

**Title.** Adipocere inside nineteenth century femora: the effect of grave conditions.

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## **ABSTRACT**

Adipocere has, infrequently, been reported from archaeological contexts normally on the external surface of bodies. In contrast to those cases this study focuses on a white, powdery and greasy substance found inside two right human femora during sampling. These samples were obtained from two identified individuals buried in the late nineteenth century who were exhumed from a rock-cut and a brick-lined grave in a steeply sloping graveyard with wet soil conditions. Both individuals were buried in coffins. Fourier transform infrared spectroscopy (FTIR) was used to test the composition of the substances, and both were found to conform to the spectrum of adipocere. This is likely to be a breakdown product of the fats in bone marrow in an anaerobic, moist environment mediated by bacteria. None of the other individuals (n=6) buried in similar graves displayed evidence of adipocere, this includes those whose femora were in a similar state of preservation (n=4). Contemporaneous data on precipitation for the month of burials do not highlight any obvious trends, but one of the individuals was found in a water-logged grave. The similar preservation of other femora buried in brick-lined graves highlights the interplay of multiple factors in the formation and degradation of adipocere. More importantly it demonstrates differential preservation which may impact on aDNA and other biomolecular research. Furthermore, this adds to the limited data currently available on adipocere found in archaeological contexts.

Keywords: Churchyard; identified skeletal collection; Fewston, North Yorkshire; Fourier transform infrared spectroscopy (FTIR); rainfall

## **Introduction**

Adipocere has been previously described in human archaeological remains from relatively recent times and, occasionally, the more distant past (Aufderheide, 2011; Fiedler et al., 2009; Mayer et al., 1997; Thali et al., 2001). These cases describe adipocere formation on the surface of the body, on the bones or under the skin in water-logged or glacial environments (Mayer et al., 1997). No cases have been published describing its presence inside human bones, as far as the authors are aware. However, adipocere has been found during sampling of recent animal bones (Upex, B. pers. comm.). This case study presents the first known finding of adipocere inside human femora from an archaeological context. Historical and archaeological evidence are used to interpret this finding, which may have implications for bone preservation, histology and biomolecular analyses in this skeletal collection.

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Furthermore this paper provides additional insights into adipocere preservation in grave contexts.

## **Materials and Methods**

The churchyard of St. Michael and St. Lawrence, Fewston, England (National Grid Reference: SE 1947 5411) was partially excavated during the development of the Washburn Valley Heritage Centre (Buglass, 2010). In total 144 skeletons were excavated. The cemetery predominantly dates from 1697 to 1896 (when it was officially closed), but there may be some earlier burials and there is one later, dated to 1921 (Henderson et al., 2013). Twenty individuals (19 adults) were identified through coffin plates or monuments. As part of an ongoing research project, bone cores were collected from the right femora of eighteen of these adults (one adult was excluded due to pathological changes in the sampling area) as well as two unidentified individuals (Table 1). The identified individuals were all buried between 1854 and 1895.

The graveyard of Fewston (Figure 1) lies on a steep slope running from north to south with a gradient of approximately 1 in 6 and a second, less steep slope with a gradient of 1 in 14, running west to east (Buglass, 2010). The underlying geology is an Upper Carboniferous millstone grit which is overlain by a well-draining loamy soil (Buglass, 2010). The soil has a low pH and most of the graves were cut into the acidic gritty, sandy subsoil below with the cut often extending down into the underlying millstone grit (Buglass, 2010). The depth of graves was variable. For those graves which extended into the bedrock, this cut often only extended a few centimetres into the rock to prevent flooding (Buglass, 2010). The grave fill was a mixture of the soil, sub-soil and underlying rock, the latter often caused post-depositional damage to the skeletons (Buglass, 2010). The soil conditions meant that the wooden coffins, textiles and flowers were often well-preserved (Buglass, 2010).

