Introduction to Peptide Mass Fingerprinting Process - Child's Boots [25-5-10/98129]

March 14, 2014 (Author: Madeline Corona, conservation research associate)

The objective of this NCPTT/NPS project is to identify collagen-based materials used to fabricate objects of cultural heritage through application of a biotechnology technique. The current work, which began in November 2013, originally developed from a prior inquiry into Alaskan Native skin-covered kayaks (see http://projects.iq.harvard.edu/pmfcm/) and provides an opportunity to focus on objects originating from Alaskan Native group for which there are standing questions. Another goal of the project is to develop further awareness about this technique's applicability by disseminating some of the ongoing results.

To these ends, we are analyzing skin, gut, and sinew samples from historical objects as well as appropriate reference materials to identify unknowns. The technique we use is Peptide Mass Fingerprinting (PMF), a sensitive, specific method that allows identification of materials to family, and sometimes, species level.

In the Spring of 2013, an undergraduate student enrolled in a Harvard course entitled 'Museum Anthropology: Thinking with Objects" was studying and researching a pair of late 19th century Alaskan Native child's sealskin boots (Figure 1). In addition to researching literature and museum documentation about the object housed at the Peabody Museum, the student consulted a visiting Alutiiq traditional skin sewer from Kodiak, Alaska with some of her questions about the boots. Together, the research and their discussions yielded suggestions about the construction techniques and generated questions about further identifying marine mammal materials (Figure 2).

Through this continuing study of the boots, we have been able to explore some of the open questions and provide additional information about the sealskin and other fur components. The PMF technique was selected due to the very small sample size and its accuracy over other methods in use to characterize such materials.

Figure 1: Pair of child's boots (25-5-10/98129). Copyright 2014 Peabody Museum of Archaeology and Ethnology.

This pair of beautifully decorated boots intricately constructed by a coastal Alaskan Native skin sewer was donated to the Peabody Museum in 1925 by John Weare (Harvard Class of 1907) in memory of his father, Charles. Charles Ashley Weare, born September 7 1852 in Iowa, was one of six directors of the North American Transportation and Trading Company which provided tools, clothing, provisions, and transportation for miners in the gold fields of Alaska during the late nineteenth century. (Ref. 1 – 3) The company's steamboats traveled from Seattle to St. Michael's Island, by way of the Aleutian Islands, and then went on to mining points along the Yukon River. Charles Ashley Weare is known to have traveled to these regions numerous times during the 1890s, and it was during these trips that he became interested in the indigenous cultures and began to collect objects created by Alaskan Natives (Ref. 4). After his father's death, John Weare generously donated his father's collection to the Peabody Museum.

The museum's collection records identify the materials of the objects simply as "leather, fur, sinew, [and] wool yarn." After examining the boots, there appeared to be at least nine different mammalian materials present. Four of these materials included:

1. Orange-brown colored skin (boot uppers)

Figure 2. Copyright 2014 Peabody Museum of Archaeology and Ethnology.

2. Thick yellowish skin (boot sole)

Figure 3. Copyright 2014 Peabody Museum of Archaeology and Ethnology.

3. Sinew (used to connect sole to uppers

Figure 4. Copyright 2014 Peabody Museum of Archaeology and Ethnology.

4. Thinner yellowish skin (straps)

Figure 5. Copyright 2014 Peabody Museum of Archaeology and Ethnology.

Samples of each material were taken from areas of damage or loss, thus minimizing impact to the object. Samples needed for analysis are no more than the size of a pinhead, however slightly larger samples are usually taken to ensure there is enough material for reanalysis if necessary (See Figure 6).

