Short running head: Historic uses of fungi for textile

Title: Fungal mycelial mats used as textile by Indigenous People of North America

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ABSTRACT

The indigenous people of the United States and Canada long have used forest fungi for food, tinder, medicine, paint, and many other cultural uses. New information about historic uses of fungi continues to be discovered from museums as accessions of fungi and objects made from fungi collected over the last 150+ years are examined and identified. Two textiles thought to be made from fungal mats are located in the Hood Museum of Art, Dartmouth College and the Oakland Museum of California. Scanning electron microscopy and DNA sequencing was used to attempt to identify the fungus that produced the mats. Although DNA sequencing failed to yield a taxonomic identification, microscopy and characteristics of the mycelial mats suggest that the mats were produced by *Laricifomes officinalis*. This first report of fungal mats used for textile by indigenous people of North America will help to alert museum curators and conservators as well as mycological researchers to their existence and hopefully lead to more items being discovered that have been made from fungal fabric.

KEYWORDS: Agarikon, biofabrication, ethnomycology, *Fomitopsis officinalis*, *Laricifomes officinalis*, mycotextile

INTRODUCTION

Recently new bioprocessing technologies have grown mycelial cultures of different Basidiomycota and engineered durable mushroom-based fabric for clothing and textile material. One application for these mycofabrics is to replace real and synthetic leather (Bayer and McIntyre 2016; Araldi et al. 2017; Haneef et al. 2017; McIntyre et al. 2018). The idea of using mycelial mats for textiles, however, was known by the indigenous people of North America for some time as evidenced by the two textiles studied in the paper reported here. These fungal textiles were made over 100 years ago. Finding examples of these objects, however, has been difficult since many cultural properties made of fungi have been listed as made from unknown material or misidentified as wood or other plant materials. One well known type of fabric made from the processed mycelium of *Fomes fomentarius* fruiting bodies has been used in Eastern European countries to make hats and other items that utilize a thick felt-like material (Pegler 2001).

A fungal textile used by the indigenous people of the Pacific Northwest Coast of North America was a Tlingit wall pocket from Ketchikan, Alaska made in 1903. This has been in the Hood Museum of Art storage at Dartmouth College for over 60 years with the note “Pair of Fungus Bags. Wedding Presents from Indian Neighbors at Ketchikan, 1903” (Figure 1). When the item was gifted to the Hood Museum in 1959, accession information indicated that the pair had been separated with one given to the Hood Museum and the other given to the Oakland Museum of California. Oakland ‘s accession information included a note: “Wall pouch made of cedar fungus”. Both of these objects were not made of animal hide or fur used for souvenirs; or cloth, typically used for personal items or gifts (Smetzer 2014). This

paper reports the results from an examination of these historic wall pockets and demonstrates they were made from mycelial felts that provided a durable long-lasting fabric.

MATERIALS AND METHODS

The wall pockets were faced with a cream colored, supple, striated material that appeared to be, as the museum notes suggest, some sort of fungal material. The wall pockets, a Victorian form, are ornamental objects used to hang on the wall with a pouch or pocket to hold household items. The two are not a matched pair. The unrecorded Tlingit maker created two different shaped wall pockets but each adorned with a variation of traditional foliate / seaweed / kelp beadwork designs. The fungal material facing, glass seed beads, black cotton trim, calico cloth lining and flannel backing are identical (Figure 1). Oakland's wall pocket is 22 x 13 cm and the Hood's is 17.7 x 12.7 cm. Small segments of the material were removed by Christine Puza, conservator, Williamstown Art Conservation Center, Williamstown, MA from the inside seams of the Hood Museum's wall pocket. These detached segments were used for DNA testing and scanning electron microscopy. Some segments were mounted directly onto stubs for observations without fixation and were mounted on aluminum stubs and coated with gold/palladium using a Cressington 108 auto sputter coater (Cressington Scientific Instruments, Watford, United Kingdom) and examined with a Hitachi S3500N scanning electron microscope (Hitachi, Tokyo, Japan).