Samples of bone from the femora were collected by drilling a core from the anterior surface at the proximal end, avoiding all anatomical landmarks (Figure 2). During this process a white substance, which was slightly powdery, greasy, and lacking smell, was found inside two of the femora (SLF09 Sk 339 and Sk 226). This substance was not in evidence in any of the other femora. Samples of this were removed from each femur using a clean wooden skewer and placed in sample bags. To test composition, the substances were sent for Fourier

transform infrared spectroscopy (FTIR). FTIR produces spectra based on the infrared absorbance of a sample, providing an indication of its molecular structure and therefore composition. This method has been widely used to test samples for adipocere (Ubelaker and Zarenko, 2011). A Perkin Elmer: Spectrum Two FT-IR Spectrometer was used to obtain a spectrum of the substance. A micro spatula sized sub-sample of the substance was placed directly onto the analysis crystal with an applied force of approximately 100 and scanned between the range of 4000 $\text{cm}^{-1}$  and 450 $\text{cm}^{-1}$ . The resultant spectra were then exported for comparison with published FT-IR spectra of confirmed adipocere samples.

This site has a wealth of associated documentary evidence including death certificates, burial register and a diary written by the son of two of the sampled individuals (John and Mary Dickinson) (Caffell, 2013). The dates of death on the death certificates and dates of burial in the burial register were used to calculate the number of days between death and burial. The interval is most commonly three or four days (Table 1). The diary was used to study the weather, as perceived by the diarist, around the time of death and burial of the identified individuals. The diary pertains to the years 1878-1879, 1881, 1884, 1889, 1891-1893 and 1897. Thus the weather data could only be collected for four of the individuals, including one of those with the white substance inside the femur. Precipitation data for Northeast England, relevant to Fewston (Alexander and Jones, 2000), were incorporated in Table 1 to determine whether this may have played a role in the formation of this substance. However, it should be noted that this information pertains to the entire North-east of England and does not reflect local rainfall or the effect of temperature.

## Results and Discussion

Figure 3 shows the infrared spectra of both samples demonstrating the absorbance bands. Peaks occur at, approximately 2940, 2900, 2870, 1700, 1570 and 1530  $\text{cm}^{-1}$ . Further peaks appear between 450 and 1500  $\text{cm}^{-1}$  and two shoulders to the higher wavenumber peaks at approximately 3020 and 2670  $\text{cm}^{-1}$ . The peak at 1700  $\text{cm}^{-1}$  is consistent with the presence of saturated fatty acids (Forbes et al., 2005a). The lower wavenumber peaks, at approximately 1570 and 1530  $\text{cm}^{-1}$ , represent salts of an unsaturated, hydroxy acids (ibid.). C-H stretching bands are represented by the peaks at 2940, 2900 and 2870  $\text{cm}^{-1}$  (ibid.). Unsaturation of fatty acids is likely to be represented by the peak at approximately 3020  $\text{cm}^{-1}$  (ibid.). Finally, the peak at approximately 2670  $\text{cm}^{-1}$  is likely to be representative of hydroxy-fatty acids (ibid.).

Adipocere is formed from adipose tissue in anaerobic, moderately alkaline, temperate conditions in the presence of water (Moses, 2012; Ubelaker and Zarenko, 2011). In human bone marrow these lipids consist of triglycerides and the fatty acids palmitic and oleic and, in smaller quantities, myristic, palmitoleic, stearic and linoleic acids (Griffith et al., 2009; Lund et al., 1962; Yeung et al., 2008). The quantity of mono-unsaturated fat is lower in bone marrow than subcutaneous fat, while saturated fats are more common (Griffith et al., 2009). No differences in quantity of the major fatty acids have been found between the sexes or in those with differing bone mineral density (Griffith et al., 2009). Therefore, sexual differences would not be expected to lead to differential preservation of adipocere from bone marrow.

Formation of adipocere is via hydrolysis and hydrogenation of adipose and is mediated by bacterial agents (Takatori, 1996; Takatori, 2001), thereby releasing fatty acids (Ubelaker and Zarenko, 2011; Vass, 2001). Adipocere is typically composed of saturated fatty acids, such as myristic, palmitic and stearic acid; unsaturated fatty acids, such as palmitoleic, oleic and lanoleic acids; and hydroxy fatty acids, typically 10-hydroxystearic acid, as well as their salts (Forbes et al., 2005c; Moses, 2012; Ubelaker and Zarenko, 2011). Burial conditions affect the ratios of these components, with wet environments producing higher concentrations of palmitic acids and lower stearic acid concentrations, and vice versa in dry conditions (Forbes et al., 2002). However, the wet condition ratios are the same as those in late stage adipocere formation. Early stage adipocere formation is characterised by high concentrations of the unsaturated fatty oleic acid (Forbes et al., 2002). FTIR cannot be used to determine the relative quantities of the constituent molecules. However, it is clear from the FTIR spectrum of these two samples that the substance found inside both femora, particularly the presence of salts of the component fatty acids, is consistent with adipocere. Of the FTIR spectra published, these two spectra most closely match the spectrum of adipocere found in a sandy-silty soil (Forbes et al., 2005b), but there are clear differences in the region of the spectrum associated with hydroxyl-fatty acids or salts. These differences may be a reflection of the different soil characteristics, described at Fewston as gritty, sandy and acidic, which would have influenced the composition of the fluid in the grave.