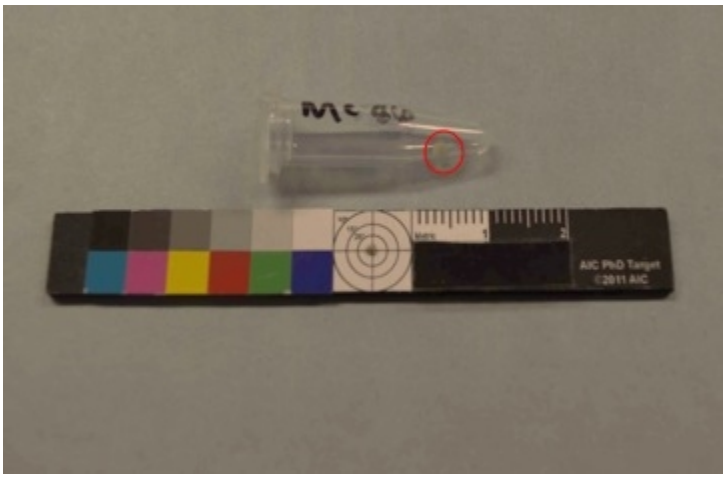

Figure 6: Example of size of sample removed from an object for analysis.

The samples are subjected to an extraction/digestion protocol in order to prepare them for analysis by Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-ToF-MS). This protocol serves to extract the collagen from a sample, break it down into proteins, and then cleave the proteins to produce predictable peptides that can be analyzed by MALDI. The digested samples are then mixed with a matrix and spotted onto a MALDI plate. When the sample/matrix solutions have crystallized, they are then ready to be analyzed by MALDI-ToF-MS.

During analysis, a mass spectrum is produced for each sample. Because each protein sequence is unique, each mixture of peptides is unique. This means that each spectrum contains characteristic marker peptides, or a "peptide mass fingerprint," that can be matched to reference spectra. In Figure 7, the blue line represents the spectrum from the yellowish skin making up the strap on the boots, while the black line represents the (flipped) spectrum of reference sample for a bearded seal. The marker peptides have been identified as Cet 1, A ion, B ion etc. in the spectrum. For the strap sample, the characteristic peptides appear at 1121 m/z, 1221 m/z, 1453 m/z, 1566 m/z, 2171 m/z, 2853 m/z, and 2957 m/z, which are the characteristic peptides found in the bearded seal reference spectrum.

Bearded seal can be identified to the species level based on its unique peptide markers (See Table below); other mammals can presently be identified only to the family, or sometimes tribe, level. For example within the Phocidae family is a tribe of earless seals (called Phocini seals) that includes many different species such as common, spotted, ringed, harp, ribbon, Caspian, Baikal, and grey seals. The markers for these species are all the same, so we are only able to identify them as belonging to the Phocini tribe.

	Cet1	(A)	(B)	(C)	(D)	(F)	(G)
Walrus	1105	1221	1453	1566	2121	2853	3003
Northern fur seal/Stellar sea lion	1105	1221	1453	1566	2121	2853	2957
Bearded seal	1121	1221	1453	1566	2171	2853	2957
Phocini seal	1105	1221	1453	1566	2171	2869	2957

Caribou 1105 1166 1427 1580 2131 2883 3093

Below are the rest of the identifications for the materials used in the boots. It is interesting to note that several different species of animal (including bearded seal (Phocidae family), caribou (Cervidae family), and a seal from the Phocini tribe (Phocidae family) were used to make this single pair of boots.

