



MICROBIOLOGY

Fungal impact on archaeological materials collected at Byers Peninsula Livingston Island, South Shetland Islands, Antarctica

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Abstract: We identified cultivable fungi present on the surface of five archaeological sealers' artifacts from the beginning of the 19th century collected on Livingston Island, Antarctica. Twenty fungal isolates were recovered and identified using biology molecular methods as taxa of *Antarctomyces*, *Linnemannia*, *Penicillium*, *Mortierella*, *Talaromyces*, and *Trichoderma*. *Penicillium* was dominant on artifacts stored at 10 and 25 °C. In contrast, *Antarctomyces*, *Linnemannia*, *Mortierella*, and *Trichoderma* occurred only on artifacts stored between 8 °C and 10 °C. Our results showed that the Antarctic artifacts harboured cosmopolitan mesophilic, cold-tolerant, and endemic psychrophilic fungal taxa. The mesophilic fungi might have contaminated the artifacts *in situ*, during sampling, transport, and/or storage in the laboratory collection or represent dormant but viable form capable to grow on the objects. However, the detection of cold-tolerant and endemic fungi shows that these fungi, when stored between 8 ° and 10 °C, continue growing on the objects, which may supply them with organic nutrients; this may accelerate degradation of artifacts in the collection. Preventive steps should be adopted to avoid further microbial contamination. Sterilised microbiological conditions can be followed during fieldwork and transportation to Brazil. The preventive protocol may represent a better alternative to avoid artifact microbial proliferation to preserve rare Antarctic archaeological heritage.

Key words: Antarctic heritage, degradation, fungi, taxonomy.

INTRODUCTION

Antarctica was the last large territory to be discovered and exploited by humans. Human presence in the region has acquired different characteristics over time. The first groups to arrive in the early 19th century were sealers from companies in the United States and England, who exploited animal resources on the South Shetland Islands to supply oil and skins to the industrial markets (Barczewski & Maddison 2015). Although historians studied the role of sealers in the discovery of the South Shetland Islands, chronicled their voyages, and discussed

the economic relevance of sealing in the region, the efforts made by archaeologists in the last 20 years to study the artifacts left by these groups have contributed to learning more about the lives of ordinary sealers who worked in the region (Zarankin & Senatores 1996, Zarankin et al. 2011).

Among the Antarctic microbial communities, fungi have been isolated from a wide variety of locations and different substrates (Rosa et al. 2019). Of more than 1,000 non-lichenised fungi reported in the Antarctic and sub-Antarctic regions, only 2-3% are considered

psychrophilic endemic species (growth capacity at temperatures $<20\text{ }^{\circ}\text{C}$) (Bridge & Hughes 2010). However, among the fungi already reported in Antarctica, those with mesophilic (growth capacity at temperatures between $20\text{-}45\text{ }^{\circ}\text{C}$) temperature profiles or wide temperature tolerance (growth capacity from $0\text{ }^{\circ}\text{C}$, with maximum growth temperature above $\geq 20\text{ }^{\circ}\text{C}$) seem to dominate different environments (Rosa et al. 2019, 2020a).

In the golden Antarctic exploratory period, called the “Heroic Era”, bases were established, and organic non-Antarctic materials were introduced in Antarctica, including wood, foodstuffs, clothes; together with these materials, exotic microorganisms may have been introduced accidentally (Farrel et al. 2011). Some evidence indicates the presence of these exotic microbes, such as the deterioration of the wood of huts and pieces in recent decades, which have highlighted the need for long-term preservation of these important historic sites (Blanchette et al. 2004, 2010, Ritchie 2006, Held et al. 2006, Farrel et al. 2011, Held & Blachette 2017). Held & Blachette (2017) identified the sequences of fungal species reported in temperate regions associated with the historic wood structures on Deception Island, Antarctica, suggesting that these species were probably introduced in construction materials and indicating that human influences and volcanic activity affected the diversity of the detected fungi. However, native Antarctic fungi also can be found in wood and objects in historic huts of Antarctica (Blanchette et al. 2004), which may contribute to the deterioration of the Antarctic artifacts deposited in collections.

