the physiological activity of these organisms and their unique ability to attack lignified wood despite the lack of lignocellulose substrates in the Ross Sea Region. The circumpolar distribution of *Cadophora* species in the Antarctic as well as the Arctic [3, 4, 9, 10, 23] suggests that these fungi are well adapted to the extreme Polar environment and may have significant effects on the dynamics of carbon cycling and ecosystem functioning. They also pose very challenging concerns for conservators of cultural heritage since these organisms can persist in the most inhospitable climate causing considerable decay to wooden structures in polar environments over time. Their relatively slow attack due to the limited time the substrates may be unfrozen can, however, provide conservators with time to plan and take action. One method currently being employed at the Ross Island expedition huts is to reduce excessive moisture from snowmelt accumulating at the foundations of the structures and to reduce relative humidity within the huts. Since moisture is essential for these decay organisms to function, limiting moisture during the short austral summers should help to reduce decay rates. New studies that examine the physiological adaption of these fungi to Polar conditions and elucidate their genomic structure and expression of genes are needed. These investigations will provide new insights to develop other measures to successfully control or limit degradative actions by these extraordinary polar fungi and more effectively preserve this important cultural heritage.

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