Even the most intact femora were slightly damaged allowing water to enter the medullary cavity. In addition, Sk 226 exhibited signs of possible post-depositional fungal damage (Figure 2a). This bone was noted as being unusually thick and harder to sample than that of

any of the other femora. The adipocere in this bone did not extend proximally to the sampling site, whereas in Sk 339 it appeared to extend both proximally and distally within the medullary cavity.

In contrast to the majority of individuals buried at this site both skeletons with adipocere were buried in more elaborate graves. Sk 226 was buried in a rock-cut grave, near a tree which caused root damage, and close to the top of the slope (Figure 1). Sk 339 was buried in a brick-lined vault near the base of the slope and below a soak-away. The grave was found to contain a lot of water at the time of excavation in comparison to the others (Figure 4). Both the rock cut and brick-lined graves would have had greater water retention than the earth-cut graves, but all were buried in low pH conditions. The burials located above and below Sk 339 in the slope (Sk 363 and Sk 408, respectively) were in earth-cut graves and neither had intact right femora. Of the other seven burials in these higher status graves, five had intact femora with slight damage while the other two had postmortem breaks in the diaphyses.

In similarity to the other burials in this part of the cemetery both Sk 226 and Sk 339 were buried clothed in wooden coffins. Due to the wet soil conditions the coffins were often well-preserved (Buglass, 2010). Most coffins were constructed from pine (*Pinus* sp.) (Buglass, pers. comm), while oak (*Quercus* sp.) was used for Sk 339's (Buglass, 2010). The soil conditions preserved some organics and textiles, including evidence of flowers (Table 1). Previous studies have found that modern (i.e. non-natural fibre) clothing enhances adipocere formation (Forbes et al., 2005c). However, the effect natural fibres have on adipocere formation has yet to be determined. Burial in a coffin is known to retard adipocere formation (Forbes et al., 2005a), but, as with other studies, this relates to the external surfaces of the body. The preservation of organics and textiles was good in the wet grave of Sk 339, but only wood from the coffin was preserved in that of Sk 226, so there are clear differences in organic preservation between the two graves.

The weather at the time of burial is only available for four of the individuals, including Sk 339. Sk 339, Sk 366 (buried in the same part of the cemetery as Sk 339) and Sk 122 (the latter two buried in earth-lined graves) were buried in cold Februaries. The latter was a wetter year and his two days of rainfall followed his death. The third burial (Sk 363, buried above Sk 339 in the slope) took place during a misty and wet November. It seems likely that the adipocere formation has occurred in Sk 226 and Sk 339 due to wet burial conditions at the

time of death and within the rock-cut and brick-lined graves. It is not possible to compare the weather or precipitation at the time of burial for all of these graves due to missing data.

Precipitation during the months of burial of the two skeletons with adipocere was 44.7mm (c. 1.5mm per day) and 58.9 mm (c. 2mm per day) (Table 1) and for the other individuals ranged from 2.5 to 81.1 mm.

The wet soil conditions and soil type (described by the excavator Buglass, 2010), the lining of the graves and the minimal postmortem damage to the femora are likely to have played a role in providing suitable conditions for the formation and preservation of adipocere inside these two femoral medullary cavities. This may have been aided by the similarity in precipitation at the time of burial for these two individuals, although comparisons could not be made with the other individuals buried in similar graves due to missing data. Nevertheless, this could explain why neither Sk 300 nor 307 (both buried in the same area of the graveyard as Sk 339 in brick vaults), had adipocere preserved, or why it may not have formed in these medullary cavities. Adipocere formation and its degradation are known to be complex, site-specific processes, involving the interplay of water, oxygen, fungi and bacteria (Fründ and Schoenen, 2009). Bacteria, such as *Clostridium*, assist adipocere formation, whereas gram-positive bacteria, fungi and oxygen degrade it rapidly (Fründ and Schoenen, 2009). Soil bacteria and the presence of fungi have not been tested at this site, but it is probable that fungal infestation affected Sk 226 (Figure 2). These are likely to have played a significant role in the differential preservation of these individuals, which may impact on biomolecular preservation.