The Byers Peninsula on Livingston Island has the highest concentration of sealing camps in the region (27 sites). Sealers’ camps consisted of stone enclosures and other structures of various shapes, the functions of which remain

unknown. In all cases, structures were built using local materials, including stone and whale ribs. Rocky outcrops or caves that provide natural shelters were integrated into the structures. The whale vertebrae served as the seating. The use of foreign materials was restricted to old sails, canvas, or seal skin (in the case of roofs), and wood or whale vertebrae (in the case of beamed structures). It is likely that wood was also obtained from wrecks found on the shores. In general, none of these structures exceeded 15 m^2 ; walls were approximately 1.2 m high. Material remains found in the camps were primarily made of wood and bone, with some textile, metal, ceramic, and glass objects.

Pioneering studies by Blanchette et al. (2004, 2010), Held et al. (2006), Farrell et al. (2011), and Held & Blanchette (2017) detected fungi on different archaeological structures and materials in Antarctica. However, there are no reports on the presence of fungi as contaminants on Antarctic artifacts stocked in museum collections outside of Antarctica. The identification of the microorganisms that act on these rare archaeological pieces may provide important information for conservation and to determine strategies for microbial control and/or suppression. In addition, knowledge of the resident microbial community colonising the objects present in Antarctica might provide interesting archaeological information, such as the detection of non-endemic Antarctic species, which might indicate that people who lived in shelters on Livingston Island (or other Antarctic regions) introduced these non-native Antarctic species in different regions.

Due to the scarcity of documents on the life of sealers in Antarctica, the preservation of archaeological remains, especially those of organised groups that are the most vulnerable, is fundamental for telling the history of this group, which has been excluded from the

master narratives (Zarankin & Senatores 1996, Zarankin et al. 2011). After the artifacts collection in the field, they take about two months to arrive in Brazil. During transport, the artifacts are stored in the ship's refrigerated chamber (at a temperature similar to Antarctic conditions of 8 °C). Upon arrival at the archaeological laboratory, the organic artifacts are immediately stored in the refrigerator between 8 °C and 10 °C. However, as the abrupt drying of organic archaeological objects is considered negative for their preservation, the materials are not immediately dried and remain moist. In the refrigerator, archaeological materials are kept between 8 °C and 10 °C with relative humidity above 60%. In contrast, glass or ceramic materials remain on the shelves at room temperature, which ranged from 13 to 28 °C. The favourable conditions of heat, oxygen, and the availability of moisture inside the artifact packaging bags can increase the proliferation of microorganisms. There are some alternatives to control and prevent microbial growth on the objects, such as the use of biocides. However, this alternative is not considered a very suitable mitigation measure because of its toxicity and interference with the interpretation of artifacts due to the addition of foreign substances. A better way to avoid biological colonisation and, consequently, degradation is preventative conservation through the control of environmental conditions. Microorganisms, especially fungi, can cause aesthetic damage to objects when using the substrate for fixation. In addition, microbes can produce pigments and stains that disfigure archaeological objects. Growth activities generate mechanical forces that often result in the detachment, softening, and cracking of the materials. Microbes also use organic material substrates as food and exert biochemical-enzymatic activities that deteriorate organic compounds (such as

cellulose, lignin, and keratin) (Urzi & Krumbein 1994). Biological disinfection is very important in the archaeological sites of Livingston Island, in which remnants of organic nature are predominant. In the current study, we chose wet and dry Antarctic archaeological artifacts from the early 19th century stored inside refrigerators between 8°C and 10°C and room temperature (25 °C) that displayed apparent mycelial growth to identify the resident fungal species and to understand how temperature and humidity conditions may affect microbial colonisation of the objects.

METHODS

Archaeological sealers' artifacts

Archaeological excavations from sealer sites from the beginning obtained of the 19th century were conducted during fieldwork at Byers Peninsula, Livingston Island, South Shetland Islands in different years (Table 1; Figure 1), which allowed the recovery of an important collection of artifacts. The conservation of these items is fundamental to preserve the histories of sealer groups; they were sampled using only physical protocols to guarantee the integrity of the recovered remains, especially in packaging and conditioning. However, the collectors did not use adequate protocols to avoid possible biological contamination of the objects. The artifacts were placed in non-sterilised polyethylene bag with flexible polyethylene foam (which is inert and provides mechanical protection and greater stability to the objects). The items were transported from the field inside rigid polyethylene boxes to Brazil, where they were stocked in the archaeological collection. The collections and studies were authorized by the Secretariat of the Antarctic Treaty and by Brazilian Antarctic Program (PROANTAR).