Additional segments were used for DNA extraction. Samples of the mycelial textile were extracted using previously published protocols (Loyd et al. 2018) and PCR protocols following Blanchette et al. (2016). In addition to PCR testing of genetic markers, a "shotgun sequencing" methodology was explored in the event the DNA was too fragmented to be PCR-amplified. These experiments were conducted in a dedicated ancient DNA facility using methods proven to be effective for degraded DNA from

archaeobotanical remains (Wales et al. 2014). The only modification to the protocol was that the specimen, a 1 mg segment of the wall pocket, was incubated in digestion buffer for 10 minutes to reduce contaminant DNA (known as a "pre-digestion" step). Subsequently the specimen and the pre-digestion buffer were separately processed, along with a water control to monitor for potential contamination. All three DNA extracts were converted to Illumina libraries for high-throughput sequencing following a method optimized for DNA recovery (Carøe et al. 2017). The libraries were amplified with sample-specific barcodes using 20 cycles in PCR and then pooled for sequencing on a portion of a lane of an illumine HiSeq4000 platform in 80bp single read mode. The resulting DNA data was explored in three ways: comparing against the NCBI nucleotide data base using BLAST (Altshulet et al 1990) and MEGAN6 (Hudson et al. 2011), comparing to a curated database of fungal genomes using MALT (Herbig et al. 2017), and mapping to Fomitopsidaceae reference genomes using BWA (Li and Durbin 2009) as implemented in the Paleomix bioinformatics pipeline (Schubert et al. 2014).

RESULTS AND DISCUSSION

Scanning electron microscopy revealed that the textile used was made from fungal mycelium that formed a dense mat. Some minute wood segments adhered to the surface of the fungal mat suggesting this was extracted from the cracks that are formed in brown rotted wood by some Basidiomycota. Fungal filaments from some parts of the textile showed smooth hyphal surfaces while others have a rough surface with granular particles (Figure 3). The mycelium also had characteristics of hyphae produced by *Laricifomes officinalis* (syn*. Fomitopsis officinalis, Fomes laricis*). This includes mycelial mats with uniform tightly woven hyphae that branch at infrequent intervals at right angles as reported by Faull (1916). These characteristics were observed in the samples of wall pockets we examined (Figure 3).

In culture, *L. officinalis* produces chlamydospores and these structures can be used for differentiating the fungus from most other brown rot fungi. However, microscopic observations of modern mycelial mats show that chlamydospores are rarely present in mats. Scanning electron microscopy observation of a segment of the wall pocket revealed that chlamydospores were infrequent in most of the historic sample sections that were examined but a few intercalary and terminal chlamydospore-like structures were seen (Figure 3). There are other brown rot fungi that produce mycelial mats in decayed wood that can be found in the Pacific Northwest including Fomitopsis pinicola (Sw.) P. Karst. sensu lato and Laetiporus sulphureus (Bull.) Murrill sensu lacto. Some of the species within both of these groups can also produce chlamydospores (Nobles 1965; Haight et al. 2019). The F. pinicola group of species grow on downed dead conifers or on dead areas of live trees and L. conifericola, the species in the L. sulphureus complex that occurs in the western United States and Canada on conifers, is restricted to the lower butt region of trees. In advanced stages of decay, cracks within the brown rotted wood can be filled with fungal mycelia. These fungal mats have been referred to as having cobweb characteristics and are thin to thick and usually rather small in size. In contrast, *L. officinalis* can occur throughout the main bole of a tree and is often well established in the tree before the tree fails and falls to the ground. Large areas of the tree may be decayed. As the brown cubicle rot progresses, the fungus fills cracks and voids in the decayed wood with thick mats of mycelium (Faull 1916; Hubert 1931; Gilbertson and Ryvarden 1986). *Laetiporus officinalis* is commonly reported to produce mats that are 5 mm thick (Allen et al. 1996). With the decay extending through a large part of the tree, the mats can be very large. In old growth trees, they commonly produce especially large sheets of mycelium that can be pulled out from the decayed wood intack in long thick segments. *Laricifomes officinalis* is the only brown rot fungus that has been reported to produce large mycelial mats that are big enough to be suitable for making objects such as the wall pockets (Figure 2). From these macroscopic and microscopic observations and

comparisons to previously published information on characteristics of mycelial mats of *L. officinalis*, we conclude that the wall pockets are most likely made from mats produced by *L. officinalis*.