## **Conclusion**

This case study highlights the potential for adipocere to be present inside trabecular bone from archaeological contexts in wet soil. The differential preservation of these skeletons has previously been reported and linked to the soil conditions (Caffell and Holst, 2010). The presence of such a range of preservation at this site is likely to have implications for biochemical analysis of these individuals. It is probable that the presence of adipocere in the femora of Sk 226 and Sk 339 will be indicative of good biomolecular preservation.

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Table 1 List of skeletons from which femoral samples were taken. Grave types and organic preservation data based on the archaeological report (Buglass, 2010). Weather data paraphrased from John Dickinson's diary (Caffell, 2013) and precipitation from the meteorological office (Alexander and Jones, 2000). All burials were in coffins, unless otherwise stated.

No.	Method of identification	Name	Sex	Age	Date of death	Interval between death and burial (days)	Right femur (sampled) intact	Grave type (earth-out unless otherwise stated)	Preservation of organics and textiles	Monthly precipitation for the set England (mm)	Weather
88	n/a	n/a	n/a	n/a	n/a	n/a	Slight damage to cortical bone at proximal end	Rock-out grave (deep)	Wood and fowlers	n/a	n/a
119	Coffin plate	Matthew Marjerson	male	38	25th Feb, 1890	4	Slight damage to cortical bone at proximal end			37.1	n/a
122	Coffin plate	Jane Marjerson	female	67	27th Feb, 1891	4	no		Wood and textile	2.5	On the day she died it was nice weather, but cold. On the day before she was buried it was very windy and wet.
130	Coffin plate	George Lister	male	66	19th Jul, 1882	3	Slight damage to cortical bone at proximal end	Brick and stone lined vault	Textile Wood and mineralised textile	81.1	n/a
138B	Partly legible monument	James Dibb	male	79	17th Mar, 1890	5	no	Coffin shared with 135A		56.4	n/a
226	Coffin plate	David Lister	male	84	16th Apr, 1888	3	Slight damage to cortical bone at proximal and distal ends	Rock-out grave	Wood	58.9	n/a
238	Coffin plate + burial register	Elizabeth Demaine	female	49	6th Apr, 1888	6	no	Grave cut by [199]	Wood and resin in base of coffin	58.9	n/a
300	Monument	Mary Dambrook	female	77	7th Sept, 1870	3	Slight damage to cortical bone at proximal and distal ends	Rubble filled, slab sealed brick vault, grave shared with SK 307	Wood and resin in base of coffin	n/a	n/a
307	Monument	Joseph Dambrook	male	77	7th Mar, 1869	4	Slight damage to cortical bone at proximal end	Rubble filled, brick-lined vault, grave shared with SK 300	Wood and textile	n/a	n/a
310	Coffin plate	Mary Dickinson	female	66	6th Mar, 1866	4	Slight damage to cortical bone at distal end		Wood	68.7	n/a
319	Monument	Sarah Dambrook	female	22	26th May, 1854	2	no	Rock-out grave (deep), in coffin with a glass window and resin and in lining	Wood and resin in base of coffin	n/a	n/a
339	Coffin plate	Richard Gill	male	41	11th Feb, 1884	3	Slight damage to cortical bone at proximal and distal ends	Brick-lined vault, with a lot of water in the grave	Wood, flowers and leaves in coffin and wig (possibly horse hair)	44.7	He died on a very wild cold day with a strong wind, two days later it was clear, and on the day he was buried it was spring-like. Afterw ards it was cold but dry.
342	Coffin plate	Beriley Dambrook	male	26	1st Nov, 1862	4	no	Rock-out grave	Wood	n/a	n/a
351	Monument	John Dickinson	male	63	18th Aug, 1875	3	Slight damage to cortical bone at proximal and distal ends		Wood	60.6	n/a
360	Coffin plate	Gill Wigglesworth	male	67	24th Apr, 1886	4	Slight damage to cortical bone at proximal and distal ends		Wood	45.6	n/a
363	Coffin plate	Sarah Gill	female	54	13th Nov, 1869	3	no		Wood	25.9	It was misty and dark on the day she died, followed by a day of dull dirty weather and two days after she was buried it was a very dull and misty day.
366	Coffin plate	John Renton New some	male	76	3rd Feb, 1892	3	Slight damage to cortical bone at proximal and distal ends		Wood and textile	65.4	He died on a very cold day with frost, which had been preceded by two days of rain. His funeral was held on a very cold day.
378	Coffin plate	Biza Wigglesworth	female	34	27th Feb, 1895	3	Slight damage to cortical bone at proximal end		Wood	25.1	n/a
408	Coffin plate	Richard Gill	male	78	18th May, 1883	4	no		Wood, Leather and textile (possibly coffin lining)	47.3	n/a
426	n/a	n/a	n/a	n/a	n/a	n/a	Slight damage to cortical bone at proximal end	Wood		n/a	n/a