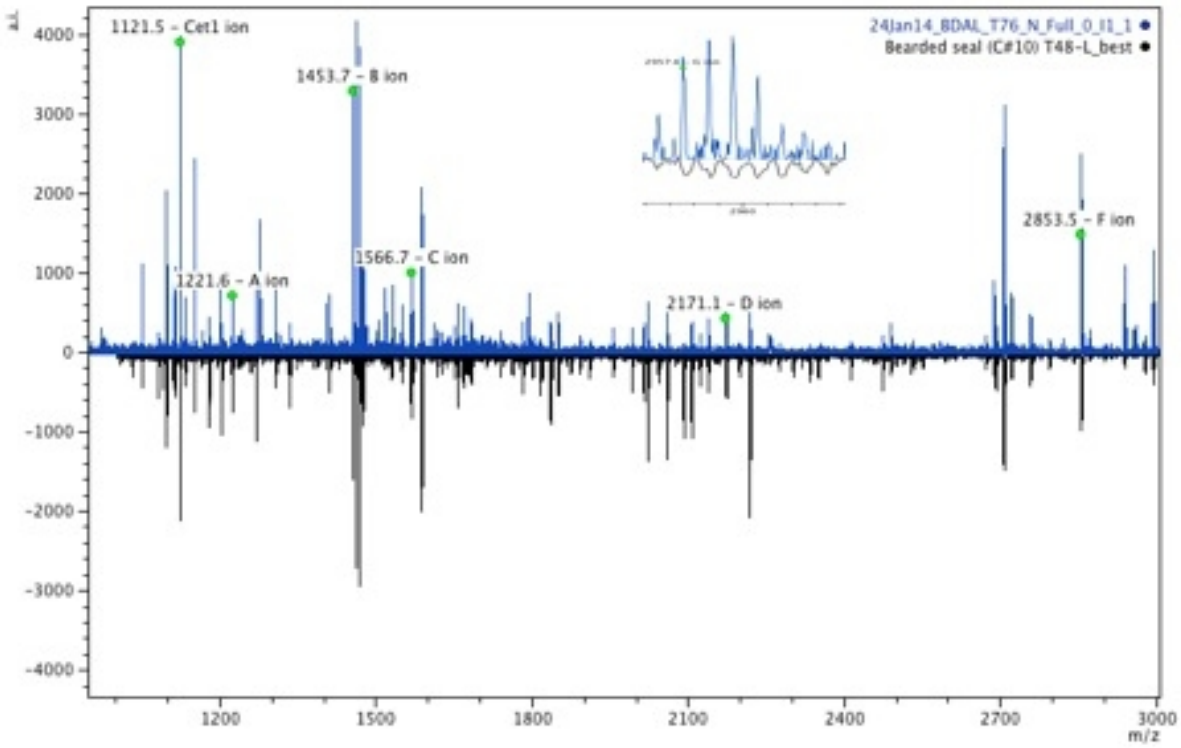

Figure 7: Thinner yellowish skin (strap) identified as bearded seal.