To confirm that the mats were produced by *L. officinalis*, segments of the wall pocket were used to extract DNA for sequencing. Unfortunately, there was no success to amplify the ITS region after several attempts. Storage conditions over the past many decades has apparently resulted in poor preservation of fungal DNA in the mycelial mats. The additional shotgun sequence approach for ancient DNA also failed to yield taxonomic identification, although DNA was successfully converted to Illumina libraries and sequenced. The sample, pre-digestion buffer, and blank produced 25.8M, 16.0M, and 210K reads, respectively. The BLAST findings, which provide a general perspective on the origins of the DNA, revealed the control was dominated by bacterial species which are known to be common laboratory contaminants (Salter et al. 2014) (Table 1). The specimen and pre-digest demonstrated some of the laboratory contaminants were also present, but neither showed a significant number of reads matching *Laricifomes officinalis* or other members of the order Polyporales (Table 1). A small number of reads matched other fungal taxa such as Pleosporaceae, Hypocreomycetidae, and Malassezia, which were likely contaminating fungi of the wall pocket. MALT analysis of fungal communities showed different fungal families in each sample tested. The control was dominated by Nectriaceae, the predigest was mostly Psathyrellaceae, and the bag specimen was primarily Saccharomycodaceae. These fungi represent taxa that also were contaminating the fungal fabric and reagents used for analyses. The final approach of mapping the sequencing data to Fomitopsidaceae reference genomes found negligible proportions (<0.02% of total reads), likely due to spurious mappings, which again precludes species identification from the DNA data.

The growth of some Basidiomycota can produce fungal mats in nature and in culture. One of the most widely known mycotextiles is amadou which is cut out of the top mycelial context of *Fomes fomentarius* fruiting bodies. This layer of mycelium is used in some Eastern European countries to make a thick felt-like fabric for making hats, purses and other items (Pegler 2001). The fibrous mycelial layer has also had a long history of use as tinder for making fires. Thin strips of the dried mycelium extracted from fruiting bodies can easily catch a spark from flint being struck to start a fire. We know from the fungi found associated with the mummified and frozen body of the Tyrolean iceman that this material was used for making fires for over 5000 years (Peintner et al. 1998; Peintner and Pöder 2000). Another fungus used historically in Europe and North America is *Laricifomes officinalis*. This fungus produces felts in the cracks of brown-rotted wood that may be one-quarter inch thick and extend several feet in length in one continuous sheet (Harvey and Hessburg 1992). The largest fungal mats are found in decayed old growth trees and this material was often collected and used as a styptic to stop bleeding. Hubert (1931) reports the indigenous People of Canada and the United States “early learned of its styptic and purgative properties, and under the name of ‘Wabadou’ it was collected and cherished by the medicine men”. Although this fungus is primarily found in the Pacific Northwest, it was reported to grow throughout the white pine range in Ontario and Quebec as well as in Michigan and Wisconsin (New York Botanical Garden accession # 575256, 01966147 and 01966762:;University of Michigan Herbarium #145810; USDA Forest Products Laboratory, Center for Forest Mycology #13208, 12920, 13179) (Neuman 1914). In addition to white pine, it has been found on larch and hemlock in this area. The name ‘Wabadou’ is an Ojibway name with ‘Wab’ meaning white and ‘adou’ a contraction from amadou. Amadou is a French word for the tinder fungus and this name was likely brought to the region by fur traders and French Jesuits (Faull 1919). The cutting of old growth forests during the late 1800’s and early 1900’s appears to have all but eliminated this fungus from the Midwestern United States, Ontario and Quebec and it is

now very rarely found in this area. However, it can still be found in western North America. Additional reports of the styptic qualities of *L. officinalis* mycelial mats have also been reported. Gilbertson (1980) indicates that *L. officinalis* "has been thought to have styptic properties and old time lumberjacks reportedly used the extensive mycelial felts in the decayed wood for dressing axe wounds" and McIntyre (1949) notes that *L. officinalis* "mycelial mats or felts had great healing properties for wounds, many veteran loggers collected these felts and stored them for emergency use in the absence of a camp doctor". Another report that appears to refer to mycelial felts of *L. officinalis* is the use by the Spokan Indians as a diaper material. Ross (2011) describes a "soft pliable growth of vegetative mycelium felt-like membrane referred to as nqa?qe?mín, which can be found beneath the bark of a buckskin tamarack snag, that was carefully peeled off to serve as a cradleboard diaper". It is possible that this diaper material could have had medicinal use.

The note with the Oakland Museum object indicated that the mat was from a cedar fungus. Hosts for *L. officinalis* in the Pacific Northwest, however, are pines, fir, larch, Douglas fir, spruce and hemlock. Although western red cedar and *Chamaecyparis* are not hosts, the term 'cedar' was likely used in a general way to indicate a conifer. Since there is no mention of cedar with the Hood Museum wall pocket and there appears to be an original note attached to the back of this object, the notes for the Oakland Museum object were apparently made in 1959 without any definitive knowledge of the type of tree the fungus was harvested from.