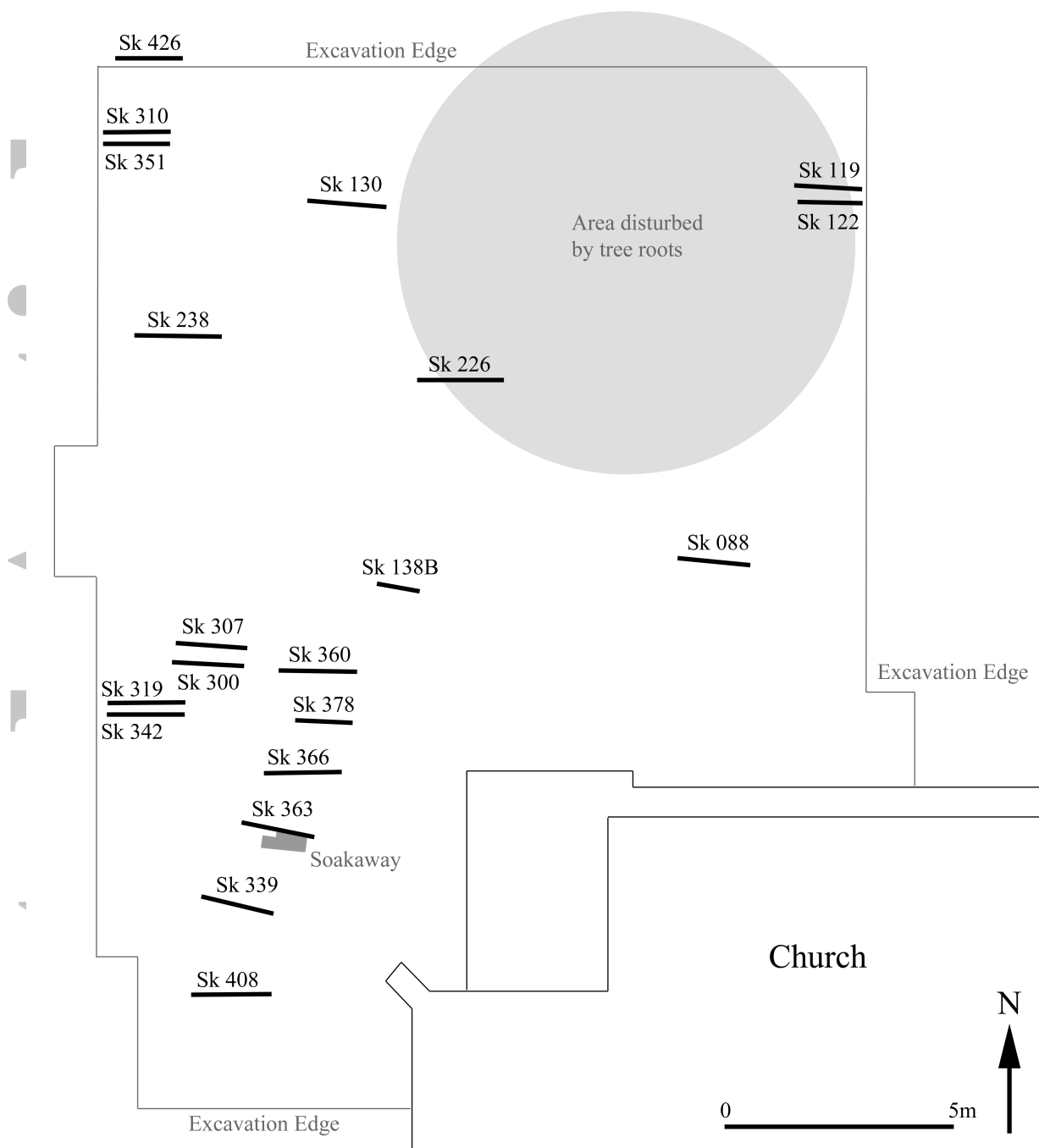


Figure 1 Plan of churchyard, only graves of the sampled individuals are indicated. Churchyard slopes steeply from North to South and less steeply from West to East. This plan is modified from that produced by Stephen Toase for John Buglass Archaeological Services.