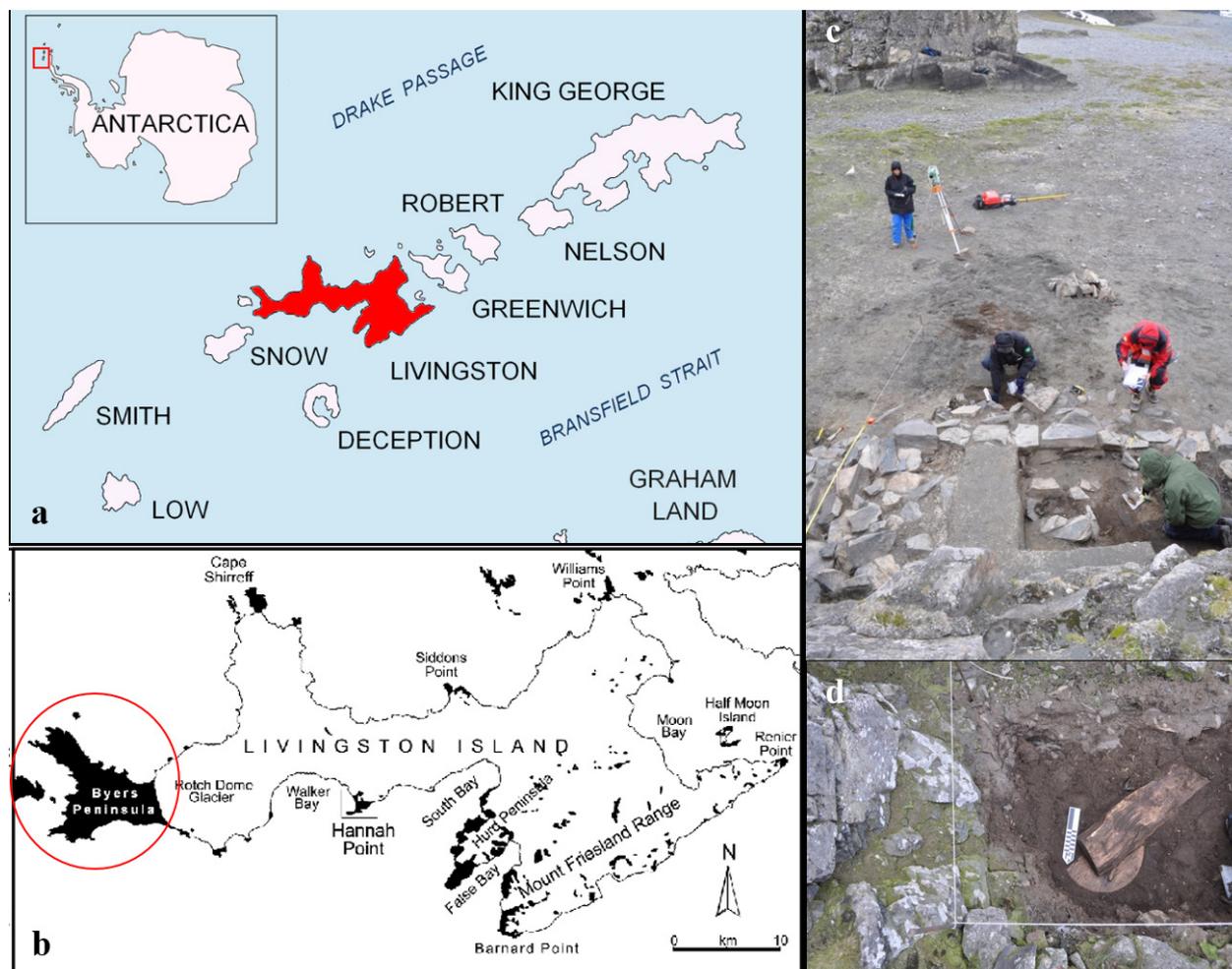


Figure 1. Location where the archaeological artifacts were sampled. (a) Antarctic Peninsula, (b) Livingston Island, and Byers Peninsula (inside the red circle). (c, d) Examples of archaeological sites where the artefacts were sampled. Photos (c) belong to A. Zarkanin.