In addition to the use as a fabric reported in this study*, L. officinalis,* commonly known as Agarikon, has a long history of traditional use as a medicine and as objects associated with native spirituality. Dioscorides reports in about 200AD that it can be used to treat many different ailments. Its importance

continued to be used over the centuries and was recognized in the early herbals of Europe and into current times (Gilbertson 1980; Stamets 2005; Girometta 2018). In North America, its use in traditional medicine by indigenous people is known from ethnology research and collections and accession notes in natural history museums (Emmons 1902; Faull 1916; Beardsley 1941; Blanchette et al. 1992). Because of its unusual appearance and effective medicinal uses, *L. officinalis* was thought to have supernatural powers (Blanchette et al. 1992). Carvings of the fruiting bodies were used by shaman all along the Pacific Northwest Coast as part of their ritual paraphernalia. The shaman would routinely use these carvings to provide an added spiritual remedy in addition to its medicinal value to help cure the sick. When the shaman died, these important possessions were placed at the head of their graves. Masks also were carved from the fruiting bodies of *L. officinalis* and used by shaman during important rituals to display supernatural powers (Blanchette 2017). The 'Fungus Dance' was one of these ritual ceremonies that was conducted during an eclipse of the sun or moon (McIlwraith 1948).

The use of *L. officinalis* mycelial mats for textile purposes by the Tlingit expands our knowledge of how this fungus was used by Native Americans in the past. Since this fungus was thought to have supernatural and spiritual attributes by indigenous people in the past, the mycelial mats may have also carried some connotations that we do not know about. Additional ethnographic investigations with indigenous elders are warranted to gather more information on past cultural uses of the mycelial mats that were used. Although currently we only know of two objects made from the fungal mats, likely there are more that exist but their identity remains obscure. This report should help in the discovery of more objects made of fungal fabric that may be in museum or other historical collections as conservators, curators and other researchers take a closer look and reexamine objects.

DNA technology was not able to provide any information that could be used to confirm the identity of the fungus that produced the mycelial mats. Instead, methods developed by early mycologists to identify Basidiomycota that do not readily produce fruiting bodies in culture were successfully used to differentiate among the few brown rot fungi that can produce mycelial mats in nature. The size and thickness of the mats and the distinctive characteristics of the fungus provide us with information that strongly suggests that these fungal textiles were made from the mycelial mats of *L. officinalis* which was extracted from the cracks of decaying trees with brown rot.

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LEGENDS AND FOOTNOTES

Figure 1. Tlingit Wall pockets made from mycelial mats. (A) Hood Museum of Art, Dartmouth College Object 159.68.14506 and (B) Oakland Museum of California object H4153.19. Made in 1903 at Ketchikan, Alaska. Wall pockets are works of art used as a receptacle for household items and designed to hang on the wall. A = 27 x 13 cm and B = 17.8 x 12.7 cm. **Provenance:** Made by an unrecorded Tlingit maker, Ketchikan, Alaska; given to Anna Elizabeth Carylon Von Hasslocher (1874- 1964) and Emil Alexander Von Hasslocher (1867-1946), as a wedding present, 1903; given to their daughter, Dorothy Vaughan Haberman (1903-1992); given to the Hood Museum of Art and the Oakland Museum of California, in 1959.

Figure 2. *Laricifomes officinalis* fungal mats. A. Transverse section of a conifer tree with brown rot (Top photo). Cracks that develop in the brown rotted wood are filled with mycelium that formsthick large mats. B and C. Large mats that were removed from an old growth tree decayed by L. officinalis. (Photo C is courtesy of Brenda Callan, Pacific Forestry Centre, Victoria BC Canada).

Figure 3. Scanning electron micrographs of fungal textile from the Hood Museum of Art wall pocket. A. Micromorphological observations show the textile consisted of a dense mat of fungal mycelium. B to D. Hyphae characteristics of the mycelial textile included hyphae that branched at right angles (arrows in B), and infrequent intercalary and terminal chlamydospores (arrows in C and D). These are characteristics of *Laricifomes officinalis*. Bar = 50 µm in A and 5 µm in B, C and D.