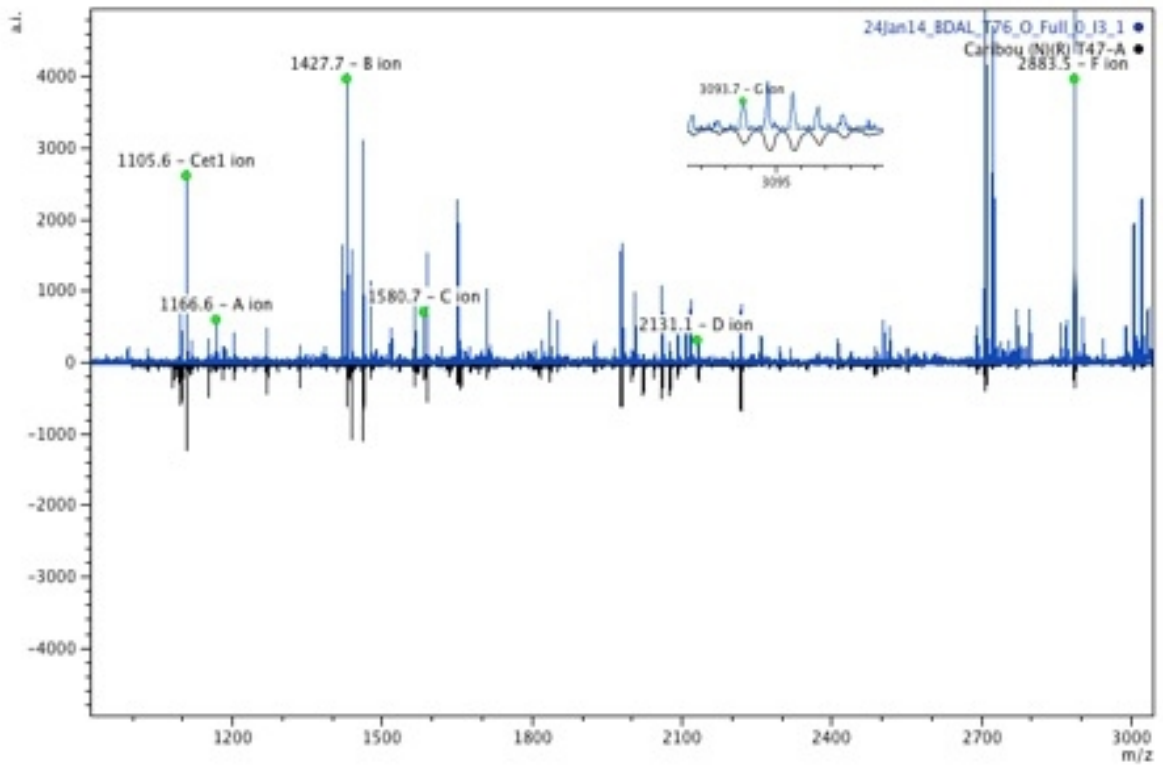

Figure 8: Sinew (used to connect sole to uppers) identified as caribou.

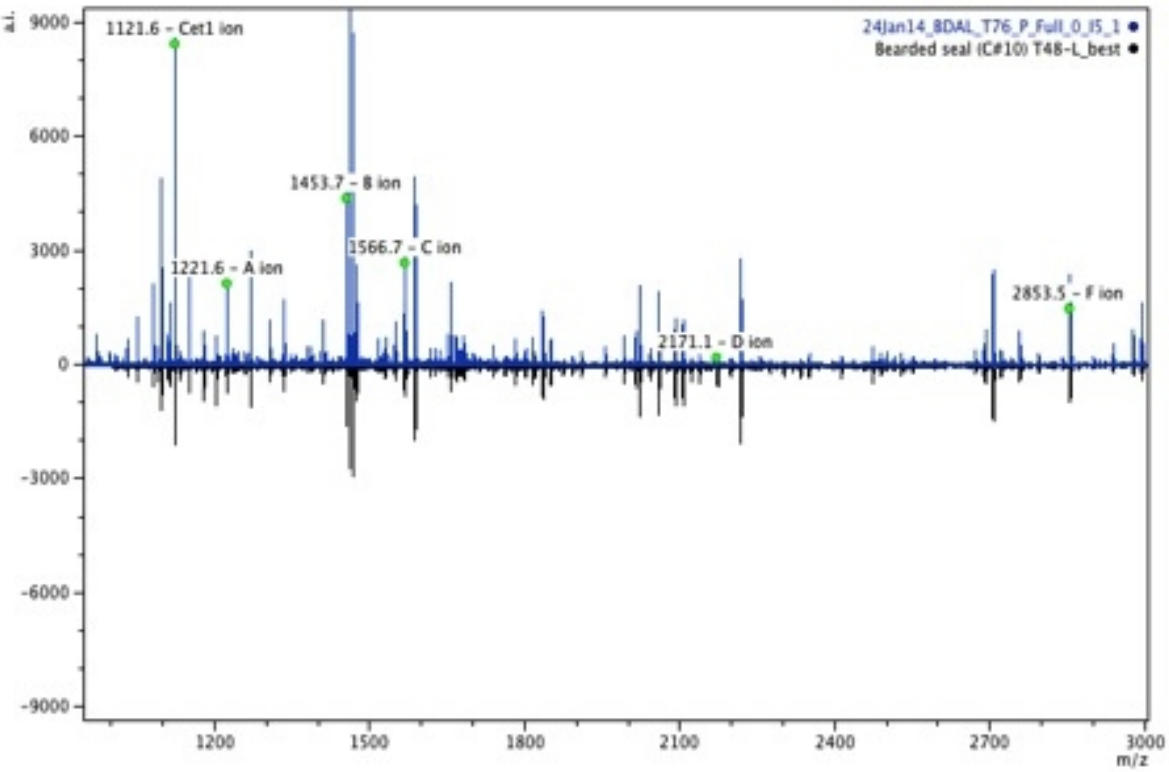

Figure 9: Thick yellowish skin (sole) identified as bearded seal.