Fungal isolation

All the archaeological materials were processed under a laminar flow hood to avoid external air contamination. Using sterile disposable loops, smears were taken from different locations of the artifact materials. The samples were inoculated on Sabouraud agar (Himedia, Mumbai, India) containing $200 \mu\text{g mL}^{-1}$ of chloramphenicol (Sigma, St. Louis, MO, USA) and incubated at 10 or 25 °C (mimicking the storage temperature of the objects) for 30 days. For each archaeological material, three different disposable loops and three Petri dishes were used. The fungi were purified in new Petri dishes

containing Sabouraud agar and deposited in the Collection of Microorganisms and Cells of the Universidade Federal de Minas Gerais, Brazil, under the code UFMGCB in cryotubes at $-80 \text{ }^{\circ}\text{C}$ and in distillate-sterilized water (Castellani 1967) at room temperature.

Fungal identification

The protocol for DNA extraction was described previously by Rosa et al. (2009). Amplification of the transcribed internal spacer (ITS-5.8S) region for filamentous fungi was performed according to Rosa et al. (2009) using the primers ITS1 and ITS4 (White et al. 1990). However, sequencing

Table I. Artefacts sampled in the archaeological sealer site on Byers Peninsula, Livingston Island, South Shetland Island, Antarctica.

Site	Coordinates	Artefact	Inventory code	Fieldwork year	Artefacts information	Storage temperature (°C)
PX-2	62°40'21.6"S; 60°55'45.9"W	Wood	2012.0888	2013	Half of barrel slap, 42 cm in diameter and 1.7 cm thick. On one of its faces, possibly the external one, are noted marks resulting from cut activities, which indicates the reuse of the parts for purposes other than which was initially manufactured. It was probably reused for food or animal processing	25
		Fabric	2012.0848	2013	Glove made of tricot with fibers of animal origin (wool). This piece integrates a set of items used as clothing, its fiber is elastic and insulating at low temperatures	10
X-1	62°68'51.8"S; 60°85'34.0"W	Wood	2014.1261	2014	Wood fragment that appears to be part of a whaling boat, due to its dimensions (length and width). The analyzed fragment has a blackened surface, possibly related to the action of fire (use as fire pit fuel)	25
Punta Varadero	62°36'49.6"S 61°04'8.06"W	Fabric/ skin/soil	2011.0316	2012	Cluster of organic remains, composed of a mix of animal skin, remains of tissues, soil and micro bone fragments	10
Sealer 1	62°36'30"S; 61°02'07"W	Whale bone	2017.1395	2017	Whale vertebrae used as internal furniture in the sealer hut, especially a chair or improvised tables. This has a blackish color that suggests its proximity to the fire-pit	10

of the ITS region may fail to recognize some fungal genera. For this reason, the ribosomal polymerase B2 (Houbraken et al. 2012) sequence, which is considered promising for a one-gene phylogeny (Malkus et al. 2006), was used to elucidate the taxonomic positions of the inconclusive taxa identified using ITS sequences. The consensus sequence was aligned with all sequences from related species retrieved from the GenBank database of the National Center for Biotechnology Information using the Basic Locus Search Alignment Tool (BLASTn) program (Altschul et al. 1997). Fungal isolates with query

coverage and identity $\geq 99\%$ were considered to represent the same taxon. However, taxa that displayed query coverage and identities $\leq 98\%$ or an inconclusive taxonomic position after the BLASTn analysis were subjected to phylogenetic ITS and polymerase II gene analysis, with estimations conducted using MEGA Version 5.0 (Tamura et al. 2011). Representative consensus sequences of the fungal taxa were deposited in the GenBank database (Table II). Information about fungal classification generally followed the databases of Kirk et al. (2011), MycoBank (<http://www.mycobank.org>), and the Index

Table II. Fungi identified from archaeological artifacts sampled on Livingston Island, South Shetland Islands, Antarctica.