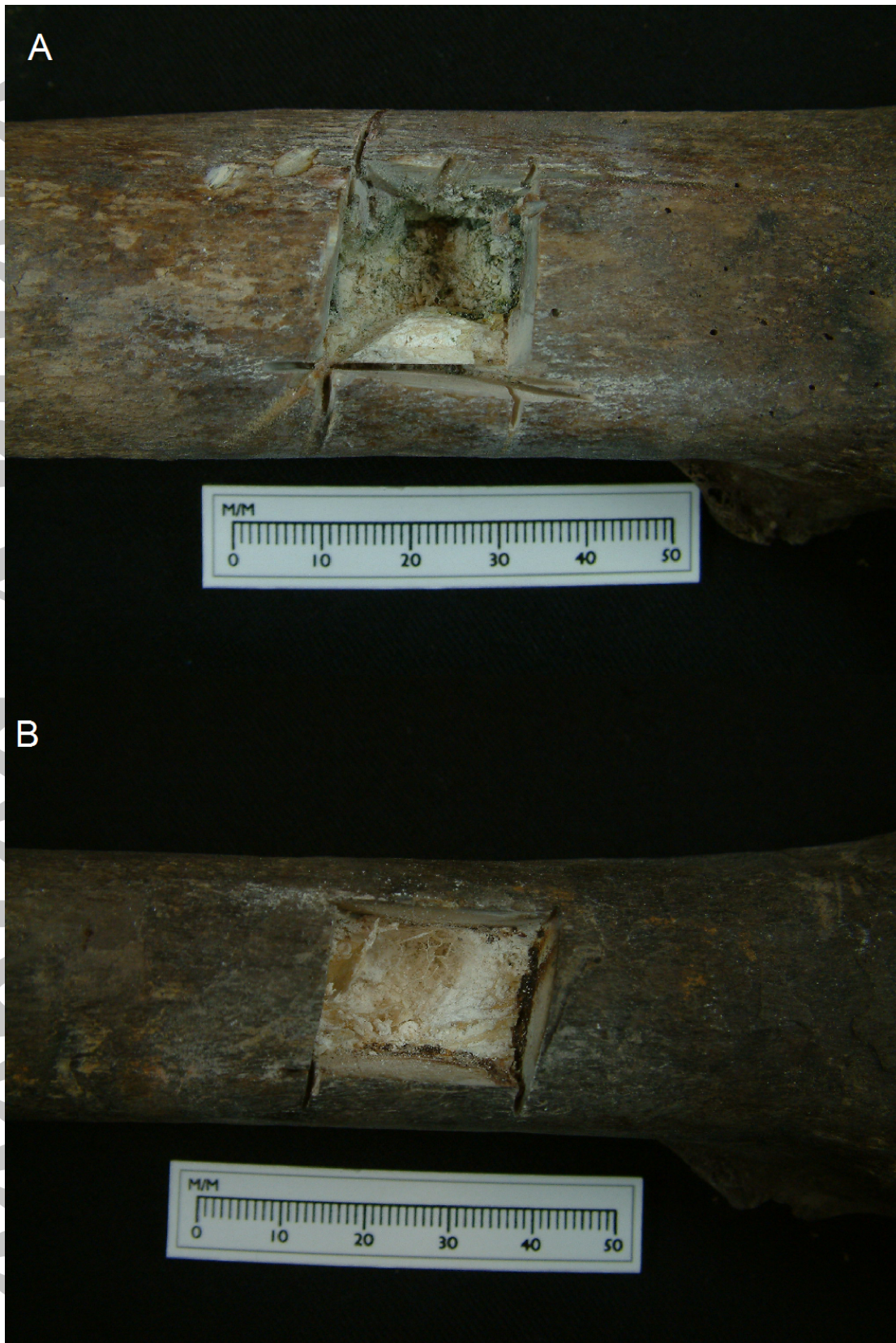


Figure 2 Sampling site and in-situ adipocere. A. Sk 226, note the circular holes proximal to the sampling site which are possibly indicators of fungal damage. B. Sk 339 (both are right femora)