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Figure 1

Figure 2

Click here to access/download;Figure;Figure 2.tiff

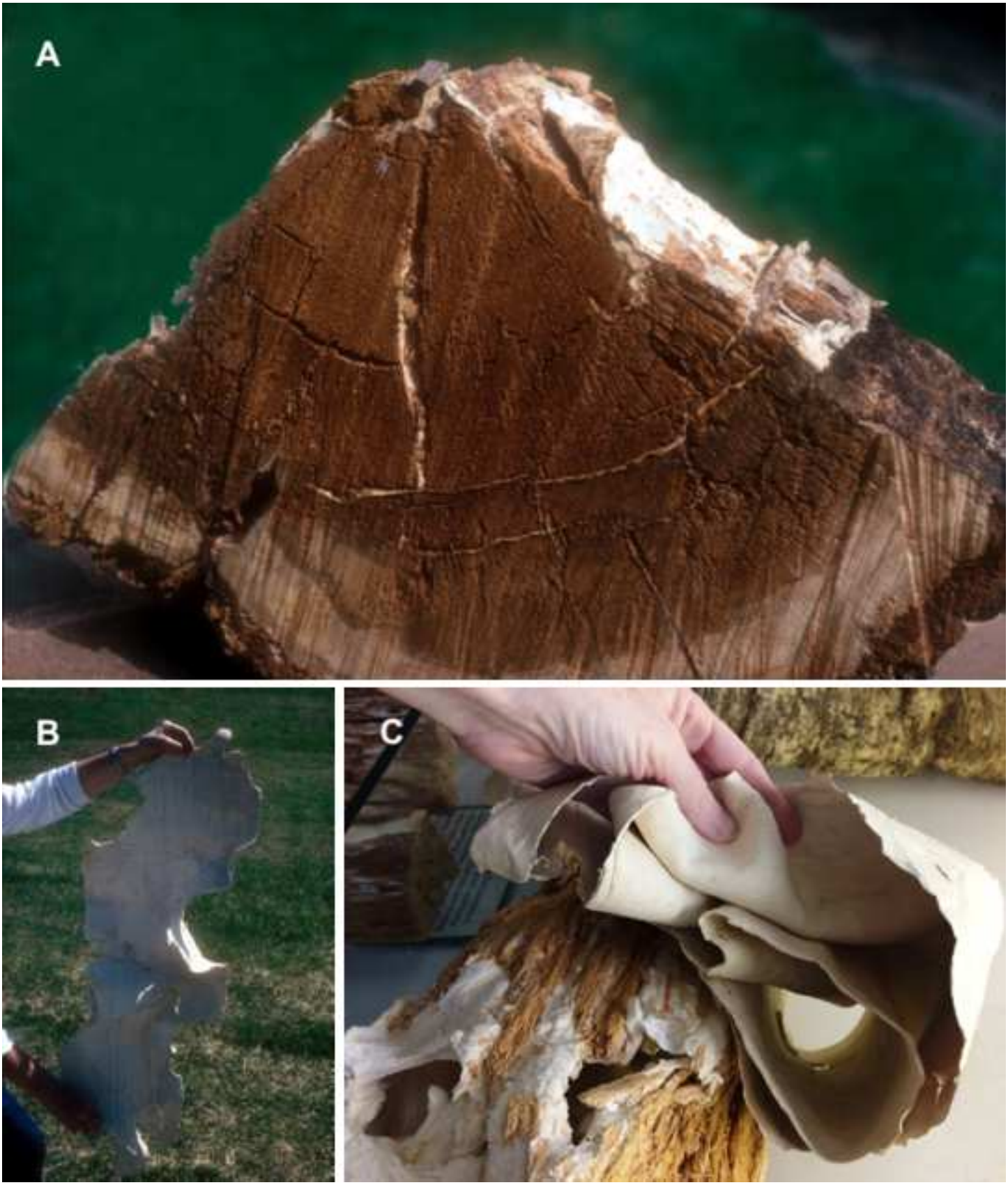

Figure 3

Click here to access/download;Figure;Figure 3.tiff

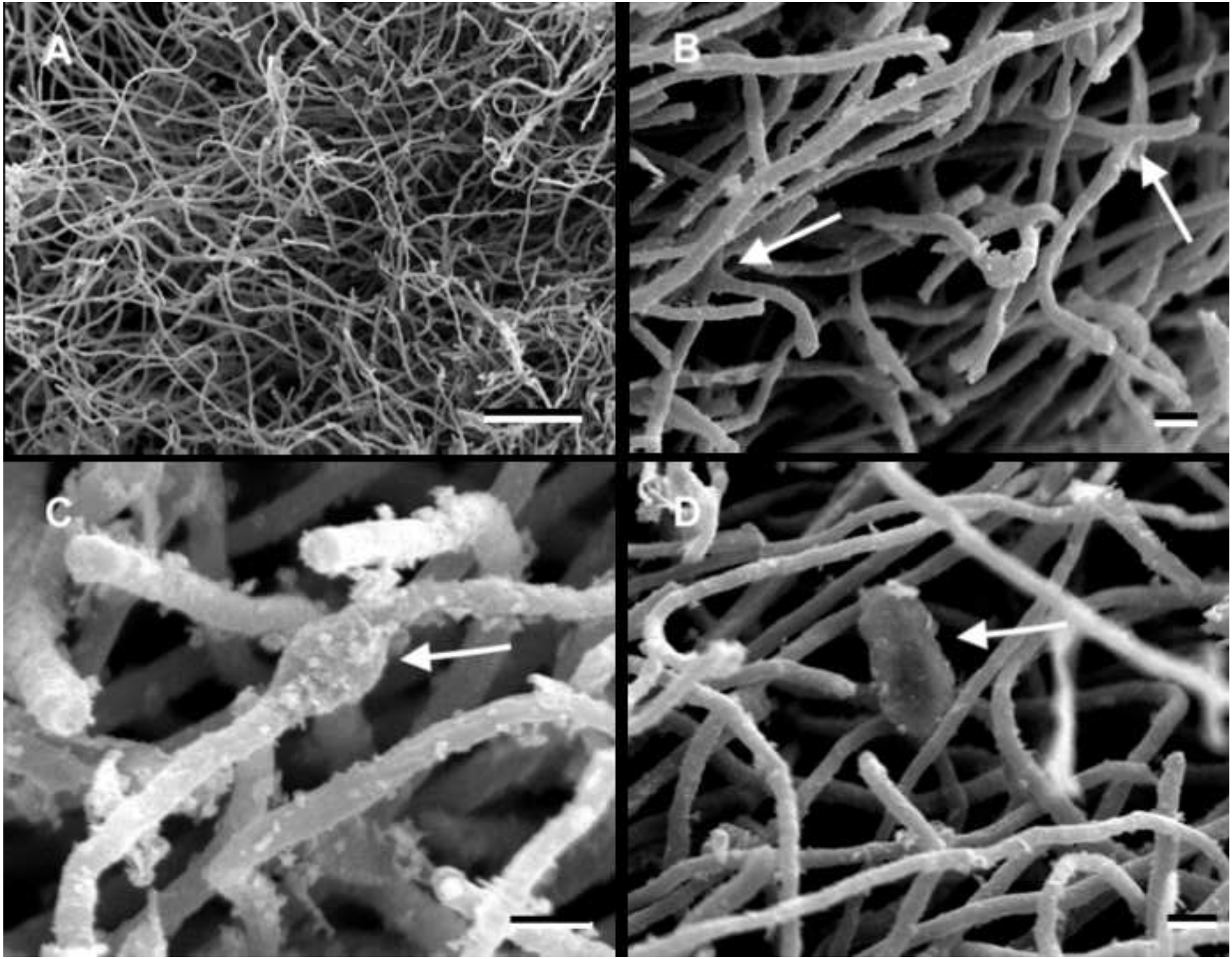

Table 1. Taxa identified in BLAST analysis. 100,000 reads were compared to the NCBI nt database to infer the origins of the DNA in each Illumina library. Very few DNA molecules from the wall pocket specimen are assigned to Polyporales, which prevented a robust taxonomic assignment using this approach.

Taxa	**Wall pocket specimen**	**Pre-digest**	**Extraction control**
Polyporales	0.07%	0.07%	0.00%
Other fungi	0.25%	0.24%	0.11%
Plant	2.17%	0.25%	2.16%
Alphaproteobacteria	2.80%	2.30%	2.11%
Betaproteobacteria	14.89%	10.48%	23.92%
Gammaproteobacteria	2.98%	2.58%	4.09%
Terrabacteria	5.32%	6.45%	1.59%
Higher taxonomic levels	3.02%	3.17%	4.09%
Not assigned	46.30%	45.68%	58.30%