Sample	Storage temperature (°C)	isolation temperature (°C)	N° of fungal isolates	UFMGCB ^a	Top BLAST search results (GenBank accession number)	Query cover (%)	Identity (%)	N° of bp analyzed	Proposed taxa (GenBank acc. n°)
Wood Figure 2, (a,c)	25	25	3	14060	<i>Penicillium rubens</i> (NR111815) ^b	100	100	434	<i>Penicillium</i> sp. 1 (MZ318091) ^d
			3	14217	<i>Talaromyces domesticus</i> (MH793055) ^b <i>Talaromyces domesticus</i> (MH793118) ^c	100 100	100 99.7	373 661	<i>Talaromyces domesticus</i> (MZ318092) ^d , (MZ223864) ^e
Fabric Figure 2, (d)	10	10	2	14205	<i>Penicillium repensicola</i> (NR153209) ^b	100	100	385	<i>Penicillium</i> sp. 2 (MZ318093) ^d
			1	14203	<i>Antarctomyces psychrotrophicus</i> (NR164292) ^b	100	100	721	<i>Antarctomyces</i> sp. (MZ318094) ^d
			2	14202	<i>Mortierella zonata</i> (JX975983) ^b	99	96.5	579	<i>Mortierella</i> sp. (MZ318095) ^d
Fabric/skin/soil Figure 2, (e)	10	10	2	14207	<i>Trichoderma gamsii</i> (NR131317) ^b	100	100	317	<i>Trichoderma</i> sp. 1 (MZ318096) ^d
			2	14215	<i>Trichoderma atroviride</i> (AF456917) ^b	100	100	375	<i>Trichoderma</i> sp. 2 (MZ318097) ^d
Whale bone Figure 2, (b)	10	10	2	14208	<i>Penicillium caseifulvum</i> (NR163685) ^b	100	100	355	<i>Penicillium</i> sp. 3 (MZ318098) ^d
			3	14200	<i>Linnemannia hyalina</i> (NR163542) ^b	100	94.5	503	<i>Linnemannia</i> sp. (MZ318099) ^d

^aUFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. Taxa subjected to BLAST analysis based on the ^bITS and ^cPolymerase II regions for elucidation of taxonomic positions. Sequences of ^dITS and ^ePolymerase II.

Fungorum (<http://www.indexfungorum.org>). Venn diagrams were prepared according to Bardou et al. (2014) to illustrate the comparison of fungal assemblages associated with artifacts with high sampling.

RESULTS

Fungal taxonomy and distribution

Twenty fungal isolates were obtained from different archaeological objects, which were identified only by molecular approach to represent nine taxa of the genera *Antarctomyces*, *Linnemannia*, *Penicillium*, *Mortierella*, *Talaromyces*, and *Trichoderma* (Table II). Despite to display ITS query coverage and/or identities $\geq 99\%$ in the BLASTn analysis, *Antarctomyces*, *Linnemannia*, *Penicillium*, *Mortierella*, and *Trichoderma* showed distant phylogenetic proximity when compared with known fungal sequences deposited in the GenBank. Due the inconclusive phylogenetic identification, these fungi were identified in genera level (Supplementary Material – Figure S1). The fungal genera varied across different artifact materials and storage temperatures (Figure 3). The genus *Penicillium* was predominant on the items stored between 8 °C and 10 °C and 25 °C. *Talaromyces domesticus* was detected only in wood stored at 25 °C. In contrast, *Antarctomyces*, *Linnemannia*, *Mortierella*, and *Trichoderma* occurred on different artifacts, but only in those stored between 8 °C and 10 °C. However, at the species level, no single taxon was detected in more than one object. In addition, when stored between 8 °C and 10 °C, the endemic Antarctic fungus *Antarctomyces psychrotrophicus* and cold-tolerant *Mortierella* sp. were detected on the tissue artifact, *Trichoderma* sp. 1 and sp. 2 on leather, and *Linnemannia* sp. on the whale bone used as a food support accessory.

DISCUSSION

The objects of the Antarctic archaeological sealer's sites were dated using different elements that indicate their national origin, manufacturing techniques, uses, and reuse. The samples chosen for our study did not have specific information that allowed us to make this association directly. However, because of their proximity to materials such as kaolin pipes, glass bottles, and metal buttons, it is possible to establish an approximate date for these pieces between 1820 and 1840 (Soares et al. 2016, 2019, Soares & Gardiman 2017). After the mycological study, our results displayed the presence of different fungal genera represented by cosmopolitan mesophiles (*Penicillium*, *Talaromyces* and *Trichoderma*), cold-adapted species (*Linnemannia* and *Mortierella*), and endemic (*Antarctomyces*) fungi on the surface of collected artifacts stored at room temperature and cold temperatures in Brazil.