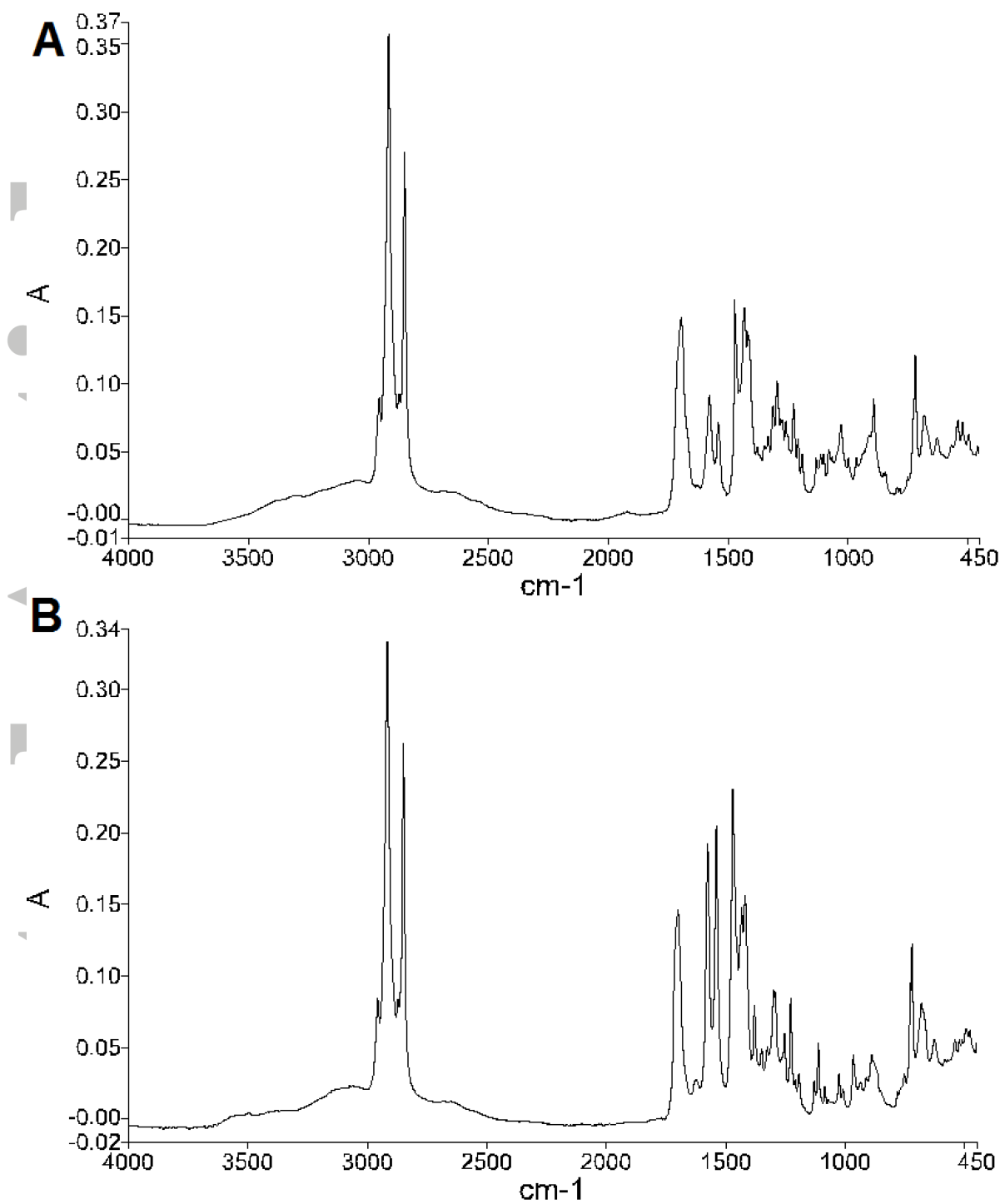


Figure 3 FTIR spectra of the samples. A. Sk 226 and B. Sk 339.



Figure 4. Comparison of graves. A Grave of Sk 339 showing water-level and preservation of the oak coffin. B. Sk 226. C. Sk 300 showing a brick-lined grave. All photographs used with permission of the excavator, J. Buglass.

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