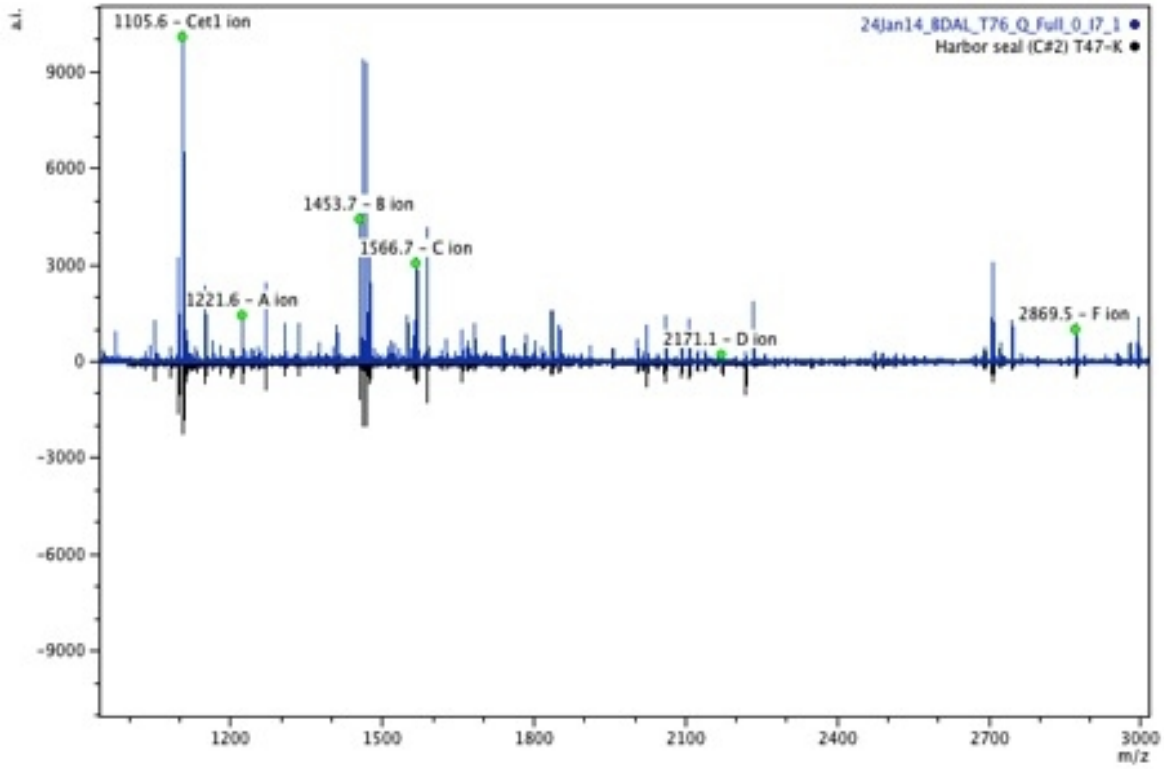

Figure 10: Orange-brown colored skin (boot uppers) identified as a seal in the Phocini tribe (Phocidae family).

Oftentimes the last ion we use for identification (the "G ion" in the high mass region of the mass spectrum) can be difficult to distinguish from other peptides. When this occurs we turn to another procedure called zip tipping which uses Zip Tips® in order to separate the ions in the upper region. In our pair of Child's Boots, the samples from the bottom skin material and the main body material needed this extra step in order to fully resolve the G ions in their respective spectra.

This pair of boots had been examined and discussed in Spring 2013 by a visiting Alutiiq traditional skin sewer with an undergraduate student enrolled in a Harvard course entitled 'Museum Anthropology: Thinking with Objects.' The student and researcher had been both confirming and debating about the boots' construction and materials used. In this case, PMF has contributed to an enhanced understanding of these objects.

References

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Madeline Corona (conservation research associate) provided the analytical work above and was the primary author of this article that appears on the Harvard/Peabody/NCPTT website at http://projects.iq.harvard.edu/pmfcm/