Penicillium includes cosmopolitan species detected in Antarctica (Rosa et al. 2020b), where they are broadly distributed, indicating their versatile adaptability to the extreme conditions of the continent; furthermore, they have been reported in soil, snow, air, ice, seawater and marine sediments, freshwater and lake sediments, plants, and animals (Rosa et al. 2019, 2020a, c). In addition, *Penicillium* has been detected in deteriorating wooden structures in Antarctica (Held et al. 2006). *Talaromyces*, also reported as *Byssochlamys*, includes cosmopolitan species present in soils and indoor environments, which have been reported in Antarctic rocks (Gonçalves et al. 2017). *Trichoderma* (*hyphomycetes*) shelter species are ubiquitous in the environment, especially in soils (Samuels 1996). In Antarctica, *Trichoderma* taxa have been detected in different environments and habitats, such as glacial ice (Jacobs et al.



Figure 2. Antarctic sealer's artifacts from which fungi were recovered. (a) Wood probably reused for food or animal processing; (b) whale bone (vertebrae) used as internal furniture in the sealer hut, especially in a chair or improvised table used as a food plate; (c) wood fragment that appears to be part of a whaling boat; (d) fabric made of tricot with fibers of animal origin (wool); and (e) fabric/skin/soil. Red arrows show the presence of fungal mycelia.

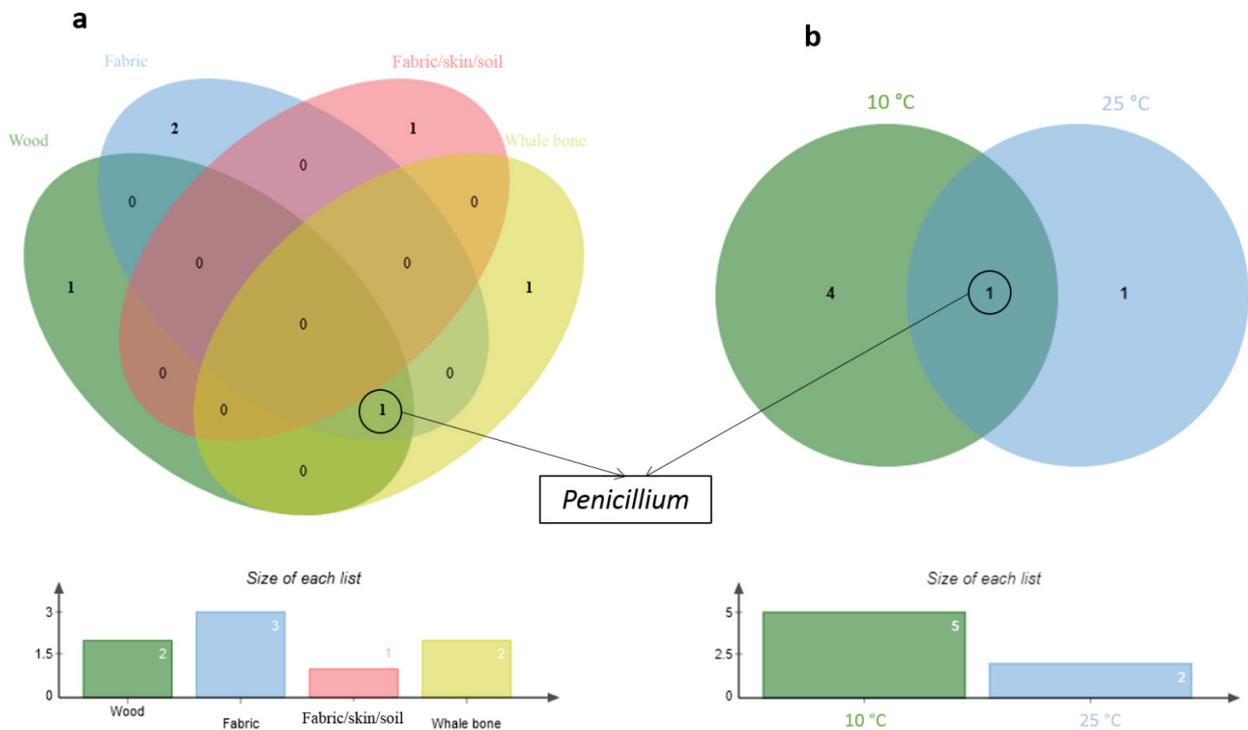


Figure 3. Similarities of fungal genera detected on the different (a) archaeological sealer's Antarctic artifact materials and (b) artefact storage temperatures.

1964), plants (McRae & Seppelt 1999), marine sediment (Ren et al. 2009), soils (Kochkina et al. 2019), freshwater lakes (Gonçalves et al. 2012), and permafrost (Kochkina et al. 2012).

The genus *Mortierella* seems to be ubiquitous in Antarctica and has been reported mainly in soil (Newsham et al. 2018, Gomes et al. 2018), snow (de Menezes et al. 2019), and plants (Melo et al. 2014, Gonçalves et al. 2016, Rosa et al. 2020d). *Antarctomyces* is an endemic Antarctic genus with two reported species: *A. psychrotrophicus* and *A. pellizariae*, which were originally isolated from soil and snow, respectively, on King George Island of the South Shetland Islands (Stchigel et al. 2001, de Menezes et al. 2017). *A. psychrotrophicus* has already been identified in different habitats in Antarctica, including soils (Stchigel et al. 2001, Gomes et al. 2018), plants (Rosa et al. 2009, Coelho et al. 2021), lake freshwater (Gonçalves et al. 2012), and lake sediments (Ogaki et al. 2020).

Furthermore, some details regarding the sampling, transportation, and storage of the objects in the collection indicate failure in the process of microbiological sterilisation, which can contribute to further microbial attack and, consequently, material degradation. Owing to logistical limitations, the boxes with artifacts did not remain refrigerated throughout the period of transportation from Antarctica to Brazil (approximately 3 months). The boxes containing pieces remained in the hold of the ship, which was affected by variations in temperature and humidity. When the artifacts arrived at the final destination in the laboratory (Brazil), they were handled with latex gloves, cleaned superficially with soft brushes, and photographed for inventory. The objects were removed from the plastic bags and placed in new non-sterilised bags. Finally, the artifacts were stored in the laboratory collection and eventually manipulated for further analysis. Some objects

were stored in refrigerators between 8 °C and 10 °C (2012.0848, 2011.0316), while others remained outside at 25 °C (2017.1395, 2012.888, 2014.1261). The storage temperature of the artifacts may have directly interfered with the fungi obtained, as well as the type of material that makes up the artifacts. However, due the capability of these Antarctic fungi survive and/or growth under the cold temperatures of Antarctica, the refrigeration above 0°C could not prevent their metabolic activities on the artifacts.

Our results showed that Antarctic artifacts harbour different fungal genera represented by cosmopolitan mesophilic, cold-tolerant, and endemic psychrophilic taxa. It is possible that mesophilic taxa originated in Europe and were transported to Antarctica via these items, where they could have undergone selection to the extreme environmental conditions and survived over the years as spores or resistant mycelia on artifacts found in the archaeological sites. The mesophilic fungi might have contaminated the artifacts *in situ* as resident taxa, during sampling, transport, and/or storage in the laboratory collection or represent dormant but viable form capable to grow on the objects, which reinforces the need for preventive and effective microbiological sterilisation throughout the artifacts recovery process to avoid exogenous microbial contamination. Moreover, the detection of cold-tolerant and endemic fungi shows that these fungi, when stored between 8 °C and 10 °C, continue growing on the pieces, which may supply them with organic nutrients and, consequently, accelerate the degradation of the objects in the museum collection. The identification of the fungi present on the archaeological artifacts represents the first step in controlling their growth, contamination, and further biological degradation, which is a common problem in the proper preservation of organic Antarctic

artifacts. As the fungal community detected on the items was represented by mesophilic, cold-tolerant, and psychrophilic aerobic species, some preventive steps should be adopted to avoid further microbial contamination. Sterilised microbiological conditions can be followed, such as the use of sterilised gloves to handle pieces during fieldwork, use of sterilised bags in which to place artifacts after sampling, sterilisation of flexible polyethylene foam and plastic boxes used to protect the objects, temperature control (≤ 10 °C) during the transportation process until arrival in Brazil (or other countries), handling of materials using sterilised gloves and bags, and storage under low air moisture and anaerobic conditions. This preventive protocol may represent a better alternative to avoid microbial proliferation on artifacts in order to preserve this rare Antarctic archaeological heritage.

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SUPPLEMENTARY MATERIAL

Figure S1.

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GCAM and BAP isolated and identified the Antarctic fungi, and revised the manuscript. GAR, FCS, and AZP collected the artifacts samples in Antarctica and revised the manuscript. LHR provided the necessary infrastructure for fungal isolation, DNA extraction and identification, wrote the manuscript, and revised all versions.

